

Langridge, James P. (2004) Correlating instrumental and sensory analyses of flavour. PhD thesis, University of Nottingham.

Access from the University of Nottingham repository:

<http://eprints.nottingham.ac.uk/11060/1/408655.pdf>

Copyright and reuse:

The Nottingham ePrints service makes this work by researchers of the University of Nottingham available open access under the following conditions.

This article is made available under the University of Nottingham End User licence and may be reused according to the conditions of the licence. For more details see:
http://eprints.nottingham.ac.uk/end_user_agreement.pdf

A note on versions:

The version presented here may differ from the published version or from the version of record. If you wish to cite this item you are advised to consult the publisher's version. Please see the repository url above for details on accessing the published version and note that access may require a subscription.

For more information, please contact eprints@nottingham.ac.uk

10 0420078 5



Correlating Instrumental and Sensory Analyses of Flavour

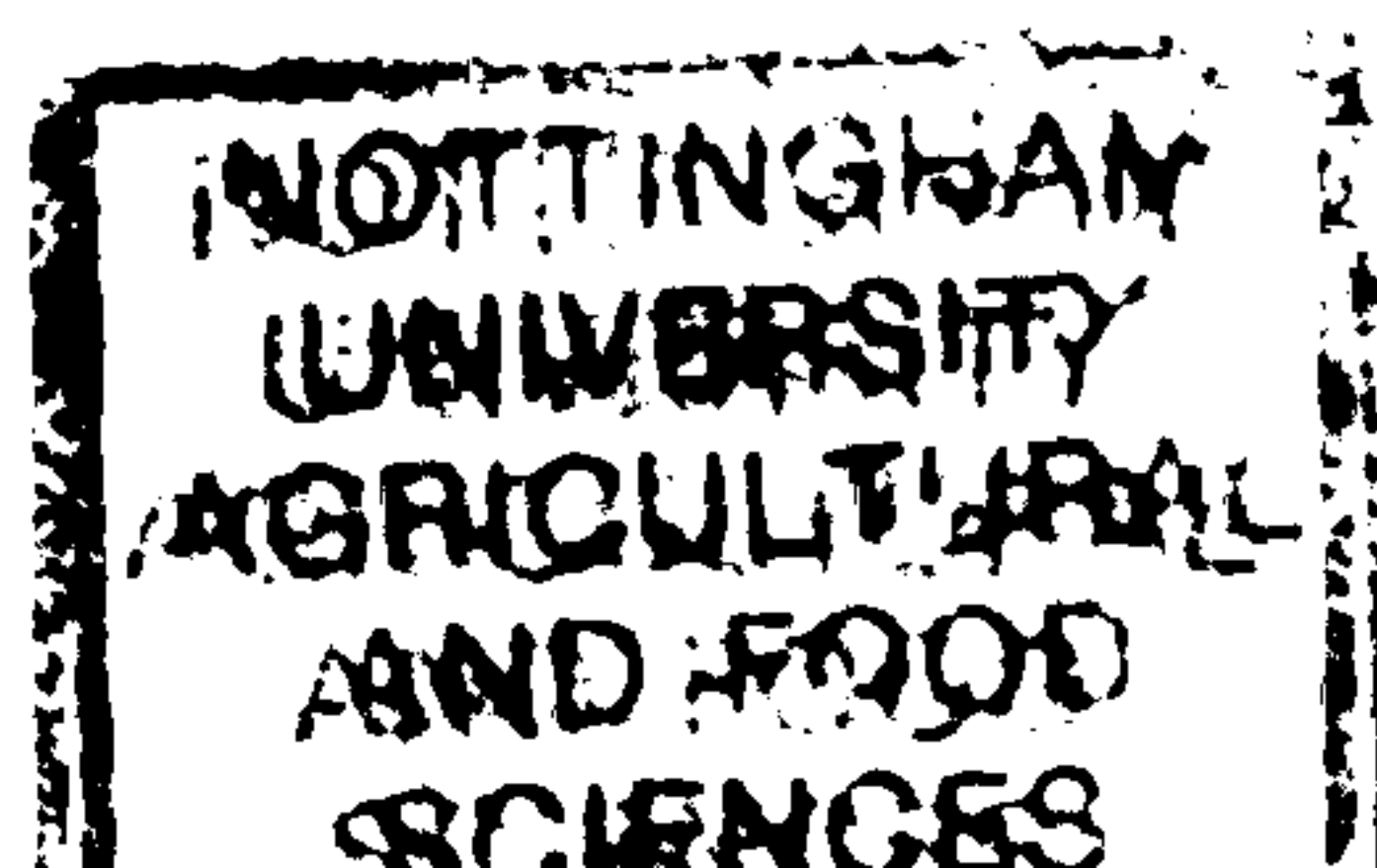
By

James P. Langridge BSc

Thesis submitted to the University of Nottingham for the degree of Doctor of
Philosophy

Samworth Flavour Laboratory
Division of Food Sciences
University of Nottingham
Sutton Bonington Campus
Loughborough
LE12 5RD

September 2004



ACKNOWLEDGEMENTS

I must first acknowledge Prof. Andy Taylor for giving me the opportunity to undertake a PhD, especially with my initial lack of food and flavour chemistry. I vividly recall my interview where in order to make an impression I decided to make what I thought was an intellectual comment. As we passed a student analysing strawberries on the mass spectrometer I said, *so the machine is measuring the ethers coming off the fruit*. Andy politely replied, *well I think it's probably esters*. Since then, his continued support, encouragement and suggestions have helped me through my PhD.

These three years would also have not been possible were it not for my sponsors; BBSRC and CCFRA (Campden, Gloucestershire). In particular, I would like to thank Dr. Inger Baek, who initially supervised the project and Chantal Gilbert, who then took over in my second year. Both of these people showed wonderful enthusiasm for the project and in general CCFRA have given me essentially a free reign without constraint. This is perhaps unusual with many industrial sponsors.

A big thank you is given to both Dr. Rob Linforth and Dr. Guy Channell, without whom the building of the different olfactometers would have been impossible and also for the flavour knowledge that Rob has passed on to me.

Other friends and colleagues I'd like to thank begin very importantly with Dr. Tracey Hollowood, Dr. Joanne Hort, Dr. Fiona Martin and Louise Hewson - sensory's answer to Grease's *Pink Ladies*! Anything I write is really an understatement in how much I have appreciated their wisdom, encouragement, friendship and saucy sense of humour. I'd also like to mention the fellow Brits with whom I began my PhD journey for always being a welcome distraction - Sarah, Mike and Chris. Additionally, I'd like to thank the French contingent in the lab, in particular Lionel, Celine (x2) and Johann, whose Gallic influence has always made me smile. I have even learnt to shrug in true French style!

Finally, I must mention my family who have fully supported me in every sense throughout my PhD, especially my parents who have shared in my enthusiasm or sometimes my despair. My brothers now have their own families but have always had time for me, which is so important to me - more than they know.

If there is anyone who reads this and decides they too deserve a thankyou, please write your name in the space here.....

TABLE OF CONTENTS

ACKNOWLEDGEMENTS	I
TABLE OF CONTENTS	III
ABSTRACT	XIII
1 INTRODUCTION	1
1.1 FLAVOUR AND PERCEPTION	3
<i>1.1.1 Olfaction</i>	3
1.1.1.1 Physiology	3
1.1.1.2 Transduction	6
1.1.1.3 Cognition	8
1.1.1.3.1 Physiological consequences.....	8
1.1.1.3.2 Odour quality, context and labelling.....	8
1.1.1.3.3 Simulation of neural response.....	9
1.1.1.3.4 Mixture perception.....	10
1.1.1.3.5 Memory	10
1.1.2 The trigeminal system	12
1.1.3 Gustation	13
1.1.4 Taste-aroma interactions	14

1.2 MAKING PERCEPTUAL MEASUREMENTS	17
1.2.1 <i>Psychophysics and threshold determination</i>	18
1.2.2 <i>Olfactometry.....</i>	21
1.2.3 <i>Time Intensity (TI)</i>	23
1.2.4 <i>Electroencephalographic techniques (EEG)</i>	25
1.3 INSTRUMENTAL ANALYSIS OF FLAVOUR	26
1.3.1 <i>Gas chromatography.....</i>	26
1.3.2 <i>Direct gas phase analysis</i>	28
1.3.2.1 <i>Atmospheric pressure chemical ionisation - mass spectrometry APCI-MS</i>	28
1.3.2.2 <i>Electronic noses.....</i>	30
1.3.3 <i>Correlating instrumental and sensory analyses of flavour.....</i>	30
1.3.3.1 <i>Compositional versus sensory analysis</i>	31
1.3.3.2 <i>The effect of matrix</i>	32
1.3.3.3 <i>Modelling the sensory response as a function of time.....</i>	34
1.4 AIMS	35
2 OLFACTOMETRY – METHOD DEVELOPMENT	36

2.1 OLFACTOMETRY - THRESHOLD DETERMINATION	38
<i>2.1.1 Volatile vaporisation olfactometer</i>	<i>38</i>
2.1.1.1 Design.....	38
2.1.1.2 Olfactometer output prediction and validation	38
2.1.1.3 Instrumental analysis	39
2.1.1.4 Results	40
<i>2.1.2 Sniff bottles: static and dynamic.....</i>	<i>43</i>
2.1.2.1 Change in headspace concentration over time	44
Results	45
2.1.2.2 Static headspace bottle calibration.....	46
Results	46
2.2 OLFACTOMETRY - METHOD OF PRODUCING VOLATILE PULSES	49
<i>2.2.1 Design.....</i>	<i>49</i>
<i>2.2.2 Validation method - Ion Trap Mass Spectrometry (ITMS)</i>	<i>50</i>
<i>2.2.3 Results</i>	<i>50</i>
2.2.3.1 Square edged pulse testing	50
2.2.3.2 Pulse time accuracy	51

2.2.3.3 The effect of olfactometer oven temperature on pulse shape and height	52
2.2.3.4 Repeatability of pulse programmes.....	53
2.2.3.5 Peak and trough resolution	55
2.2.4 <i>A note on olfactometer output concentration</i>	57
2.3 CONCLUSION	58
3 RETRONASAL AND ORTHONASAL THRESHOLDS: CORRELATING PANEL VARIATION WITH <i>IN VIVO</i> ANALYSIS	59
3.1 MATERIALS AND METHODS	61
3.1.1 <i>Subjects</i>	61
3.1.2 <i>Selection of compounds</i>	61
3.1.3 <i>Sample preparation</i>	61
3.1.4 <i>Calibration of the sniff bottles</i>	62
3.1.5 <i>Nosespace concentration</i>	63
3.1.6 <i>Mouth-Nose Partition Coefficient (MNPC)</i>	64
3.1.7 <i>Volatile persistence in the breath</i>	65
3.1.8 <i>Peak height: area ratio</i>	66

3.1.9 <i>Threshold determination methods</i>	67
3.1.10 <i>Data analysis</i>	68
3.2 RESULTS	68
3.2.1 <i>Repeatability of the psychophysical and instrumental method</i>	68
3.2.2 <i>Psychophysical results analysis</i>	69
3.2.3 <i>Relationship between orthonasal and retronasal thresholds</i>	71
3.2.4 <i>Regression analysis of instrumental measurements versus psychophysical</i>	73
3.2.5 <i>Relationships between instrumental and psychophysical measurements: variation between the volatiles.</i>	74
3.3 DISCUSSION	76
3.3.1 <i>Relationships between the orthonasal and retronasal thresholds</i>	76
3.3.2 <i>Regression of psychophysical and instrumental data</i>	77
4 PERCEPTION OF PULSED AROMA: DISCRIMINATION TESTING	79
4.1 PRELIMINARY STUDIES	80
4.1.1 <i>Discrimination testing</i>	80
4.1.1.1 <i>Method</i>	80

4.1.1.1.1 Olfactometer programme	80
4.1.1.1.2 Sensory procedure	81
4.1.1.1.3 Choice of aroma.....	82
4.1.1.2 Results	83
4.1.2 <i>Simultaneous breath measurement and discrimination test</i>.....	85
4.1.2.1 Method	85
4.1.2.1.1 Procedure.....	85
4.1.2.1.2 Simultaneous instrumental measurement.....	86
4.1.2.1.3 Instrumental data extraction method.....	86
4.1.2.2 Results	90
4.1.2.2.1 Sensory result.....	90
4.1.2.2.2 Relationship between sensory choice and instrumental parameters	90
4.1.2.3 Discussion.....	92
4.2 SIMULTANEOUS DISCRIMINATION TEST AND INHALATION BREATH BY BREATH STUDIES.....	93
4.2.1 <i>Method</i>.....	94
4.2.1.1 Olfactometer programme	94
4.2.1.2 Sensory procedure.....	95
4.2.1.3 Instrumental measurement	95
4.2.1.4 Instrumental data extraction method	96
4.2.2 <i>Results</i>	99

4.2.2.1	Sensory results	99
4.2.2.2	Instrumental results - experimental control	100
4.2.2.3	Instrumental ratios and their effect on certainty rating.....	102
4.2.2.4	Ratios of instrumental parameters with respect to sensory choice when the same stream was presented twice	102
4.2.2.5	Ratios of instrumental parameters with respect to sensory choice in tests where A2 was paired against C _{0.6} in either direction	104
4.3	DISCUSSION.....	106
5	PERCEPTION OF PULSED AROMA - TIME INTENSITY	109
5.1	METHOD	111
5.1.1	<i>Olfactometer output programme</i>	<i>111</i>
5.1.2	<i>Sensory procedure.....</i>	<i>112</i>
5.1.3	<i>Instrumental analysis and data extraction</i>	<i>112</i>
5.1.3.1	APCI-MS settings.....	112
5.1.3.2	APCI-MS trace interpretation	113
5.1.3.3	Data point time standardisation.....	113
5.1.4	<i>Statistical analysis.....</i>	<i>114</i>

5.2 RESULTS	114
5.2.1 <i>Raw results</i>	114
5.2.1.1 Instrumental	114
5.2.1.2 Sensory analysis	116
5.2.2 <i>Exhalation measurement: principal component analysis and assessor grouping.</i>	118
5.2.2.1 Sensory TI.....	118
5.2.2.2 Comparing sensory principal curves to instrumental data.....	123
5.2.3 <i>Inhalation measurement: principal component analysis and grouping.</i>	126
5.2.3.1 Sensory TI.....	126
5.2.3.2 Comparing sensory principal curves to instrumental data.....	130
5.2.4 <i>Analysis of data sets according to the minor component (PC2).....</i>	134
5.3 DISCUSSION.....	139
5.3.1 <i>Summary.....</i>	139
5.3.2 <i>Comparison of time intensity and paired comparison experiment.....</i>	140
5.3.3 <i>Relevancy to previous and current work</i>	141

6 PHYSICAL DISRUPTION OF THE AROMA STIMULUS.....	144
6.1 METHODS AND MATERIALS	145
6.1.1 <i>Breath volatile measurement</i>	145
6.1.2 <i>Breath flow measurement</i>	145
6.1.3 <i>MASTICATION MEASUREMENTS (ELECTROMYOGRAPHY; EMG)</i>	146
6.1.4 <i>Swallowing measurements</i>	147
6.1.5 <i>Aroma presentation.....</i>	147
6.2 RESULTS	148
6.2.1 <i>The effect of air flow on the volatile concentration measured by the APCI-MS in the inhalation configuration</i>	148
6.2.1.1 <i>Controlled flow of nitrogen past the interface</i>	148
6.2.1.2 <i>Different breathing rates and velocities</i>	149
6.2.1.3 <i>The relationship between breathing rate and breath flow rate</i>	150
6.2.2 <i>The disruption of the stimulus from retronasally presented aromas by mastication.....</i>	152
6.2.3 DISRUPTION OF THE STIMULUS FROM RETRONASALLY PRESENTED AROMAS BY SWALLOWING.....	154

6.3 DISCUSSION.....	155
7 GENERAL DISCUSSION	157
BIBLIOGRAPHY	162

ABSTRACT

The relationship between *in vivo* captured data from an atmospheric pressure chemical ionisation mass spectrometer (APCI-MS) and sensory/psychophysical analyses was investigated. The stimuli used were mainly single volatiles under gas phase control or calibration by development of different olfactometry methods.

Gas phase concentration retronasal (via the mouth to the nasal cavity) and orthonasal (via the nostrils) thresholds were determined for a trained panel of 13 individuals. Four volatiles were used with different sensory/physico-chemical properties and an adapted staircase method was employed to measure the individual thresholds. The data showed good repeatability over short durations of one week and also longer ones of eight months. It was used to test the hypothesis that thresholds varied between people due to differences in their in-nose concentration as measured or estimated by the APCI-MS. The analysis did not support this theory but relationships between orthonasal and retronasal thresholds were shown, in which the latter were ~50 times lower than the former. Threshold determination of a larger group of 20 individuals revealed clusters of individuals.

Methods of producing square edged pulses of aroma compound in the gas phase were developed using a modified chromatograph autosampler with a gas flow of $5 \text{ mL}\cdot\text{min}^{-1}$ and pulse rate of 0.6 secs. A trained panel of 23 individuals performed two types of sensory test using pulsed and constant olfactometer outputs of isoamyl acetate. The original intention was to reveal whether pulsed odorants were perceived as the same as or different to constant concentration. Initial experiments yielded results that were difficult to interpret, although the nature of the results was clarified when simultaneous breath by breath analysis techniques were employed. Here it was shown that each individual in different repetitions disrupted the olfactometer output pattern in unpredictable ways. This pattern disruption was measured in two instrumental configurations, as either volatiles in an exhalation or volatiles as they were inhaled together with two types of sensory test. In both sensory tests the pattern

of aromas in an inhalation revealed a relationship with perception. In particular, the sensory response in the time intensity study was related to differences in the inhalation profiles between people, which in turn was related to an individual's breathing. This shows that physiological differences such as breathing and the structure of the nasal cavity have an impact on perception.

1 INTRODUCTION

The senses of smell and taste have often been considered relatively unimportant in comparison to the other modalities. If this were true, then it seems illogical that the production and market for flavours and fragrances is one of the fastest growing industries. For example, in the US this industry is forecasted to be worth \$6 billion by 2007. The increase is mostly driven by the ongoing consumer preference for natural ingredients, and rising consumer interest in more authentic flavours and fragrances (Flavors & Fragrances, Global Information, 2004). This then drives technology and large investment into research and development in order to crack the secrets of receptor function, flavour release and sensory processing. Despite all the vast areas of research, a number of gaps in the knowledge remain.

The main problem in understanding the mechanisms behind olfaction and gustation is the fact that they cannot be reduced to single dimensions. Chemoreception describes the process, where exposure to disconnected chemical stimuli is converted into a neural response. This is in contrast to sight, which can be reduced to physical measurements such as amplitude (level of grey) and wavelength, for light and colour perception respectively that in turn provide a continuum. The perception of sound can also be reduced to just two dimensions; vibrational frequency (pitch) and amplitude (strength). Olfaction, however, can only be reduced to a response dimension, where two million olfactory receptors are subjected to the vast range of chemical stimuli available (Jaubert, *et al.*, 1995).

After transport to the receptors, the psychological responses to these chemical stimuli can be recorded and interpreted by psychophysical and sensory techniques such as threshold determination and time intensity. However, correlation between these analyses and instrumental measurements is generally inconsistent and ambiguous in its interpretation.

Modelling chemoreception is problematic before it is possible to understand the relationships between the stimulus, the receptor and the response, because the chemicals must be first released from their source and transported to the appropriate areas (Taylor, 2002). This means that the chemical stimuli in their original and generally stable form (distal stimulus) are subjected to a total disruption on their path to the receptors (proximal stimulus). This is in contrast to the stimuli pertaining to perception of vision and sound, where disruption along the path from source to sense is relatively minor. Furthermore, disruption of chemical stimuli is not just restricted to food matrices, as simple air borne odours will also be subject to state change as they are inhaled and differentially absorbed into the mucosal regions of the nose (Keyhani, *et al.*, 1997). For this reason, instrumental techniques that monitor aroma compounds in the breath as a simulation of the proximal stimulus, have revolutionised our understanding of flavour perception (Taylor, *et al.*, 2000).

This thesis introduces new ideas about the relationship between proximal measured stimuli and the sensory response. It is therefore important to first describe how the various disciplines of flavour research can combine to try to explain the complex nature of this relationship.

1.1 FLAVOUR AND PERCEPTION

1.1.1 Olfaction

1.1.1.1 Physiology

The organs associated with olfaction are depicted in Figure 1.1.

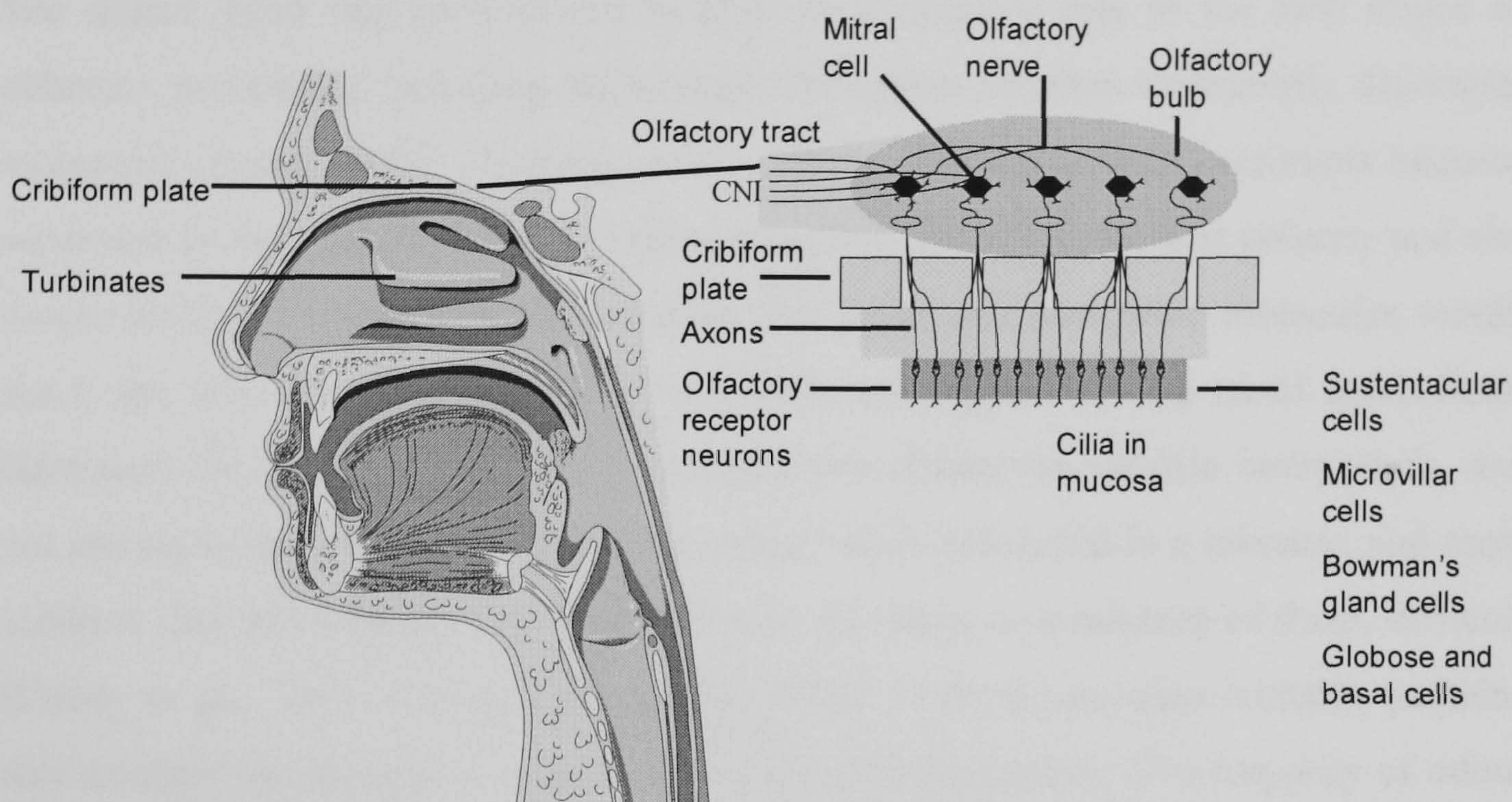


Figure 1.1 Cross section diagram of face showing organs associated with the olfactory system¹.

The cells responsible for olfaction are situated in three areas in the upper recesses of the nasal chamber: the cribriform plate, the superior/middle turbinates and the septum. These areas are covered with a mucosal epithelium, which contains six morphologically and biochemically distinct cells. The first and most important cell is the bipolar sensory receptor neuron, which transduces signals to cranial nerve I (CNI). The second cell type is known as the sustentacular or supporting cell. These cells have been shown to have a number of functions including the regulation of mucus production and they may be responsible for the deactivation of odorants by producing xenobiotic enzymes containing cytochrome enzymes P450. The function of the remaining cells; microvillar, Bowman's gland, globose and basal, is generally less understood and is thought to be mainly concerned with repairing damage to the nasal epithelium (Doty, 2001). The receptor cells have recently been shown by Nobel

¹ Leffingwell & Associates: Olfaction - A Review - www.leffingwell.com/olfaction.htm

prize winners Richard Axel and Linda Buck (1991) to be coded by a large family of about one thousand genes, from which each olfactory cell expresses only one odorant binding receptor each.

The mucus itself has been shown to play an invaluable role in the first stages of olfactory processing including differential absorption between structurally dissimilar molecules (Laing, 1988; Hornung, *et al.*, 1987). During a 'sniff', odorants become separated as they are absorbed in different regions according to their polarity and air-mucus partition coefficient. This means that polar and non-polar molecules would reach the appropriate receptors in very different regions of the nasal epithelium. However, the degree of proximity between two dissimilar volatile compounds was not shown to have an effect on the perception when presented in a mixture, and most subjects find identification of more than two odorants, in a mixture of three, difficult (Laing, *et al.*, 2002; Laing, 1988; Laing, 1986). The mucus also contains proteins that enhance the process of odorant transport to the receptors. The majority of odour active molecules are hydrophobic and therefore only a small portion of the compounds passing through the nasal cavity would diffuse through the mucosa to the receptors. Odorant binding proteins (OBP's) are present in the mucosa that reversibly bind to volatile chemicals and transport them to the bipolar receptor cells (Tegoni, *et al.*, 2000). These proteins of the lipocalin family have a low but wide range of ligand binding properties as shown in spectroscopic binding assays with compounds from heterogenous families (Lobel, *et al.*, 2002).

The bipolar receptor cells play the most important role in the onset of signal transduction. These cells are lined with cilia, which contain a seven domain transmembrane receptor. These proteins comprise the largest family of G-protein-coupled-receptors and function by initiating a biochemical cascade after interaction with an odorant, which leads to cell depolarisation and transduction to the olfactory bulb via olfactory fila (Dryer and Berghard, 1999). These fila are comprised of the 6 million receptor cell axons as coalesced structures that traverse the cribriform plate

and subsequently make connections with the olfactory bulb, where the information processing begins.

Anatomical computational fluid dynamics of the nasal cavity have been used to model the air flow in order to show the effects of the different structures such as the turbinates on odorant transport. Research has mainly used scanning techniques to produce three-dimensional models of the nasal cavity, which can then be re-created on a far larger scale. Some important aspects of olfaction have been discovered in this way such as the flow of air through the nasal cavity being laminar and not turbulent (Keyhani, *et al.*, 1995; Keyhani, *et al.*, 1997), that different areas will be subjected to very different flow rates and also the effect on different volatiles. One recent study showed that at different anatomical conditions, air flow rate through the olfactory region and odorant uptake rate in olfactory mucosa can possibly differ by up to two orders of magnitude even though the flow rates remained similar (Zhao, *et al.*, 2004). Other studies have investigated these structural differences with regards to the problem of pollutant control and damage to the nasal cavity, where it has been postulated that low flows were observed in higher olfactory regions in order to protect the olfactory bulb from particles (Kelly, *et al.*, 2000; Martonen, *et al.*, 2001; Martonen, *et al.*, 2002).

1.1.1.2 Transduction

Although genetic research has shown that only one type of receptor is derived from a single allele, each one is generally responsive to a wide range of chemicals. It is generally acknowledged that different receptors will have a range of binding coefficients to the same odorant, which in turn provides a neural code that is then processed by the higher brain cortices. Currently, there is extensive research into understanding this characteristic odour signature from the binding of odorant to receptor, and there are three main theories: vibrational, molecular shape and functional group theory. Vibrational theory assumes that odorants elicit an individual signature frequency that is decoded in the brain centres and corresponds to a certain sensation. An example of this has been demonstrated in an experiment where differently labelled analogues of benzaldehyde were perceived as significantly dissimilar in duo-trio tests (Haffenden, *et al.*, 2001). Molecular shape-receptor 'lock and key' theories that mirror the known affinities between enzyme and substrate have been studied for many decades and have been limited in providing any substantial evidence (Turin, 2002). It is more acknowledged that a combination of molecular size, disposition of the functional groups and chirality are responsible for the interaction between stimulus and receptor to be deciphered (Laing, *et al.*, 2003; Laska, 2002). The transduction process is summarised in Figure 1.2.

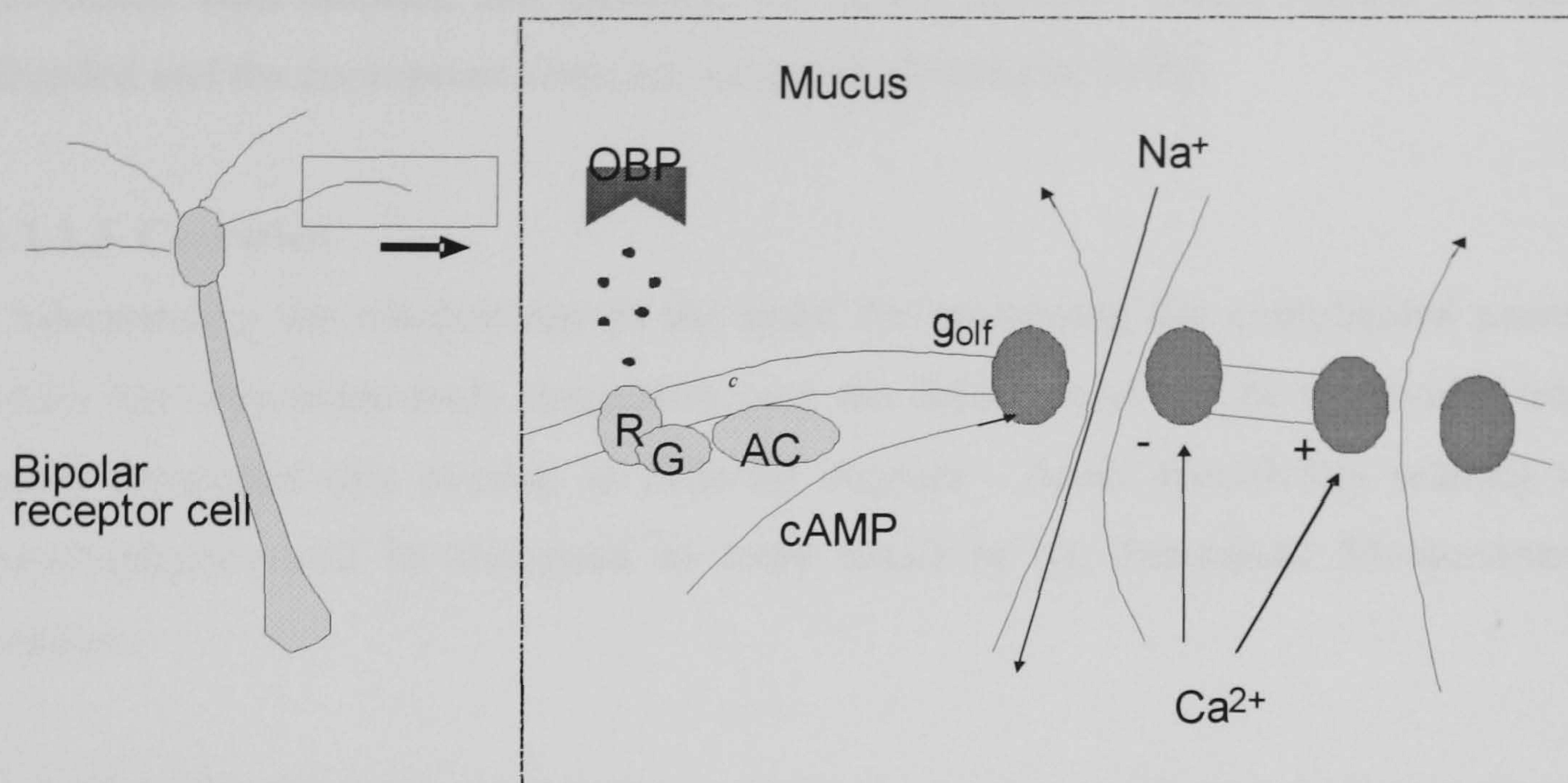


Figure 1.2 Olfactory receptor transduction. Odorant binding protein (OBP) transports molecules to the receptor (R). G-protein (G) activation then triggers adenylyl cyclase (AC) to produce intracellular cyclic AMP. This elicits opening of the channel allowing Ca^{++} to flow in, which activates a chloride current that helps depolarise the cell.

Odorant binding causes the G-protein subunit to induce cyclic AMP (adenosine monophosphate) production, a molecule that is ubiquitous in cell signalling. This in turn creates a depolarised state in the cell and opens up ion channels (Firestein, 2002). The level of cAMP activity has been shown to be proportional to the degree of electrical activity in animal epithelium preparations (Dryer and Berghard, 1999), and also to the perceived intensity of the odorant in humans (Doty, 2001). The cascade process of cAMP is not the sole transduction mechanism, and it is generally acknowledged that a variety of cell signalling molecules regulate, inhibit or enhance the overall response in the olfactory bulb (Breer, 2003; Gold, 1999; Lowe and Gold, 1995).

After stimulation, signals from the receptor axons pass into the olfactory bulb, where second order neurons, known as mitral cells, modulate the signal using inhibitory processes. The signal is then transferred to a number brain centres in the corticomedial amygdala via the olfactory nerve tract, and also to lower brain centres

associated with emotion and memory; the limbic system. These signals are then decoded and the appropriate response executed (Goldstein, 1999).

1.1.1.3 Cognition

Understanding the mechanisms in the brain for processing the complicated neural codes has been extensively researched over the decades and can be subdivided into many categories that overlap to different degrees. Areas specifically relating to psychophysics will be discussed in more detail in the Perceptual Measurement section.

1.1.1.3.1 Physiological consequences

Unlike vision and sound perception, odour perception is also governed by the mechanisms of other parts of the body involved with latent inhalation and active sniffing. In other words, the sense of smell also requires the use of lungs and diaphragm to function. Many researchers have shown that these physiological aspects have an influence on the optimisation of odour perception. One area is the role of active sniffing in two contexts; optimisation of odour intensity and activation of the brain in preparation for incoming odour. The former showed that a sniff between 0.39 and 0.64 seconds optimised the intensity of certain polar molecules (Laing, 1985b; Laing, 1985a), the latter of which indicated that the act of sniffing odourless air would sometimes activate the brain differently from respiratory inhalation of odour alone, indicating that the brain prepares to receive an aroma (Hamada and Yamaguchi, 2001). Other research includes the study of reaction times to recognise an orthonasally delivered aroma. In two such studies it was shown that typical reaction times fall between 600 to 2000 ms (Laing and Macleod, 1992; Lorig, 2000).

1.1.1.3.2 Odour quality, context and labelling

This incorporates cross modality studies such as the relationships between verbal descriptor, associated colour or previous exposure with quantitative/qualitative aspects of odour perception.

A famous example of the effect of colour context was shown in an experiment where enology students were provided with white wine, to which red wine colouring was added. The students then proceeded to provide descriptors of the wine that would normally have been associated with red wine, demonstrating the effect of visual contextual cue (Gottfried and Dolan, 2003). It was also shown that neural activity to an odour was higher when a congruent picture was provided than when an incongruent one was.

There have also been studies of a philosophical nature regarding the sparse nature of odour descriptions compared to the 1,400 odorous chemicals that can be discriminated by a subject. One interesting theory suggests that olfaction relies more heavily on implicit memories that an odorant elicits, supported by the fact that many people report the ability to imagine complex sounds and images whereas the evidence for this in olfaction is weak (Stevenson and Boakes, 2003). In order to find a solution to this problem of insufficient language, researchers have attempted to analyse the proximity of odour labels and their sensations by creating mapping systems such as odour prisms (Lorig, 2000). One such study asked individuals to rate different odours as a function of their similarity, in which each subsequent odorant became the control. This created a map of commonality between the elicited sensations (Carrie, *et al.*, 1999). Another system, which attempts to express odour description in universally understood terms, is the Field of Odours (Jaubert, *et al.*, 1995). This system teaches people a series of very dissimilar odorants that in turn form reference points to which other odorants can be compared and located upon related scales.

1.1.1.3.3 Simulation of neural response

This area of research includes the highly complex artificial neural networks, which mimic the brain's ability to store information as patterns rather than binary codes. They have been used to demonstrate the perceptual learning process and the transformations between the first and second olfactory processing stages, which are transduction to the olfactory bulb and then the cortical regions, respectively (Goldstone, 1998; Laurent, *et al.*, 2001). This system has also been used to try to

understand the sensory responses to foods as well as odours with limited findings (Wilkinson and Yuksel, 1997; Boccorh and Paterson, 2002).

1.1.1.3.4 Mixture perception

The discrimination of odours in binary or multi-component mixtures has long since been known to be relatively basic in humans. It has been shown that the maximum number of odours in a mixture to be differentiated is only three to four (Laing and Francis, 1989). The reason for this limitation has been attributed to the slow processing of the short term olfactory working memory (Jinks, *et al.*, 1998; Jinks and Laing, 1999b). Furthermore, it was shown that a person's ability and also the time required to discriminate two different odours is the inverse of the degree of similarity, i.e. two dissimilar odourants will be discriminated more quickly and with greater accuracy than those of closer perceptual proximity (Jinks and Laing, 2001; Jinks and Laing, 1999a). However, there is no evidence to suggest the presence of transitivity in the order of which odourants are processed first, i.e. if odorant A is perceived before B and B is perceived before C, then A should be perceived before C (Jinks and Laing, 1999b).

The problem with mixture component experiments concerns the process of perceptual learning. If odours are repeatedly administered to subjects in a mixture, they might acquire perceptual qualities of each other. In one experiment, when different aroma compounds were presented together with either cherry-like or smoky-like smells, the original odours alone became rated as significantly more similar to their partnered aromas after exposure than before (Stevenson, 2001). This highlights the dynamic nature of cognitive olfaction, where experiments can be easily biased and yield a false result.

1.1.1.3.5 Memory

As mentioned previously, humans can only discriminate a maximum of 3-4 odours in a mixture. This suggests that odour mixtures are processed as perceptual 'wholes', which are formed by comparisons to memory templates. A similar process is indicated in vision perception, where in a field of co-occurring stimuli, some features

must be grouped into perceptual objects that are distinct from the other stimuli (Wilson and Stevenson, 2003). However, olfactory perception differs from vision in that it lacks the ability to then be analytical of these perceptual 'wholes' and the entire system is heavily affected by perceptual learning. An example of this is that individuals can improve their discrimination abilities of unfamiliar odours on repeated exposure. Synthetic processing in the olfactory cortical regions of the brain, predominantly the piriform cortex, is said to be responsible for this type of memory template fluidity (Jehl, *et al.*, 1995).

The role of a specific gene in the human genome is often only exposed when there is a fault or expression error which results in a mutancy. This concept of abnormality being used to understand normality can be transferred to memory studies and olfaction, whereby a specific trauma, brain lesion or illness leads to decreased perceptual abilities. One recent study investigated the olfactory abilities of patients who had undergone resection from a temporal lobe (including primary olfactory regions) (Dade, *et al.*, 2002). They showed that the olfactory memory requires input from left and right temporal lobe regions for optimal odour recognition, which compared to verbal and non-verbal visual material, is far less subject to functional lateralisation (tendency of which side of the brain is performing the task). This was noted by the fact that although patients generally performed less well than normal subjects in odour recognition tests, their ability was not shown to be a function of the side of the resection.

Memory has a far more important role in olfactory processing at a higher level in the hedonic response. Research has shown that odours trigger memories that are more emotional than memories emanating through the other modalities (Herz and Cupchik, 1992; Herz and Zeitlin, 1995; Herz and Cupchik, 1995). The close proximity of primary olfactory neural regions with structures associated with emotional influenced memories is assumed to be the reason for this link, where the olfactory tract makes direct synapses with the amygdala-hippocampal complex (Herz, *et al.*, 1999). There are further consequences of this close relationship between odour and emotion to the extent that the heart rate fluctuates in response to the perceived pleasantness of

certain aromas and the skin increases in conductance with perceived arousal to the stimulus (Bensafi, *et al.*, 2002).

Perhaps this close connection to a hedonic response partly explains the large variation between humans so often observed in the different research areas of olfactory perception.

1.1.2 The trigeminal system

Approximately 70% of all odourants are said to elicit a trigeminal response (Coren, *et al.*, 1999). This somatosensory system transduces through cranial nerve V, and is highly important in its relationship to olfaction as it conveys information on texture, temperature, tingling and irritation. These sensations are frequently used in food and beverage products to enhance our enjoyment such as carbonation, addition of chillies, crunchiness of crisps and even the heat of coffee (Carstens, *et al.*, 2002). Nevertheless, this sensation has had far less attention to its functions than olfaction.

The system is innervated by the mandibular branches of the trigeminal nerve, which in turn are comprised of two major fibre systems situated in all areas of the oral and nasal cavities. The first type of 'receptor' are known as unmyelinated C-fibres and elicit types of burning painful sensations, the second are the myelinated A_{delta} fibres that mediate sharp and stinging sensations (Hummel, 2000a). The former are also responsible for nociception (mechanical touch and pressure), when they are situated inside the tongue tissue. Both types of fibre project first to the trigeminal complex known as the subnucleus caudalis, which is then followed by transduction to other areas of the brain such as the somatosensory thalamus and cortex (Carstens, *et al.*, 2002).

The most interesting fact of the trigeminal system is the way in which the central and peripheral mechanisms are controlled. These have been studied mainly by electrophysiological measurement such as event related potentials (Kobal and Kettenmann, 2000). There are three main consequences of oral irritation; sensitisation, desensitisation and stimulus induced recovery. Sensitisation occurs after repeated application of capsaicin, citric acid or concentrated NaCl that causes an increase in intensity across trials, in terms of both electrophysiological and

psychophysical measured activity (Stevens and Lawless, 1987). Desensitisation occurs after re-application of the irritant when a longer rest period is given between stimuli, but repeated dosage further increases the level of irritation (stimulus induced recovery) (Carstens, *et al.*, 2002).

Research has shown that the trigeminal system may interact with olfactory perception, as it was shown in one experiment that the application of mustard oil component (allyl isothiocyanate) could decrease the odour threshold of both phenyl ethyl alcohol and butanol (Jacquot, *et al.*, 2004). This example introduces the idea of modality interactions that are frequently observed in flavour perception research, which is discussed in section 1.1.1.6.

1.1.3 Gustation

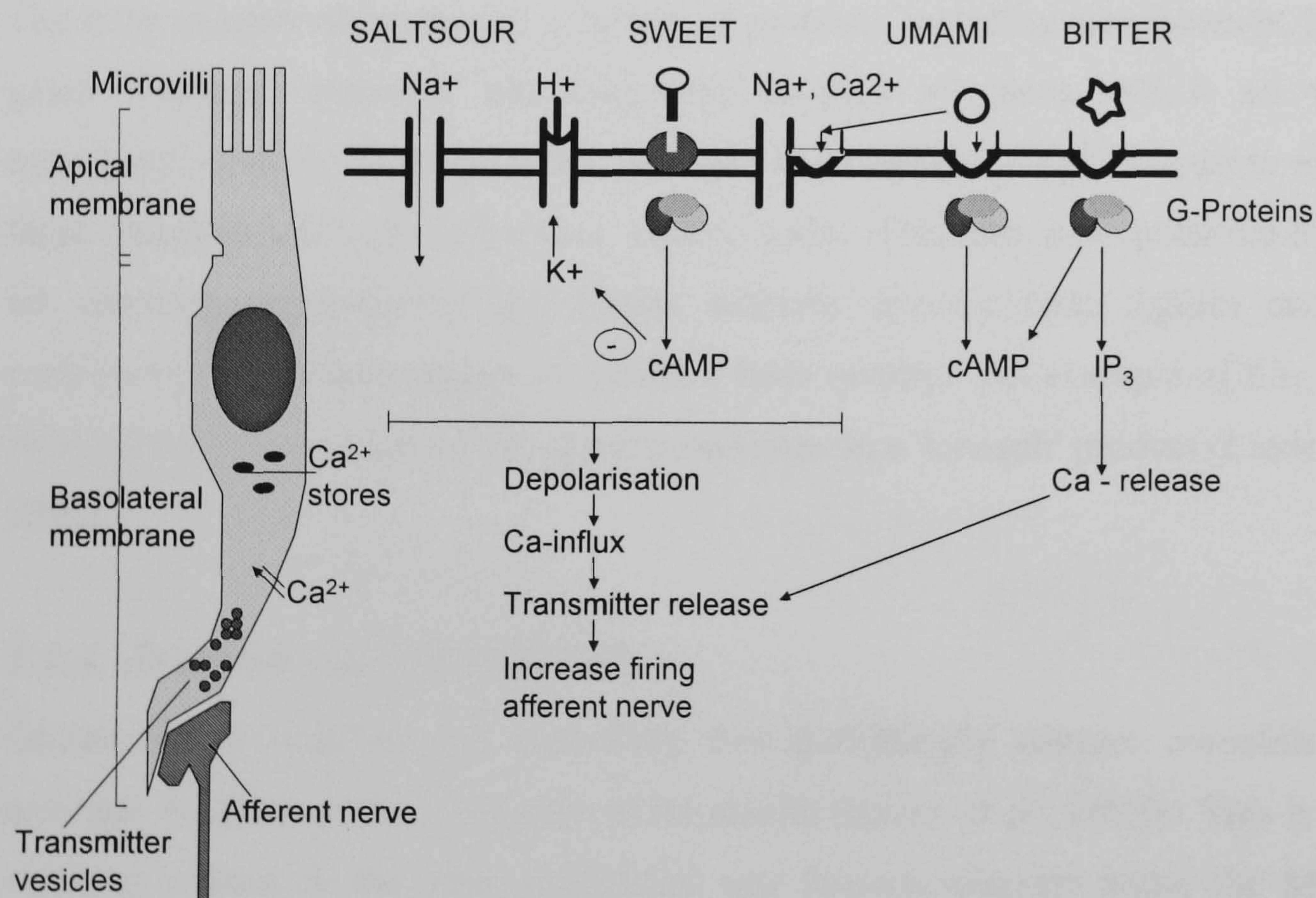


Figure 1.3 Gustation: overview of structure and mechanism.

A review on flavour and perception cannot be complete without mentioning the sense of gustation, which includes; sweet, sour, salty, bitter and umami.

Figure 1.3 depicts a taste bud and an overview of the different, albeit related, mechanisms that result in the respective sensations (Goldstein, 1999).

The main difference between gustation and olfaction is the former detects soluble molecules and the latter detects air borne ones. Furthermore, the transmission of signals differs greatly between the two systems. Whereas vertebrate olfaction transmits information about chemicals from bipolar receptor cells to the central nervous system via cranial nerve I, gustation uses specialised epithelial cells to transmit information via the cranial nerves: VII (facial), IX (glosopharyngel) or X (vagal) (Breslin, 2001). Other differences include the apparent innate nature of gustation, such as a baby's obvious expression of pleasure or displeasure when given something sweet or bitter, respectively. This is in contrast to olfaction, where it is thought that no hard wiring to hedonic responses is present at birth (Stillman, 2002). Hard wiring theory of gustation is also supported by the fact that our terminology for the sensations is so easily transferable to different languages and cultures.

The taste receptor cells contain a variety of proteins, including ion channels, ligand-gated channels, enzymes and G-protein coupled receptors, which all trigger transduction events. In recent years, there has been much research in order to identify these receptors and unscramble their genetic code. This may have potential benefits for consumer acceptability and health, whereby specific taste ligands could be constructed that either enhance or inhibit a taste quality, An example of this would be a molecule that enhanced the apparent saltiness in a 'low-salt' product (Lindemann, 2001).

1.1.4 Taste-aroma interactions

Gustation and olfaction are essentially two peripherally distinct modalities that combine to give a unified sensation in the mouth (Keast, *et al.*, 2003). This is also a common feature of the other modalities, one famous example being the McGurk effect, which is obtained by dubbing an incongruent articulatory movement on an auditory phoneme (McGurk & MacDonald (1976), Colin, *et al.*, 2002). The interaction is so strong that even after gaining knowledge of the phenomenon, most individuals still perceive the effect in exactly the same way as before (Shimojo and Shams, 2001). Studying these interactions in flavour perception is more complicated: if you have trained a panel to separate taste and odour attributes, it might be more

difficult to measure their perception as a combination of the different modalities (Delwiche, 2004).

Many researchers have investigated the taste-aroma interactions between sweetness and fruity aromas in various forms including; sucrose/aspartame-orange aroma (Nahon, *et al.*, 1998), sweetener-strawberry aroma (Nahon, *et al.*, 1996), sub-threshold saccharin-benzaldehyde integration (Dalton, *et al.*, 2000), sucrose chewing gum-menthone (Davidson, *et al.*, 1999) and sucrose-banana aroma in continuous delivery (Hort and Hollowood, 2004). Other pertinent investigations include the relationships between sweetness and acidity on the perception of champagne aromas (Martin, 2002) and even an interaction between bitterness and cut grass aromas in olive oil (Caporale, *et al.*, 2004).

The theory of multi-modal interaction is therefore widely accepted, but the mechanisms behind the effect are poorly understood and hypotheses often contrast one another. Some of these theories relate back to section 1.1.1.3.5 on memory, such as the idea that the interaction is cognitively learnt and is subsequently a dynamic process where, for example, an associated sweetness perception with certain aromas can be learnt. Furthermore, if the effect can be created, it can also be removed (Stevenson, *et al.*, 1999; Goldstone, 1998). Functional magnetic resonance imaging has been used to gain peripheral evidence for the convergence of taste and aroma stimuli and this type of research will undoubtedly become more popular once effective stimulus delivery strategies have been established. One recent study showed that certain areas in the human brain became activated in response to combined olfactory and taste stimuli that were significantly greater than the sum of activation produced by each alone. These activated areas, such as the caudal orbitofrontal cortex (OFC), are associated with higher processing centres of the brain. Other relevant regions included the nearby medial OFC, which was shown to correlate with the hedonic responses of the combined stimuli of sweet and fruity aromas (de Araujo, *et al.*, 2003). This further supports the taste-aroma convergence theory to be under cognitive control and thus the sensory responses of two different individuals are unlikely to be the same. This is highlighted by the use of Dynataste

experiments of solutions of sucrose and banana aroma, which demonstrated that the responses of assessors formed distinct perceptual clusters rather than a consensus effect (Hort and Hollowood, 2004).

1.2 MAKING PERCEPTUAL MEASUREMENTS

The measurement of an individual's perception is not just dependent on the questions they are asked, but also on the nature of the stimulus and the way in which it was analysed. Figure 1.4 shows the flow of information from the original product to the perceptual outcomes.

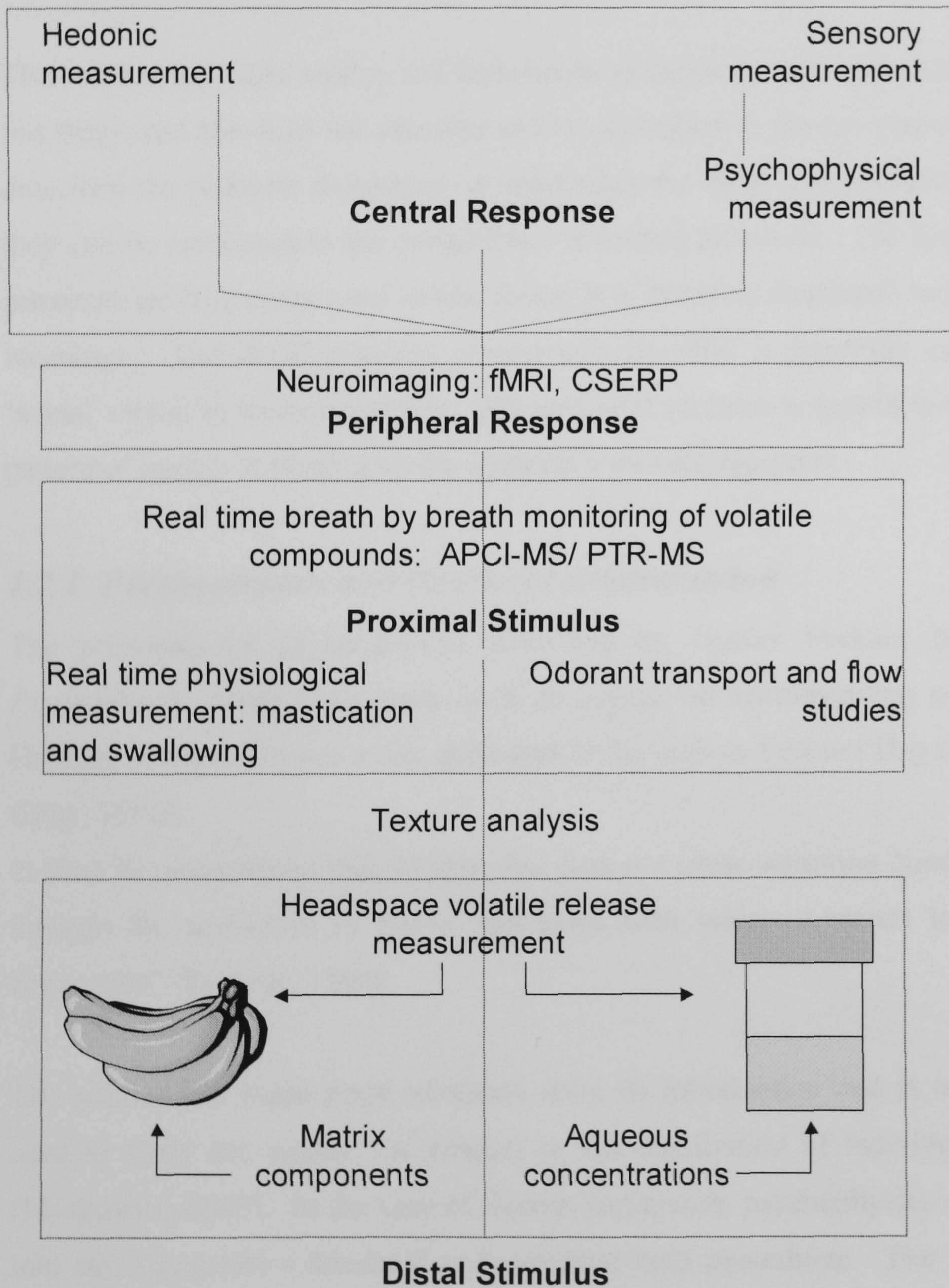


Figure 1.4 Flow of information on the overall flavour of a product and the stages at which instrumental or perceptual measurements can be made.

This schematic was constructed in order to understand the relevancy and stimulus stage of the parameters measured and used in this thesis, which were mainly focussed on; (vi). Breath by breath data, (viii). Psychophysical measurement and (x). Sensory measurement. Other stages involved to a lesser extent were; stage (ii). Headspace concentration and (iv). Physiological measurement.

This section describes aspects and techniques of the perceptual measurements used in this thesis and also how the stimulus can be controlled in the gas phase. Section 1.3 describes the different techniques in measuring the distal and proximal stimuli and they can be correlated to the peripheral and central processes. The terms distal and proximal are frequently used in this thesis, it is therefore important to describe their meanings. The distal stimulus corresponds to what is generally considered the 'actual' object in the environment. The proximal stimulus is generally defined as the pattern of energy impinging on the observer's sensory receptors.

1.2.1 Psychophysics and threshold determination

The principles of psychophysics described by Gustav Fechner (*Elemente der Psychophysik, 1860*) have made such an impact on understanding perception that Germany even celebrates a day dedicated to the author, Fechner Day (October 22nd) (Doty, 2002).

'It must be remembered that the stimulus does not cause sensation directly, but rather through the assistance of bodily processes with which it stands in more direct connection'. (Fechner, 1860).

The science has made great advances since its introduction and is now frequently used to study the senses, the product or the distribution of individual differences (Moskowitz, 2003). In the case of flavour perception, psychophysics can be divided into two categories - threshold and suprathreshold procedures. The former can be further subdivided into tests of detection sensitivity, recognition sensitivity and differential sensitivity. Suprathreshold tests include measures of odour identification, discrimination, memory and perceived odour intensity (Doty, 2001).

Threshold procedures and theories are being constantly improved for the reason that they are now considered highly effective diagnostic tools and indicators of degenerative conditions. Examples of this include the effects of old age, smoking, Alzheimer's, depression, alcoholism, AIDS, head trauma, ADHD and noxious chemical exposure (Hirsch and Trannel, 1996; Lehrner, *et al.*, 1997; Murphy, *et al.*, 1998; Bacon, *et al.*, 1998; Elsner, 2001). Other practical uses for olfactory thresholds include the control of environmental taints, most notably the control of swine odours (Chen, *et al.*, 1999) and drinking water contamination (Macrae and Falahee, 1995).

However, the constantly changing opinion of threshold methods means that the practical uses can frequently become outdated. The driving force behind these changes can often be due to research on the variability of threshold determination, from which new methods are developed or improved. The majority of research on this threshold variability was conducted between the late 1980's and late 1990's, one important study being the test-retest reliability of ten commonly used olfactory tests (Doty, *et al.*, 1995b; Doty, *et al.*, 1995a). They showed that detection thresholds were significantly more reliable than recognition thresholds due to the contamination by response bias, which is based on conservatism or liberalism in reporting the presence of a weakly perceived odour. Other authors reported that the variability of thresholds was due to the fluctuations of any individual's threshold over time, which then subsequently obscured the mean values that would be recorded for a population (Stevens, *et al.*, 1988). Additionally, this individual variation was tested as a function of time and showed that thresholds showed relatively good short term reliability but that this drifted over time (Lawless, *et al.*, 1995).

The most accepted reason for variability in threshold measurements is the person's ability in making a decision. In one study, which characterised the different styles of decision making, it was shown that people who procrastinate in making their decision not only take longer to complete a task, but also seek more information about the alternative choices and thus have generally a higher threshold for certainty (Ferrari and Dovidio, 2000). It is therefore of no surprise that the majority of psychophysical method developments incorporate techniques that can measure the decision based criterion levels.

Signal detection theory accounts for the changes in decision criteria over time and represents them as a probability distribution based on proportions of hits, false alarms, misses and correct rejections (Coren, *et al.*, 1999). Other methods that incorporate the treatment of decision use binomial distributions between the probability of a 'Yes' response by chance alone being treated as the inverse of the detectability (Walker, *et al.*, 2003). There are also techniques that assume that the effect of decision can be totally omitted from the test such as unforced-choice tasks (Kaernbach, 2001) and maximum-likelihood adaptive staircase procedures (Linschoten, *et al.*, 2001). The most frequently used technique remains the Thurstonian based R-index which has also been successfully transferred to sensory applications such as difference testing and ranking in both objective and subjective measurement (O'Mahony, 1986; O'Mahony, 1992; O'Mahony and Rousseau, 2003).

With established psychophysical techniques, researchers have been able to form models between thresholds, aroma compounds and their physico-chemical parameters. One method has been to investigate molecules from the same structural families but with increasing chain lengths, and generally indicates an inverse relationship between the two, i.e. as chain length increases, the threshold decreases (Takeoka, *et al.*, 1996).

Other examples of this type of study include differences between branched and unbranched esters and the effect of double bond (Takeoka, *et al.*, 1998). The conclusions are generally limited and the models are not easily applied to different structural families of molecules. Modelling of thresholds has also been attempted by assessing all aroma compounds and their partitioning behaviour in various situations. One such study formed relationships between the aroma compound and regions of partition during olfaction such as the air, the mucus membrane and the receptor (Abraham, *et al.*, 2002). Even though their proposed model could account for a high degree of variation between aroma compounds, it could not be transferred to all structural families, most notably aldehydes and carboxylic acids.

1.2.2 Olfactometry

It has been mentioned that threshold determination is prone to a high degree of variation, and results may vary depending on the test used. The method of presenting the aroma stimuli is also a common source of variation and researchers still use a variety of designs ranging from the simplistic to the elaborate and expensive.

Table 1.1 describes the ranges of olfactometer available and also the methods frequently used in research.

Table 1.1 Commonly used methods of producing gas phase concentrations of aroma compounds

Method of odorant presentation	Gas phase production	Examples
Static techniques	Glass/Teflon Headspace Generation bottles	Glass and Teflon Squeeze using mineral oil solvent (Doty, <i>et al.</i> , 1995b) Teflon squeeze bottles (Takeoka, <i>et al.</i> , 1996) Glass bottle with mineral oil solvent + Butan-1-ol intensity matching (Sulmont, <i>et al.</i> , 2002)
	Static vaporisation	'Sniffin Sticks': felt tip pens-Propylene glycol as solvent for 112 odours (Wolfensberger, <i>et al.</i> , 2000; Tateyama, <i>et al.</i> , 1998) Perfume strips: serial dilution + GC calibration (Tsukatani, <i>et al.</i> , 2003)
	Active vaporisation	UPSIT. Matching of verbal labels to a 'scratch & sniff' encapsulated odour. (Doty, <i>et al.</i> , 1995b)
Dynamic Techniques	Bubbling for volatile capture	Bubbling air through dissolved odorant, into Teflon gas sample bags. Assessor actively inhales contents of bag (Owen and Patterson, 2002)
	Bubbling+Mass flow control for direct presentation to assessor	Rotometer based flow dilutions: volatile saturated air becomes diluted to form different conc. Flow ranges: 140 mL/s - 43 L.min Calibration usually based on vapour pressures. Mostly used in neuro imaging studies. (Walker, <i>et al.</i> , 1990; Evans, <i>et al.</i> , 1995; Murphy, <i>et al.</i> , 2000) (Murphy, <i>et al.</i> , 2000; Castle, <i>et al.</i> , 2000; Pause and Krauel, 2000; Lorig, 2000)
	Bubbling + Mass flow control + online calibration	Odour pulse duration as indication of concentration (Wang, <i>et al.</i> , 2002) Olfactometer output calibrated by infra red spectroscopy (Walker, <i>et al.</i> , 2003) Pure volatilisation and air dilution olfactometer (Vuilleumier, <i>et al.</i> , 2000)

The odorant presentation methods listed in Table 1.1 range from distal stimulus measurement (solvent phase measurements, sniffin sticks and perfume strips), to proximal stimulus estimation or measurement (GC , infra-red spectroscopy and direct volatilisation). It is therefore probable that each method will generate a slightly different psychophysical result. Furthermore, the choice of method appears to be slightly dependent on the academic institute attended and consensual opinion on olfactometry has still not been fully established. Commercially available olfactometers are generally quite standard in their design and mostly incorporate air dilution control of saturated vapour. However, they can be quite expensive (up to \$15,000), cumbersome and can be difficult to accurately calibrate. For example, olfactometers that use sniffing masks may be inaccurate due to the difficulties in confirming the dilution ratios at the time when the assessor is sniffing (Jiang 2004, www.environodour.com.au).

The gas phase concentration accuracy of the olfactometer is not the only difference between the various methods. Researchers frequently use air dilution olfactometers at different gas flow rates, temperatures and humidities. Laing suggested that the optimal flow rate for an olfactometer should be $10 \text{ L}\cdot\text{min}^{-1}$ (Laing, 1985b), however, it is also believed that gas flows of this rate may cause trigeminal stimulation and should be avoided (Owen, *et al.*, 2002; Lorig, 2000).

Variation in olfactometer design would be less of a problem if the concentration of aroma delivered to an assessor was always instrumentally confirmed.

1.2.3 Time Intensity (TI)

Psychophysical measurements are recorded as relatively static functions, whereas time intensity methods can capture perception with respect to the true dynamic properties of sensory events. This means that perceptual conversions to a ratio scale are recorded by an assessor for the entire duration of an eating/sniffing event rather than just an average based on the sensory experience as a whole, i.e. magnitude estimation. It was originally developed in order to study the persistence of tastes such as sweetness, bitterness and astringency (Cliff and Heymann, 1993). However, in recent years it has become more popular for the sensory analysis of other attributes, mostly retronasal aroma, and has become invaluable in instrumental correlation studies, such as those involving the online monitoring of volatiles in the breath (Taylor, *et al.*, 2000).

The analysis of time intensity curves can be problematic because simple averaging can obscure the true response signatures of each assessor, although in most cases TI curves follow an inverted U shape. For this reason, many researchers have developed different statistical tools in order to extract summary measures from sets of TI curves. One of the first techniques extracted the means of normalised parameters from the ascending and descending portions of the curves such as time to reach maximum intensity (T_{\max}) and maximum intensity (I_{\max}) (Overbosch, *et al.*, 1986). Other extractable measures such as plateau time depend on being able to identify the appropriate landmarks on each individual curve (Piggott, 2000).

Figure 1.5 shows the extractable parameters from a typical shaped TI curve (Dijksterhuis and Piggott, 2000).

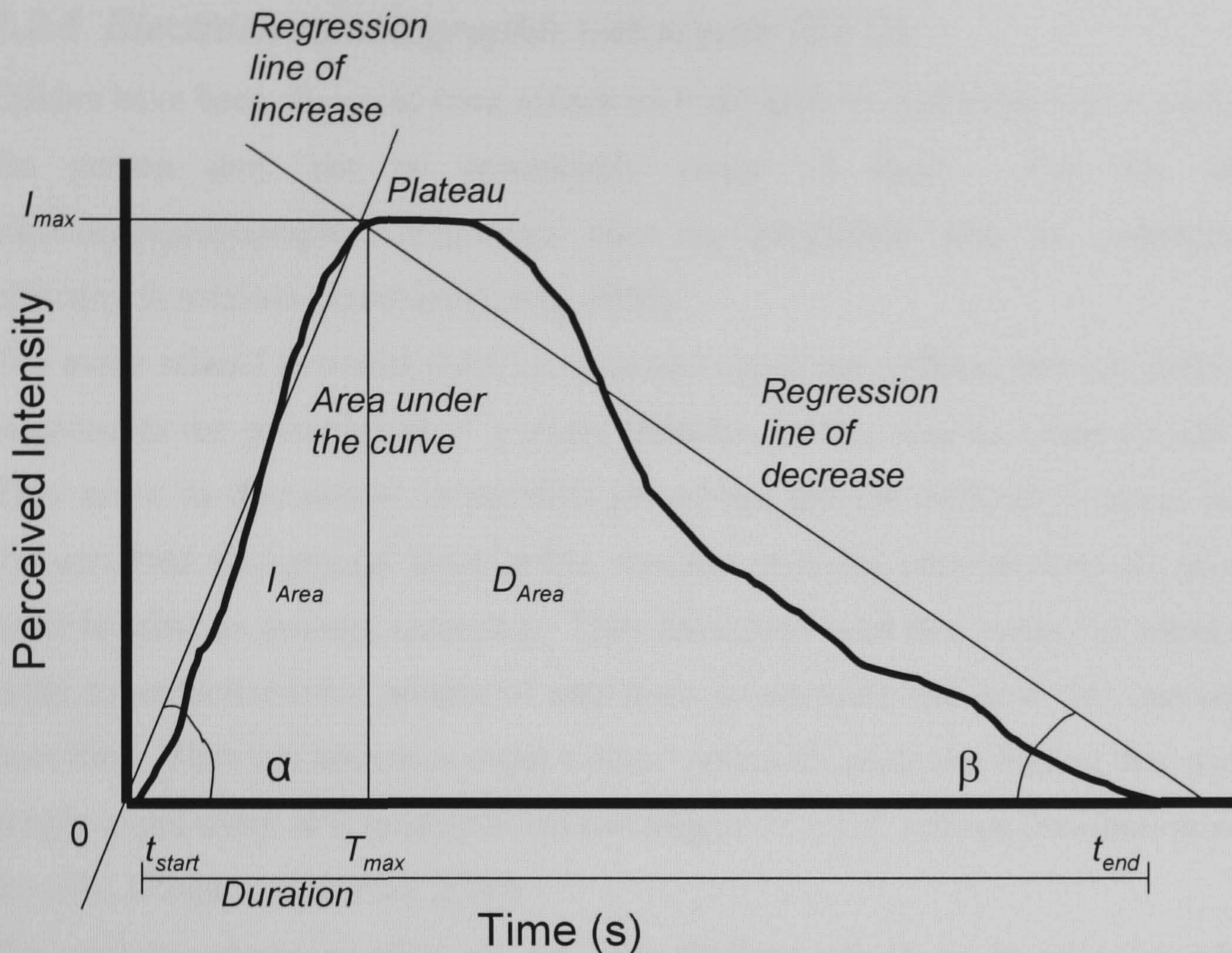


Figure 1.5 Typical time-intensity curve showing the common parameters taken from the curve (Dijksterhuis and Piggott, 2000).

The method of extracting curve parameters is perhaps the most commonly used technique, and is employed as standard in sensory analysis software packages such as FIZZ (Biosystemes, Paris, France). There are also methods that enable multivariate analysis of these parameters when two or more product attributes are monitored, such as the STATIS method (Chaya, *et al.*, 2004).

However, it is becoming more popular to fit models to these curves as a simulation of the actual physiological/neurological processes as a person eats. Most of these models stem from ideology from Overbosch and co-workers, which is discussed in section 1.3. One recent model splits a typical TI curve into two parts, and then fits a sigmoidal curve onto the first half and a decay curve on the other. In both cases, the response is assumed to be logarithmic (Garrido, *et al.*, 2001).

Principal component analysis can also be used to analyse TI curves, which yields information on assessor agreement and deviations from the principal curve (Piggott, 2000).

1.2.4 Electroencephalographic techniques (EEG)

Odours have been shown to have effects on brain activity and behaviour even though the person may not be consciously aware of them. For this reason, electroencephalographic techniques play an invaluable role in understanding olfactory perception processes (Lorig, 2000).

The event related potential (ERP) is a neural signal that reflects network activity in response to the presentation of a single stimulus, in this case an olfactory stimulus. They occur as fluctuations in the EEG recordings that are difficult to detect against the unrelated background noise unless repeated stimulus presentations are given in order to yield an average recording. They have been used in a variety of methods in order to understand the peripheral responses to odorants and how this can change over time. They are known as event 'related' potentials since the finding that even the simple expectation of a sensory event can trigger a typical voltage distribution within the EEG (Pause and Krauel, 2000).

The work has shown a variety of interesting findings, which can be related to a better understanding of the higher processes as well. Examples include the confirmation that brain activity changed in relation to increasing stimulus concentration, in particular the ERP latency was indicated (Tateyama, *et al.*, 1998).

The processes of adaptation and habituation of hedonically different odorants have also been described by using olfactometer pulsing technology. It was shown that the olfactory system adapts/habituates more rapidly to malodours than to pleasant odours and that the degree of adaptation is inversely proportional to the stimulus strength (Jacob, *et al.*, 2003). Other related findings include a theoretical prediction for the time constant of adaptation where there is no perceived gap between the pulses, although not calculated. Additionally, after repeated odorant presentation it was shown that cognitive perception may cease entirely yet the receptors may still relay the information to the brain. This suggested that habituation is a far more rapid process than neural adaptation (Wang, *et al.*, 2002).

Other neuroimaging techniques of relevance include functional magnetic resonance imaging (fMRI). These have confirmed theories of the location of olfactory higher

processing areas of the brain that ERPs alone cannot provide, such as the lack of lateralisation of orbitofrontal cortex, (Kobal and Kettenmann, 2000).

1.3 INSTRUMENTAL ANALYSIS OF FLAVOUR

As depicted in Figure 1.4 food and beverages can be analysed as a function of their distal stimuli or their proximal stimuli components. For relationships to be formed between the aroma compounds and the sensory responses, it is more relevant to be able to analyse the relevant volatiles in the gas phase. This can be either headspace studies or *in vivo*, and this can be conducted using gas chromatography or direct mass spectrometry.

1.3.1 Gas chromatography

Two different methods can be used to sample the volatiles; static or dynamic headspace. They both measure and identify aroma compounds as a consequence of their partitioning from the solvent phase into the gas phase: static as a closed system, dynamic as more of an indication of mass transfer. Static headspace simply involves removal of a small gas aliquot from the air above the sample, and dynamic headspace actual forces inert gas through the sample and thus is capable of trapping a higher concentration onto adsorbant materials such as diphenyl phenylene oxide (or Tenax) (Snow and Slack, 2002). Molecules can then be separated by gas chromatography and their identities deduced by electron impact mass spectrometry (GC-EIMS) (Taylor, *et al.*, 2000). Additionally, gas chromatographs can be configured as to partially elute the fractions into an odour port (GC-olfactometry), where an assessor can simultaneously describe the quality of the aroma components (Schieberle, 1991). This can give an indication of the identity of the odour active compounds in an unknown mixture, however, there can be problems in the assessor missing important elutions (Hanaoka, *et al.*, 2001). Moreover, GC-olfactometry can give a false impression of the importance of some of the eluted aroma compounds in regards to the entire spectrum. This can be partially compensated for by using odour activity values in terms of ratio of the concentration of the odorant in the material to the odour

threshold. Nevertheless, it is suggested that only 5% of the volatiles identified in foods contribute to the overall aroma (Grosch, 2001). Other drawbacks of GC-MS include the convoluted nature of the chromatograms, where low volatile peaks may be obscured by high and abundant species. Additionally, the fragmentation of ions can increase the overall complexity and requires an extensive library database (Snow and Slack, 2002).

As a tool to understand the process of flavour release in the breath, it is highly impractical, as its lengthy sampling runs would not be efficient in capturing data from the fast and dynamic events observed in *in vivo* studies (Taylor, *et al.*, 2000).

Regardless of the incurred problems in GC-MS analysis, it still retains much of its popularity especially in volatile loss and shelf life trials, and is generally more affordable than the direct mass spectrometry techniques such as atmospheric pressure chemical ionisation (APCI-MS) and proton transfer reaction (PTR-MS).

1.3.2 Direct gas phase analysis

1.3.2.1 Atmospheric pressure chemical ionisation - mass spectrometry APCI-MS

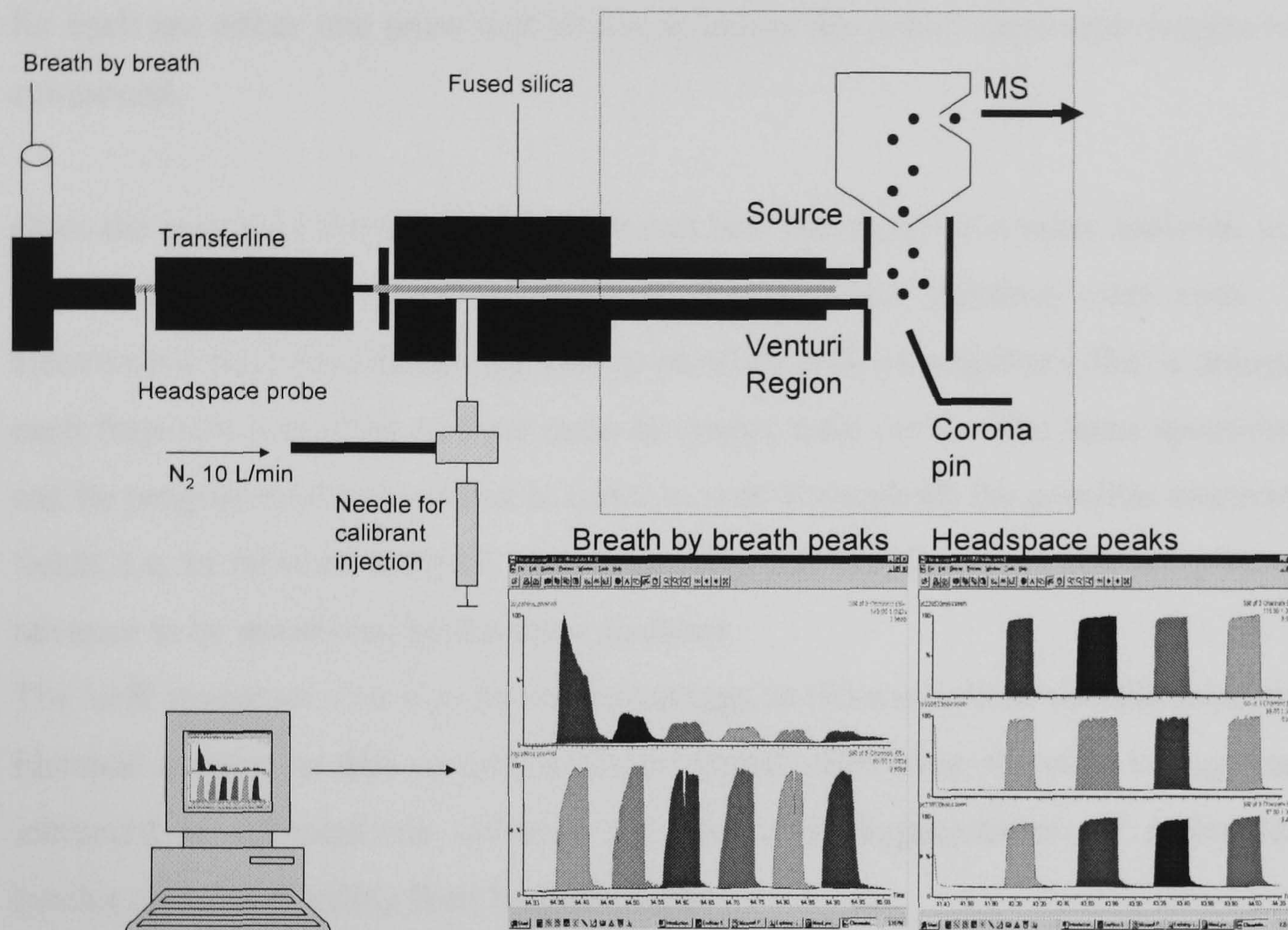
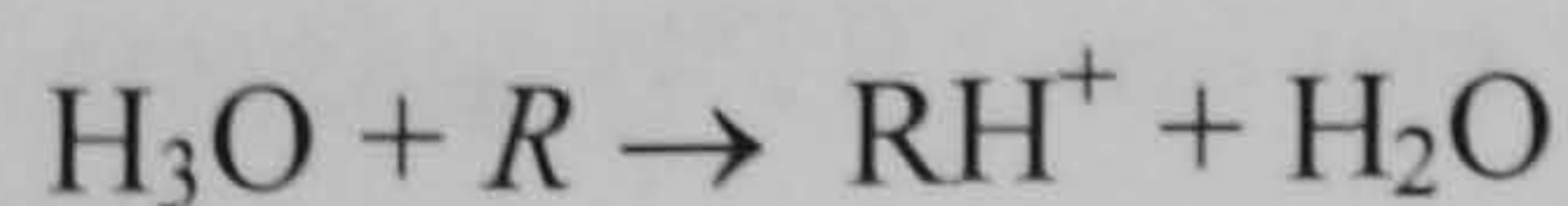


Figure 1.6 Schematic diagram of the APCI-MS source and type of chromatograms obtained.

Direct mass spectrometry such as APCI-MS were designed to overcome some of the problems associated with GC-MS techniques such as spectra complexity, time limitations and the presence of water. A schematic of the APCI-MS source and the corresponding types of breath by breath and headspace chromatograms are shown in Figure 1.6.

The APCI-MS functions by first forming an initial reactant ion from water and the discharge from the corona pin. Using water means that its inevitable presence in food matrices, headspace and naturally in the breath are not a hurdle, as would be in GC-MS analysis, but is in fact a requirement of the process. This ion can then transfer its charge to any, as in the following equation:



This type of ionisation is known as 'soft', in that there is very little or no fragmentation of the molecules. This means that upon mass analysis, aroma compounds mixtures will appear as single ions where the mass to charge ratios (m/z) for each are either one mass unit above or below the actual molecular weight of the compound.

Once the ions have been formed they travel into the region of a mass analyser, in this case a quadropole, which consists of four parallel stainless steel rods. An electrostatic field then causes the ions to oscillate with an amplitude that is unique for each fragment according to their mass to charge ratio (m/z). The mass spectrometer can be programmed beforehand in order to scan through all the possible electrostatic fields, i.e. in mixture analysis. Alternatively, just one ion mass can be selected in advance to be monitored by the mass analyser.

The 'soft' ionisation can also be a disadvantage in the analysis of volatile mixtures of identical masses, as they cannot be discriminated. However, the cone voltage can be increased to subsequently enhance the degree of fragmentation of molecules of similar mass, thus aiding their identification.

Breath by breath (*in vivo*) measurement functions well with APCI-MS as ions can be scanned at sufficiently high speeds for real time events to be captured in the chromatograms, even the effects of chewing. Furthermore, a simplistic method can be used to follow the patterns of an individual's breathing by selecting the volatile acetone ($58 + H^+$) to be monitored. Acetone is naturally present in the breath as a fatty acid metabolite that is expired in the lungs and appears as peaks coinciding with exhalations. Many food and beverage products have been analysed for their behaviour during consumption including strawberries, thickened solutions, gelatine, chewing gum and boiled sweets (Taylor, *et al.*, 2000; Linforth, *et al.*, 1999; Harvey and Barra, 2003).

Direct mass spectrometry of the gas phase can also be achieved by proton transfer reaction (PTR) and ion trap (IT). Both of these methods are variations of a similar

principle to APCI-MS. PTR has similar sensitivity to APCI-MS and also incurs the problems of poor differentiation between aroma compounds of the same nominal mass but different composition. Further selectivity between compounds can be achieved using time of flight (TOF) analysis instead of a quadropole (Linforth, 2000).

1.3.2.2 Electronic noses

The term 'electronic nose' is used to describe devices consisting of various odour sensors, which are becoming popular in quality control in the food industry. The chemical sensors react to particular agents by means of a reversible interaction, from which a signal can be then be transduced and reflect either quantitative or qualitative analysis. There are a number of different sensor types such as catalytic or tin oxide, conducting polymer and acoustic wave and they will generally have different specificities for odorants in simulation of the natural receptor-ligand interactions in the nasal epithelium. The conducting polymer sensors are the most popular as they can function at room temperature, the odorant specificity is easily altered and they are not easily inactivated by contaminants (Deisingh, *et al.*, 2004).

Data processing is more complex as it requires a form of pattern recognition, which basically means categorisation of different species upon a variety of vectors that can be measured within the capabilities of the sensors employed. The recognition programmes form multivariate data plots from principal component analysis or artificial neural networks, upon which new product information can be fitted. This normally means that electronic noses are essentially 'naïve' when they are first constructed and are then 'trained' to discriminate libraries of different odorant molecules. They generally lack sensitivity, and their slow response rate makes them unsuitable for following rapid changes in breath volatile concentration (Gopel, 2000; Linforth, 2000).

1.3.3 Correlating instrumental and sensory analyses of flavour

A researcher's success in determining psychophysical values is partially governed by the degree to which they can control the aroma stimuli (see section 1.2.2). In contrast, the behaviour of stimuli in sensory experiments is more difficult to control

because the food/beverage matrices are far more complicated. Therefore, understanding, monitoring or modelling the aroma behaviour in a product may be far more conducive to sensory analysis than knowing the chemical composition alone. A stage further is to understand how the behaviours and consequences of these aroma release patterns differ from person to person and how this impacts on perception. This section reviews the types of research into correlating instrumental measurements to the sensory responses.

1.3.3.1 Compositional versus sensory analysis

This popular type of correlation study characterises a product by certain compositional measurements and then matches them to the sensory attributes. An example of this is where an agricultural product has different cultivars and varieties according to the way in which it is farmed or where it is grown, and these lead to different sensory attributes. The most common approach is to analyse the composition by gas chromatography techniques, either by direct extraction or headspace analysis.

In one study on the attributes of cooked oats, more than fifty volatiles compounds were detected by GC-MS from purged headspaces, yet just six of the total peak areas could account for 43-94% of the variation between products according to the sensory attributes measured (Zhou, *et al.*, 2000). This type of analysis has also been applied to the classification of coffees, where GC-MS peaks and attribute scores were subjected to principal component analysis. Results showed that the main source of variation between the coffees according to both sensory and instrumental analyses was due to the botanical variety and the minor source was due to the type of roast method (Maeztu, *et al.*, 2001).

There are many food products characterised in this manner and often include simultaneous textural analysis such as sensory measurements of hardness or grittiness in the case of apples (Karlsen, *et al.*, 1999).

Establishing the source and formation of common product taints has also been investigated, one good example being of 'warmed-over' flavour in cooked meats. This undesirable taint occurs when lipid oxidation occurs in cooked meats kept in

refrigerators forming volatiles such as hexanal. This has been characterised for a number of meat types by a combination of GC-MS, electronic nose measurement and sensory description. Results showed that an additional number of oxidation products were responsible including; pentanal, octanal, nonanal and 1-octen-3-ol. and the electronic nose measurements could discriminate samples even on the basis of muscle types (O'Sullivan, *et al.*, 2003).

1.3.3.2 The effect of matrix

The food matrix is known to effect the delivery profile and the overall perception of the retronasal aroma (Taylor and Linforth, 2001). This physical and often multi modal interaction has been frequently investigated using emulsion, hydrocolloid or gelatine systems.

The release of aroma from emulsions has been extensively studied and modelled from a purely theoretical angle and from sensory perspectives in real products. Harrison and co-workers postulated the theories behind the mass transfer of aroma compounds from emulsion into the nosespace by predicting the various phase partition coefficients based the molecular hydrophobicity and the lipid fraction. This was then used to calculate the rates of release of aroma compound from the matrix and suggested that release of hydrophobic (lipophilic) molecules decreased with increasing lipid fraction due to a limit in the rate of mass transfer at the emulsion-gas interface (Harrison, *et al.*, 1997). However, these formulas were not directly applied to *in vivo* studies and were inappropriate for correlation with sensory studies. A better way of understanding the release mechanisms compared differences between headspace and breath by breath measurements, which demonstrated that salivary dilution in the mouth resulted in a change in the partition coefficient (Doyen, *et al.*, 2001; Linforth, *et al.*, 2002). In terms of sensory perception, this reduction in aroma release caused by the increasing lipid fraction results in a decrease in sensory intensity. The effect is less than that observed in headspace studies (Roberts, *et al.*, 2003). Flavour release studies from yoghurts and sensory TI revealed that hydrophobic compounds were more affected by lipid fraction for the maximum intensity, but not time-to-maximum intensity or persistence (Brauss, *et al.*, 1999).

Sensory analysis of flavoured hydrocolloid thickeners showed that perception is not always related to the aroma release but can be more indicative of certain rheological measurements. Both taste and aroma perception can be suppressed by increasing concentrations of hydrocolloid, which was originally assumed to be determined by the thickener concentration relative to its coil-overlap concentration (c^*). However, Cook and colleagues showed that a different rheological parameter known as the Kokini oral shear stress (τ) was far more indicative of the sensory response (Cook, *et al.*, 2003). The oral shear stress is a theoretical calculation based on fluid dynamics in the mouth when a sample is forced between the tip of the tongue and the roof of the mouth and demonstrates a multi modal interaction between somatosensory stimuli and aroma signals (Cook, *et al.*, 2002; Hollowood, *et al.*, 2002).

Flavoured gelatine samples were also used to model the sensory response from the release of aroma compounds in real time. It was demonstrated that, despite consuming identical samples, assessors obtained very different volatile concentrations in the nose. However, this was not shown to be related to the perceived maximum intensity (Hollowood, *et al.*, 2000). The different volatile release profiles were considered to be a consequence of the rate of breakdown of the gelatine, which was to some degree reflected in the TI sensory response (Baek, *et al.*, 1999). However, only a minimum of arbitrary parameters were extracted from the time intensity and instrumental curves, namely the maximum intensity (I_{\max}), time to reach I_{\max} and rate of volatile release (gradient on the up-slope).

1.3.3.3 Modelling the sensory response as a function of time

The original Stevens law ($I=k(S-S_0)^n$) described the relationship between the stimulus concentration and the sensory response in reflection of entire integrated events. $I =$ *the perceived intensity*, $S =$ *stimulus concentration*, $S_0 =$ *stimulus threshold concentration*, $k =$ *scaling constant*.

However, over the course of an eating event these stimuli-response relationships are highly subject to change due to the phenomena of adaptation and habituation. For this reason, in the mid 1980s Overbosch and colleagues began to construct modified version of Stevens law in order to incorporate a time dependent adaptation term, which would compensate for its two main effects:

- The shift in the sensitivity range of a system
- The increase in the threshold value.

The new equation calculated that at any point in time there would be a different relationship between the actual stimulus value and the threshold value. These studies have influenced the methods of correlating sensory and breath by breath data over time, because whereas the sensory response is constantly readjusting itself to the stimulus, the mass spectrometer cannot (Overbosch, *et al.*, 1991; Overbosch, *et al.*, 1986). See equation 1.1 for the revised Overbosch equation.

$$\text{Equation 1.1. } S = e^{-\int A / S dt} * A \int e^{\int A / S dt} dt .$$

This integral equation postulates that at any point in time, the adaptation to the stimulus will have a different relationship to the threshold. Therefore, time (dt) and an adaptation (A) constant are applied to the original equation.

Applying the modified equation to real studies is an arduous task and requires many repetitions and highly complicated mathematical analysis. Therefore, attempts to do this have focussed on model foods that have previously been intensively studied, such as flavoured gelatine (Hollowood, *et al.*, 2004). In this study, trained assessors made time intensity measurements of four different volatiles (carvone, anethole, isoamyl acetate and limonene) in gel samples and their individual retronasal and orthonasal measurements were also determined. The Overbosch equation was then applied to the data sets but was only relatively significant for carvone and anethole. However, an alternative function was developed, which included a time-weighted average of the

stimulus. This refinement stemmed from knowledge gained about vision perception and persistence, yet was also applicable to flavour perception. This approach improved the model and yielded significantly higher correlations between the sensory and the instrumental data.

1.4 AIMS

Recent studies have improved our understanding of the capabilities of the APCI-MS. Nevertheless, the true relationships between the breath by breath instrumental data and the sensory responses remains ambiguous. The aim of this thesis was to use simple aroma delivery systems in order to assess relationships between the instrumental measurements and the sensory responses using a combination of psychophysical and sensory techniques.

Initially, methods were developed in order to control and measure aroma delivery in the gas phase for determination of both retronasal and orthonasal thresholds. Thresholds were chosen as the starting point of this work for two reasons firstly, the large variation between people has been described in many previous psychophysical research studies. Secondly, threshold tests are far less prone to assessor psychological errors associated with sensory analysis. Therefore, the variation in the results would be assumed to be real differences in human sensitivities rather than cognitive issues, which could be then compared to the APCI-MS *in vivo* data.

Further studies were designed to gain a better understanding of the true relationship between breath by breath data obtained from the APCI-MS and the sensory response. The initial intention was to investigate the perception of pulsed odorants, which has been described in Gestalt theory for the other modalities, such as *flicker fusion frequency* in vision perception of moving pictures. This was conducted orthonasally using two olfactometer configurations with an APCI-MS as a measurement of volatile compounds in the exhaled breath or as a novel measurement of their disruption as a subject inhaled. This provided further evidence of physiological differences between people and how this impacted on perception.

2 OLFACTOMETRY – METHOD DEVELOPMENT

The aim of these studies was to develop a method to generate repeatable concentrations of volatile compound in the gas phase, in order that orthonasal thresholds could be determined. The other part of this study was to develop a method of pulsing aroma compounds in the gas phase. The specification was that any design should incorporate best practice from standard and commercial olfactometers and be simple to validate using the Atmospheric Pressure Chemical Ionisation Mass Spectrometer (APCI-MS). An introducing section (1.2.2) gives an overview of the types of olfactometer, which are currently available on the market or have been independently constructed for use in olfaction research.

The use of APCI-MS in olfactometer validation in this study meant that the designs could be proven for their efficacy by real measurement rather than purely on mathematical prediction, which can be based on vapour pressure, density or equilibrium coefficients. The main problem in threshold research has often been the degree of assumption on stimuli concentration in the gas phase, such as basing orthonasal threshold values on volatile concentrations in the aqueous phase alone assuming equilibrium exists between the liquid and the gas phases. Different aroma compounds at identical concentrations may differ greatly in the headspace as the mass transfer depends both on the partition coefficient of the compound and the interaction between the solute and solvent (Brockerhoff and Grant, 1999). Thus, the measurement of thresholds based on aqueous concentration can give a false representation simply due to vaporisation differences alone. This study shows that even simplistic olfactometry designs can be effective when the actual gas phase of a volatile can be instrumentally confirmed.

When breath by breath data of food consumption is recorded on the APCI-MS, the ion traces are seen as tidal peaks and troughs as the person exhales and inhales the

volatiles down the MS-interface. Nevertheless, time intensity curves of perception of the same food stuffs will generally be smoothed and independent of the subjects respiration. This can be assumed to be comparable to the aspects of depth perception in vision, whereby if one object is partially occluded by another, our perception completes the missing object rapidly and automatically. With flavour perception, the entire signal between aroma laden exhalations becomes integrated and the gaps are 'filled in' in a similar way (Coren, *et al.*, 1999). However, the responsible mechanisms for this integration are not fully understood and may be more linked to adaptation, habituation, or even signal response time to the stimulus, rather than a cognitive effect alone.

Various authors have begun to utilise aroma pulsing techniques in order to investigate some of these perceptual areas and related aspects. Producing artificial pulsing of aroma would naturally be difficult and somewhat dangerous retronasally, thus the research is generally conducted orthonasally. The main area that requires the use of aroma pulsing is in brain imaging studies such as chemosensory event related potentials (Pause, *et al.*, 1999; Pause and Krauel, 2000; Tateyama, *et al.*, 1998), and investigations into reaction times (Laing and Macleod, 1992). Regardless of technique, a common feature in these studies is the absence of absolute instrumental confirmation of the aroma stimulus concentration and time to reach the nasal cavity. In this study a pulsing olfactometer was constructed and validated using on line techniques that outline the potential uses and limitations.

2.1 OLFACTOMETRY - THRESHOLD DETERMINATION

2.1.1 Volatile vaporisation olfactometer

2.1.1.1 Design

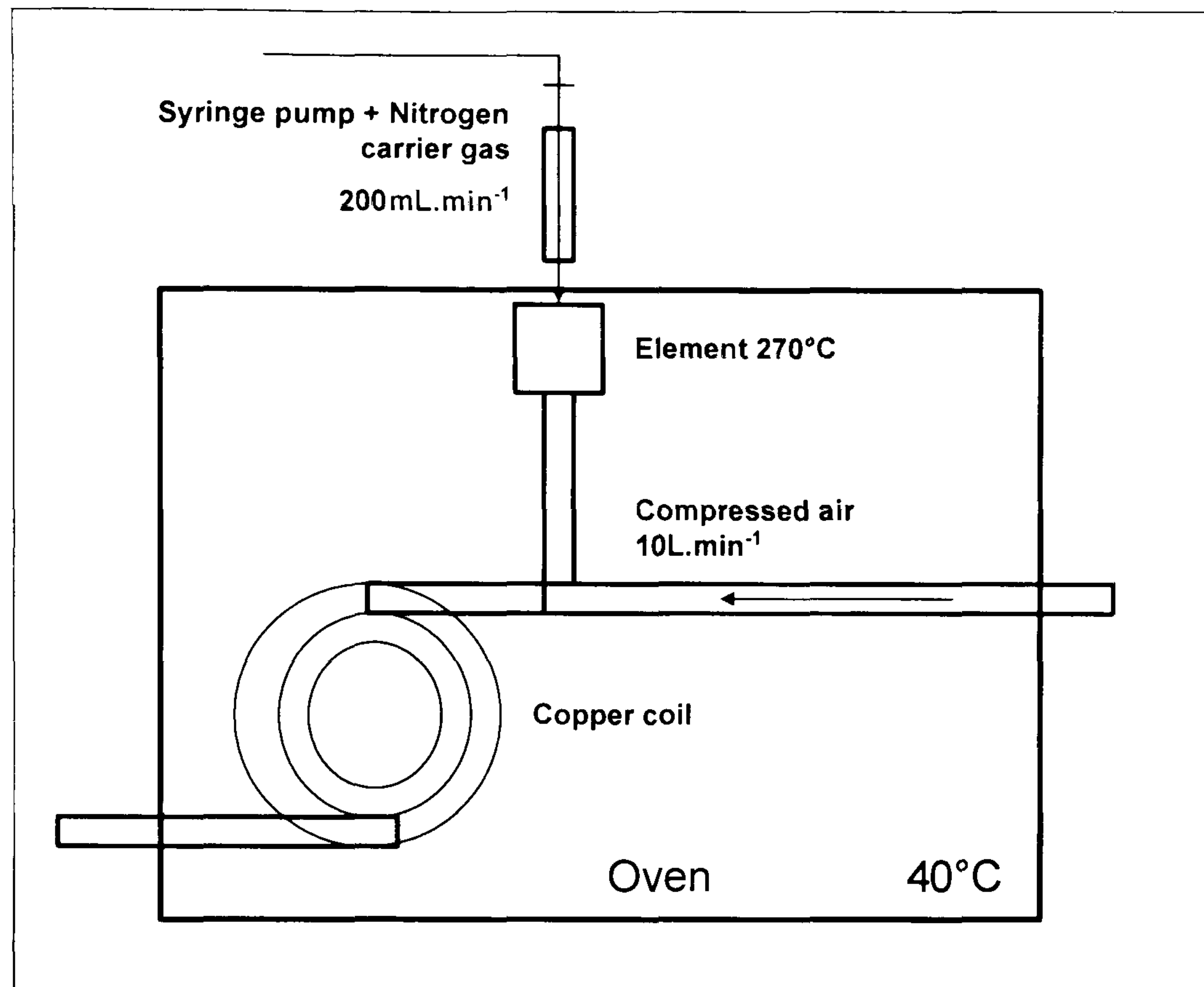


Figure 2.1. Volatile vaporisation olfactometer

Figure 2.1 shows a diagram of the design. Preparations of volatiles dissolved in ethanol were loaded into a 100 μL syringe (SGE, Melbourne, Australia). Injection of the syringe was controlled by a pump (Harvard Apparatus, Edenbridge, UK), which ran at rates between 2-40 $\mu\text{L}\cdot\text{min}^{-1}$. The solution was vaporised by a copper element (gas chromatography injection port at 270°C) and then carried into the oven by a stream of nitrogen gas (200 $\text{mL}\cdot\text{min}^{-1}$). The concentrated vapour was then diluted by a stream of compressed air. The gas flow rate (5-10 $\text{L}\cdot\text{min}^{-1}$) was controlled by a needle valve (Swagelock, Nottingham, UK) and measured by a GFM air flowmeter (Aalborg, New York, USA).

2.1.1.2 Olfactometer output prediction and validation

A formula was used to predict the output volatile concentration in terms of solution concentration, syringe injection rate and air flow.

$$C_{output} = \frac{F_p \times C_s \times 23.9}{F_a \times \delta \times M}$$

Where: C_{output} is the concentration in the olfactometer emission,

F_p is the flow rate in $\mu\text{L}\cdot\text{min}^{-1}$ of the syringe pump

F_a is the total sum of air dilution in olfactometer in $\text{L}\cdot\text{min}^{-1}$

23.9 dm^3 is the occupancy of 1 mole at 40°C Standard Pressure.

δ is the density of the compound

M is the weight (g) of 1 mole of the compound.

Formula was derived by assuming that in 1 minute the syringe pump would inject a known number of moles into the olfactometer. Inside the oven at 40°C , the vapour concentration was the molar occupancy at 40°C divided by the density and the molecular mass. This was then diluted by the overall gas flow. Therefore, when a setting was changed (either flow of syringe pump, or gas flow), a predicted concentration emission could be compared to an actual measurement from the APCI-MS.

2.1.1.3 Instrumental analysis

Olfactometer emission was sampled in real time at a rate of $10 \text{ L}\cdot\text{min}^{-1}$ using the APCI-MS (Micromass, Manchester, UK). The volatile molecules were ionised (4 kV-corona discharge) and the profile followed by monitoring the appropriate molecular ion (MH^+) (see Table 2.1). The dwell time was 0.2 s. The APCI-MS was calibrated by introducing known concentrations of each volatile (100 ppb) in solutions of hexane by using the syringe pump (Taylor, *et al.*, 2000).

Table 2.1: Molecular masses monitored by the APCI-MS from olfactometer emission

Volatile compound	Ion (MH^+)Mass
Ethyl butyrate	116.7
Benzaldehyde	106.8
Isoamyl acetate	130.2
Linalool	136.9

2.1.1.4 Results

An example of a trace from the APCI-MS is given in Figure 2.2. This shows the stability of the volatile signal. Graphs were plotted of predicted versus actual concentration emissions from the olfactometer in Figure 2.3. The R^2 values of each line were above 0.95, and the gradient relatively close to 1 (see summary Table 2.2). Deviations from the predicted 'Y=X' line equation could be attributed to two potential sources of experimental error: the preparation of the calibrant and also that of the volatile in ethanol solution. This was most prevalent with ethyl butyrate and isoamyl acetate because they have higher boiling points, which can cause inaccuracies when rapid vaporisation occurs inside Gilson pipettes.

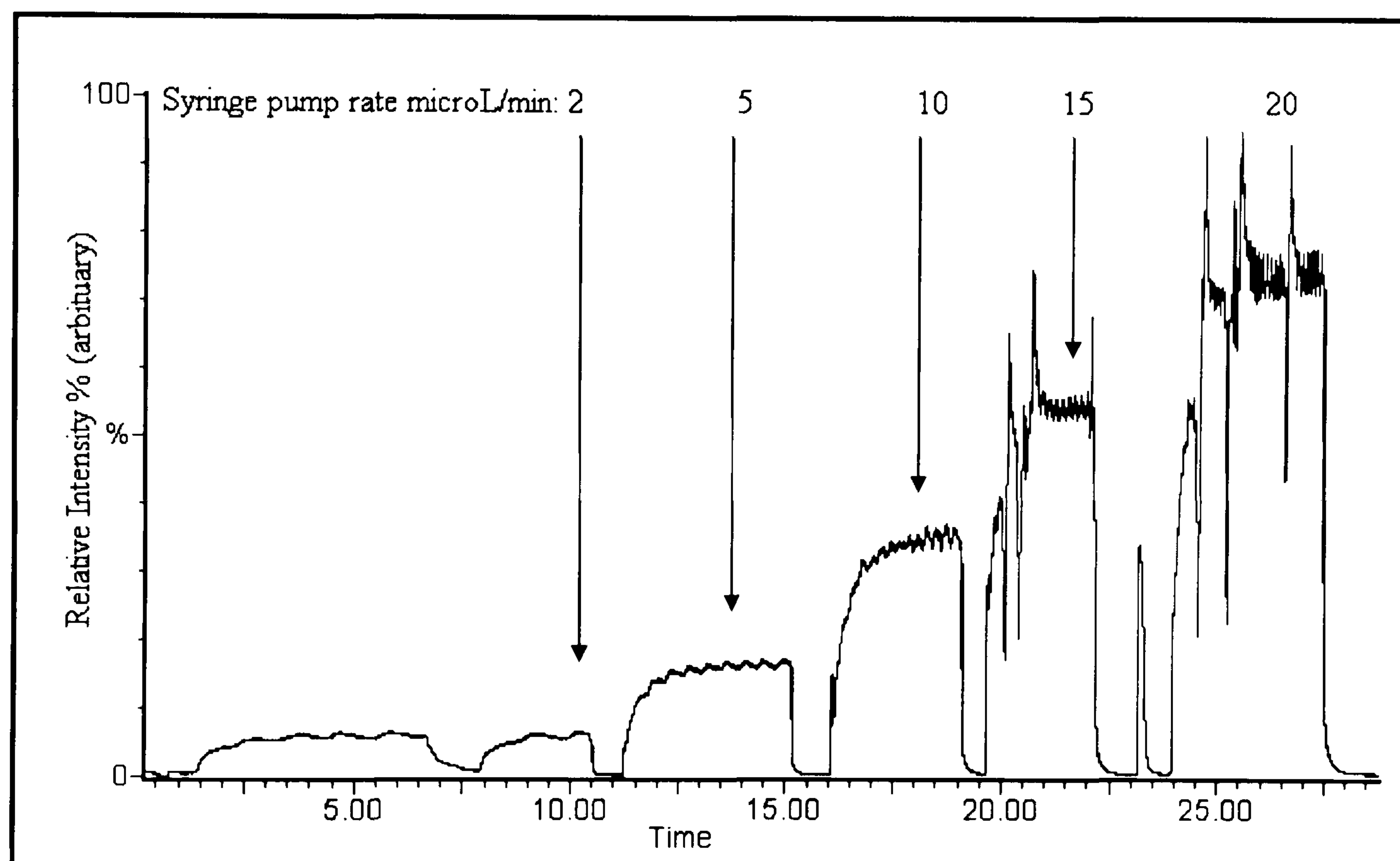


Figure 2.2. Atmospheric pressure chemical ionisation - mass spectrometer (APCI-MS) trace of ethyl butyrate emission from olfactometer. Concentration in the syringe 1000 ppm.

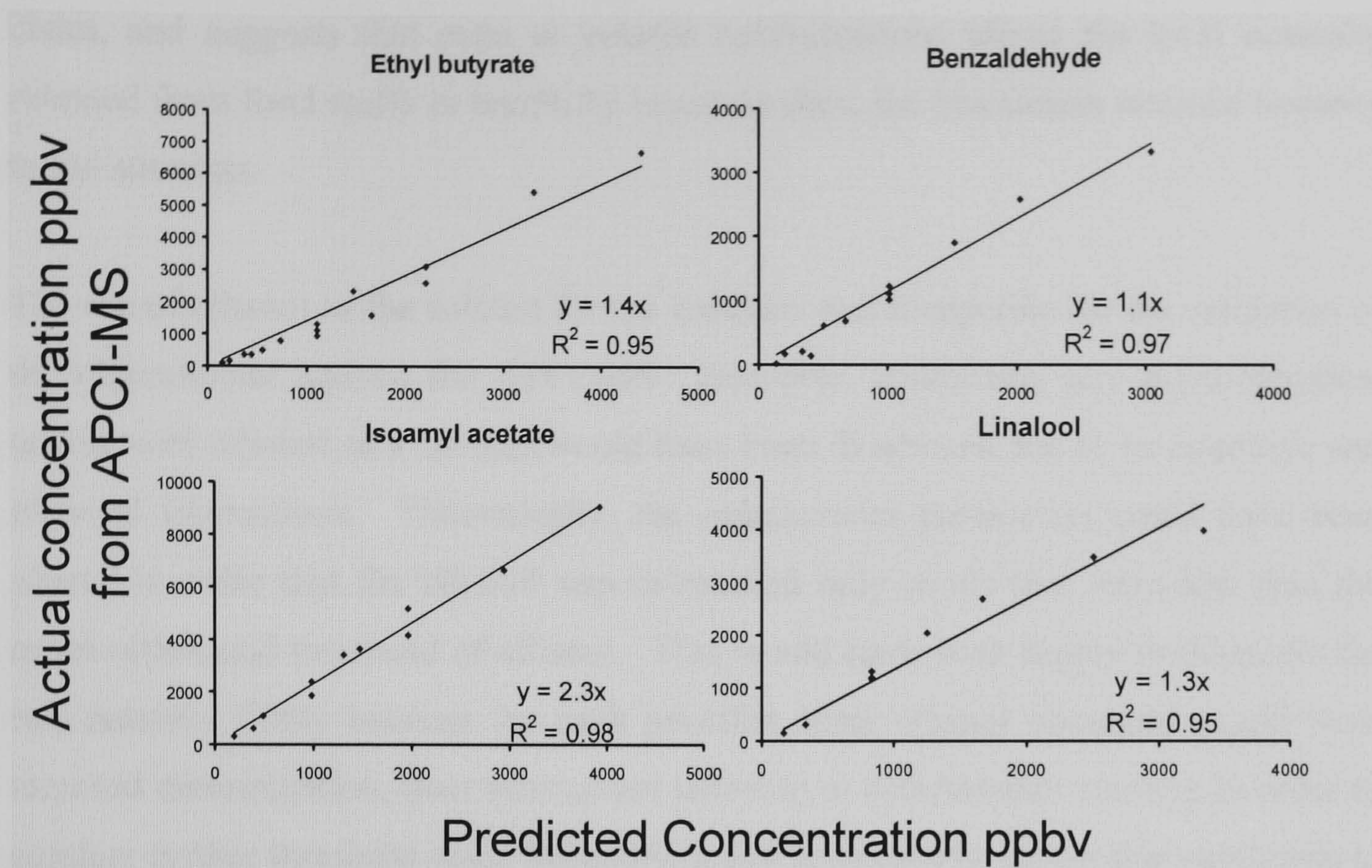


Figure 2.3. Predicted olfactometry gas phase concentration versus actual APCI-MS measured

Table 2.2. Summary of R² values and equations of the line: predicted concentration versus actual concentration.

Volatile compound	R ² value	Gradient
Ethyl butyrate	0.95	1.5x
Benzaldehyde	0.97	1.1x
Isoamyl acetate	0.98	2.3x
Linalool	0.95	1.3x

The results of this design validation showed two main points: the first being the accuracy of the predicted olfactometer output, and secondly that the APCI-MS demonstrated linearity in measuring volatile compounds within the volatile concentration range used in this study. There have been recent claims that absolute measurement of flavour intensity using mass spectrometry techniques is unreliable due to problems with the chemical ionisation process and the transfer of ions to the source (Normand, *et al.*, 2004). The results from this study would not support this

claim, and suggests that even at volatile concentrations above the level normally released from food stuffs in breath by breath studies, the instrument retained linearity to the stimulus.

The use of ethanol as the solvent for the volatiles was acceptable for the validation of the olfactometer against the APCI-MS. However, conducting any psychophysical testing with ethanol as a solvent would have been ill advised due to its cognitive and physical interactions. Theoretically, the olfactometer parameters could have been altered in order that the ethanol vapour reached only levels that were less than the mean orthonasal threshold of ethanol. This would have been highly problematic for two reasons, firstly because for each panellist, their ethanol threshold would have required determination, then subsequent tailoring to olfactometer settings in order to conduct further threshold tests. Secondly, it has been clearly shown that subthreshold levels of volatiles can have an additive effect to the overall threshold, thus biasing results (Keast, *et al.*, 2003; Dalton, *et al.*, 2000). The only suitable solvent would be water, yet even this would have been a compromise as the humidity would have changed with changing volatile concentration. Humidity has been previously assumed, but not proven to affect sensory threshold (Philpott, *et al.*, 2004). Furthermore, most volatile compounds are hydrophobic and therefore solubility is limited at the solute concentrations required in the syringe. Figure 2.4 shows the APCI-MS-olfactometer trace of ethyl butyrate in water (1000 ppm).

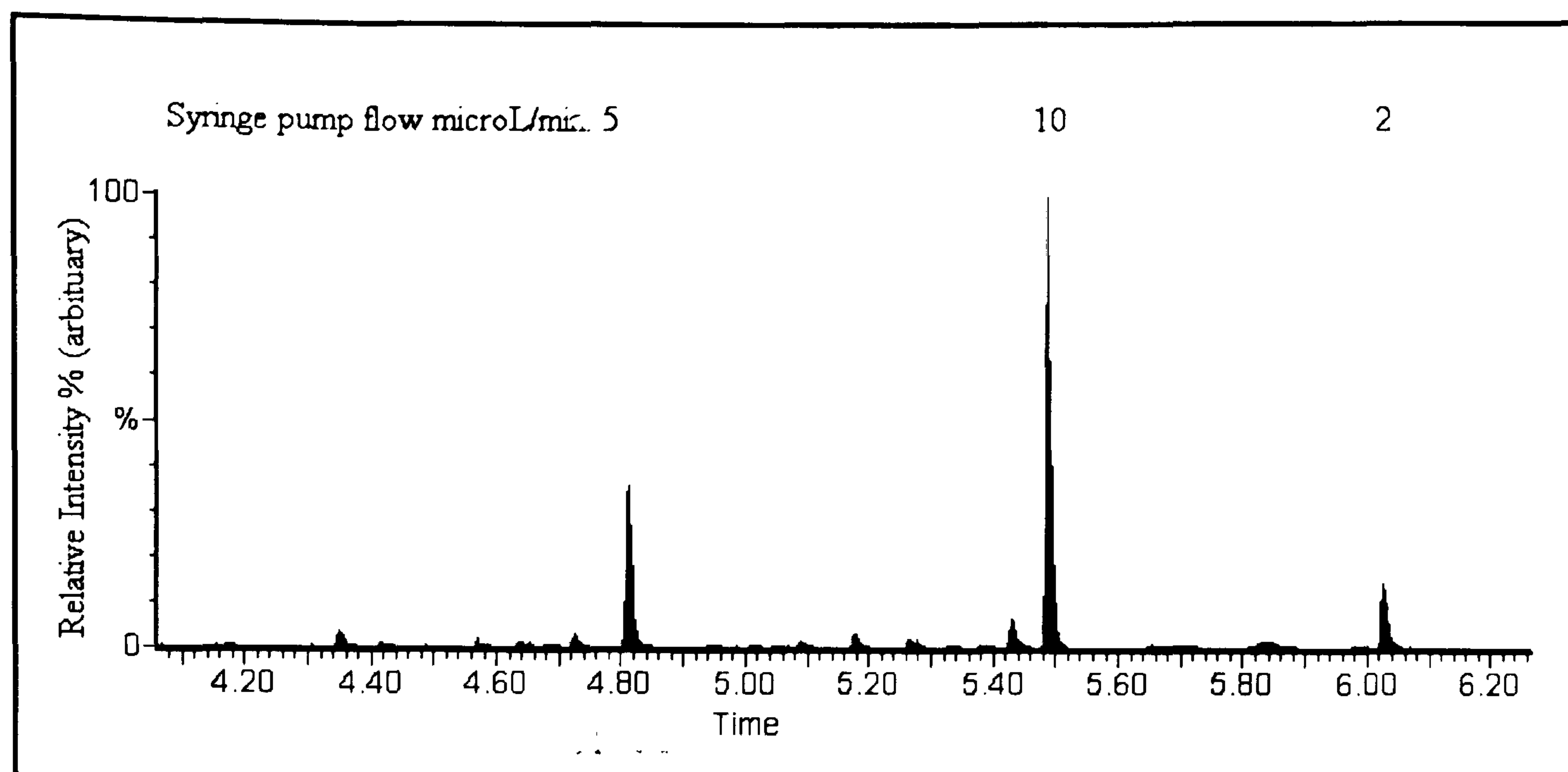


Figure 2.4. APCI-MS trace of ethyl butyrate gas phase concentration from olfactometer. Aqueous concentration in syringe was 1000 ppm.

The trace showed that the normally smooth signal became very spiked and unstable. This was probably due to the injection of random coalesced volatile droplets rather than a homogeneous solution. Introduction of neat volatile to the syringe would not have been a viable option, because even at minimal syringe pump flow rates and maximum gas flow, the concentration emission would have been many magnitudes stronger than the required threshold level.

To conclude, this design was simple and fast to control and produced highly reliable outputs but was totally inappropriate for use in sensory testing due to the requirement of a solvent. It was however useful in demonstrating the linearity of olfactometer volatile concentration in the gas phase.

2.1.2 Sniff bottles: static and dynamic.

An alternative delivery system was required, which would not have issues with the volatile solvent. One option was the use of sniff bottles. Scientific opinion on the use of such techniques in olfactometry research varies greatly, and beliefs appear to be discipline dependent. In medical areas focussed on olfaction, as an indication of a physiological, neurological or even pathological problem, simplistic sniff bottles are in favour (Lucchini, *et al.*, 1997; de Kruijk, *et al.*, 2003; Hornung, *et al.*, 1998; Lehrner, *et al.*, 1997). Although in recent years this is beginning to lean towards the

use of controlled flow olfactometry (Welge-Lussen, *et al.*, 2002) (see section 1.2.2 on olfactometry). Conversely, fundamental disciplines that focus mainly on the perceptual aspects or the mechanism of olfaction often regard sniff bottles as unreliable (Vuilleumier, *et al.*, 2000). However, corroborations of the claims are usually based on conjecture rather than experimental data.

In this study, two types of sniff bottle (static and dynamic) were compared for their ability to produce stable and reliable concentrations of aroma compounds using APCI-MS validation.

2.1.2.1 Change in headspace concentration over time

The static sniff bottles were simply glass bottles (123 mL, Fischer scientific, Loughborough, UK) containing 50 mL of either limonene, anethole, carvone or isoamyl acetate in water (1000 ppm). The dynamic sniff bottles were modified 500 mL solvent wash bottles (Nalgene, Hereford, UK) containing each of the same four volatiles at 250 ppm (Figure 2.5). Concentrations were chosen according to detection capabilities of the mass spectrometer: both types of bottle produced gaseous aroma concentrations that were detected mid range.

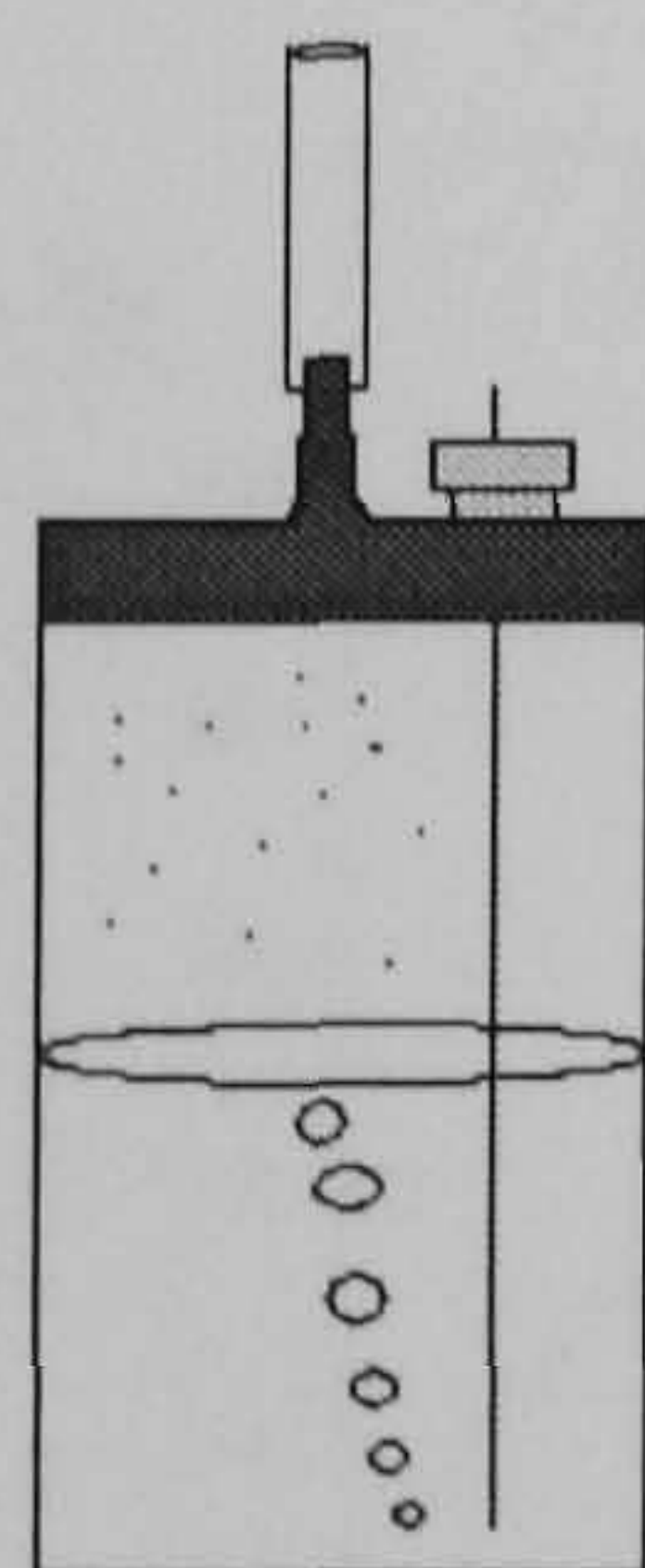


Figure 2.5. Dynamic headspace sniff bottle. As subject inhales through nose piece, laboratory air is forced through the solution producing constant output.

The headspace concentrations of the two types of bottles were recorded using APCI-MS over a period of ~80 minutes. Measurements were taken every 10 minutes (laboratory temperature 20°C). The measurement of the static sniff bottles involved vigorously shaking the solution, removing the lid and holding the bottle under the APCI-MS probe until a steady signal was recorded on the trace. For the dynamic sniff bottles, nitrogen gas was blown through the inlet tube (1 L.min⁻¹), which

bubbled through the solution. This simulated a controlled version of volatile release when an individual inhales the air through the bottle, where air would also be sucked through the liquid in the same direction and pass into the nosepiece. The nosepiece was then held under the APCI-MS probe until a steady signal was recorded.

The APCI-MS operating conditions were as follows: cone voltages; carvone 15, anethole 23, isoamyl acetate 18 and limonene 20; corona pin voltage at 4 kV; ion dwell time = 0.25 s; flow 20 mL min⁻¹. The mass spectrometer was calibrated as in section 2.1.1.3.

Results

Figure 2.6 shows the outputs of four volatiles from the static and dynamic sniff bottles normalised relative to the concentration at 2 min.

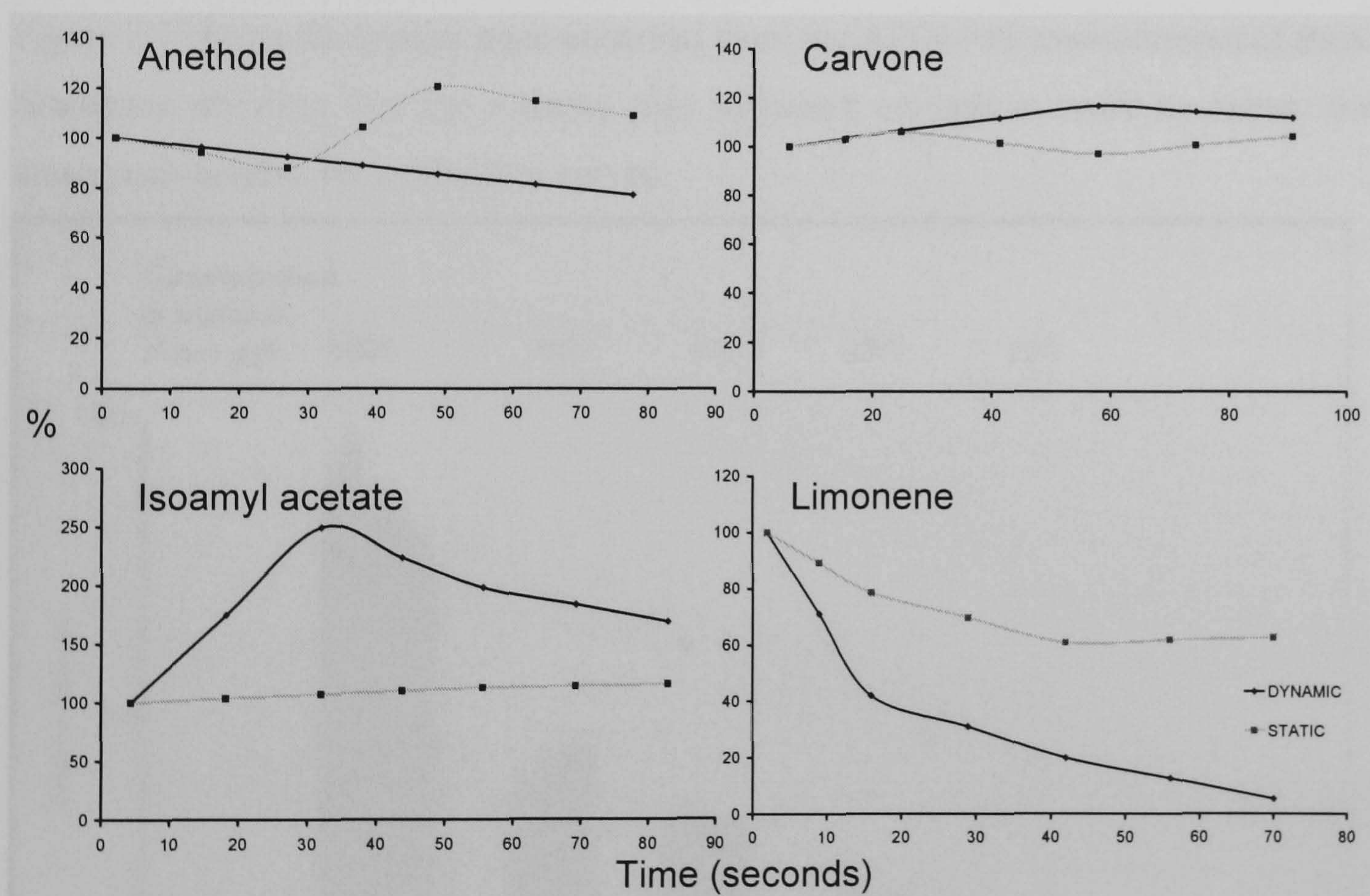


Figure 2.6 Volatile concentration deviation over time of dynamic and static sniff bottles as normalised.

The graph shows that although both bottles showed deviations in the headspace concentration over time, the static headspace bottles were shown to be more stable over time. Unlike the other volatiles limonene showed a decay in headspace concentration over time. This was likely to be due to its higher hydrophobicity and

volatility in comparison to the other volatiles. The aqueous reservoir of limonene was depleted at a faster rate due to equilibrium driving molecules from the solution into the gas phase to maintain the higher air-water partition coefficient.

2.1.2.2 Static headspace bottle calibration

Using the same protocol as in section 2.1.2.1, the headspace of 4 replicates of each of the 4 volatiles at increasing aqueous concentrations (100-1000 ppb) were measured in order to calculate the air-water partition coefficients. These coefficients were effectively a measure of equilibrium between the concentration of aroma compound in the gaseous phase and that in the aqueous phase.

Results

Figure 2.7 shows the typical trace observed from the APCI-MS measurement of static headspace showing that the stability was sufficient enough in order to extract the mean peak heights for calibration curves.

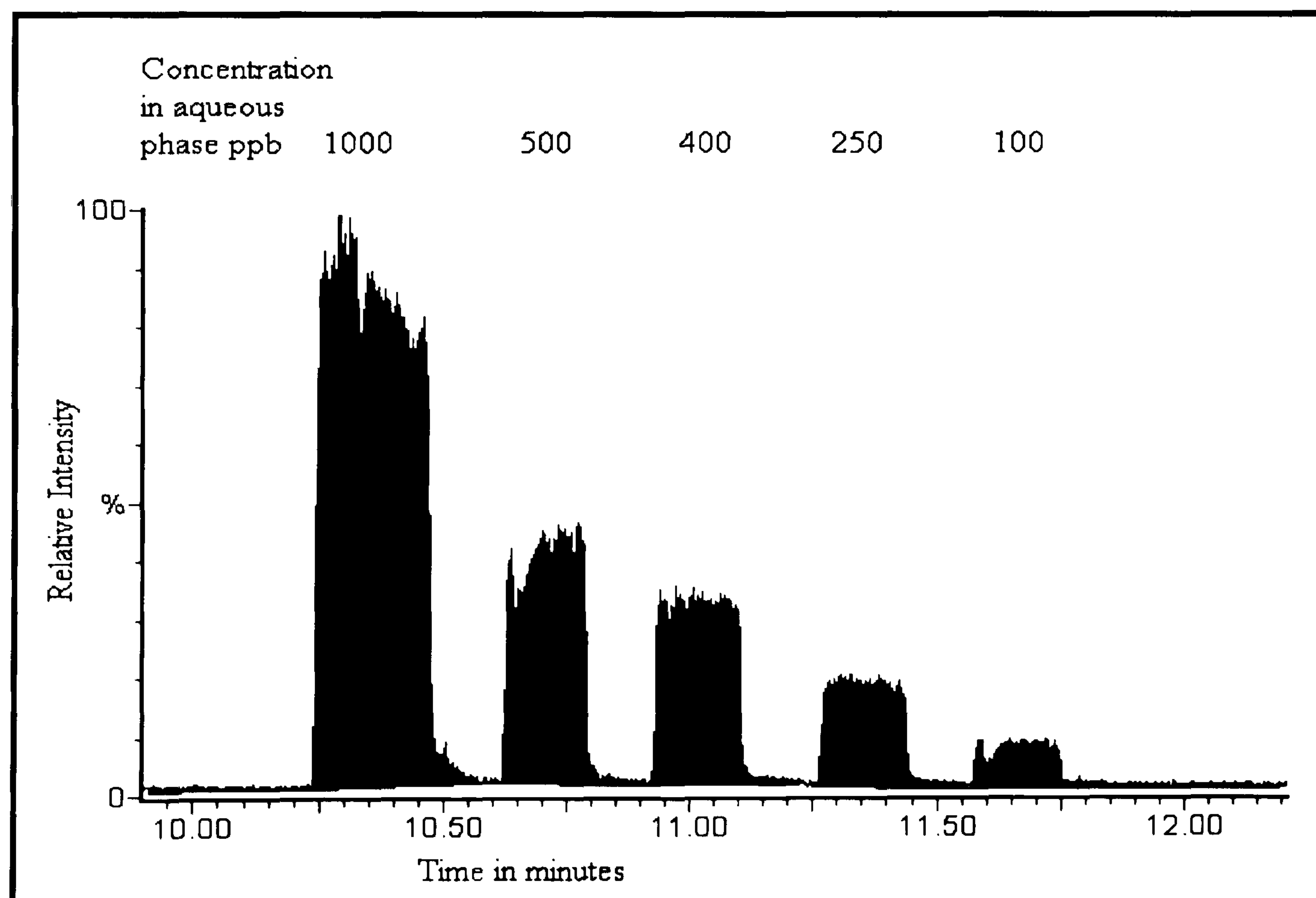


Figure 2.7 APCI-MS trace of open static headspace of carvone at different aqueous concentrations.

Isoamyl acetate, anethole and limonene all showed similar traces, although limonene had the least stable signal. Figure 2.8 shows the four calibration curves and Table 2.3 summarises the R^2 values and equations of the lines.

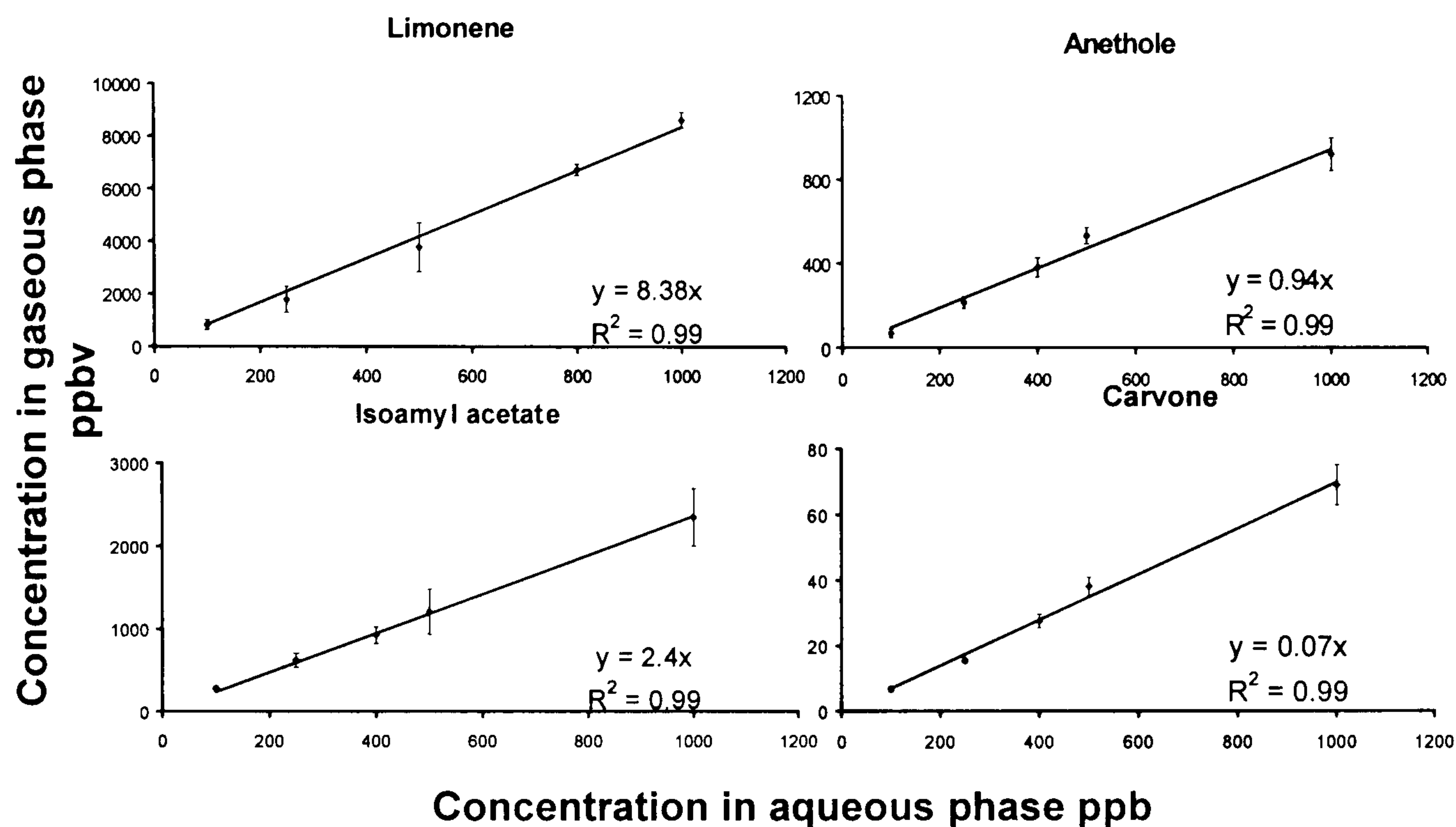


Figure 2.8 Static sniff bottle calibration curves of 4 volatiles with 4 replicates. Gradient is an indication of the air-water partition coefficient of each volatile..

Table 2.3. Summary of R^2 values and equations of the curves of static sniff bottle calibration with APCI-MS.

Volatile	R^2 values of curve	Equation of the line (air-water partition coefficient) $Y=$
Limonene	0.99	$8.38x$
Anethole	0.99	$0.94x$
Isoamyl acetate	0.99	$2.4x$
Carvone	0.99	$0.07x$

These results show that the headspace concentrations of these volatiles remained suitably linear and stable even though the entire cap had been removed from the glass bottle. Standard headspace measurement would normally introduce the APCI-MS probe via a small aperture in the cap to minimise headspace disruption.

Sensory thresholds exist at concentrations much lower than those measured. Instrumental limitations make it difficult to measure low concentrations but it was assumed that interpolation of the calibration curves would have remained linear. As mentioned in 2.1.1.4, results showed the mass spectrometer response was linear to the stimulus.

In summary, static headspace sniff bottles demonstrated a very simplistic yet stable method of producing reliable concentrations of volatile in the gas phase. The bottles were also considered suitable for future psychophysical testing. It must be mentioned that the calibration method did not take into account any assumptions on disruption of the headspace when a panellist sniffs. Whereas, the APCI-MS probe removed a small quantity of the headspace ($20 \text{ mL}\cdot\text{min}^{-1}$) with little or no effect, a person inhaling the headspace would naturally have a far greater impact on the equilibrium. The average monorhinal inhalation rate for humans during a normal sniff is 16.2 L min^{-1} (Laing, 1985b), which is ~ 800 times higher than the APCI-MS flow. This effect on the headspace would be difficult to test and far more difficult to model due to the extent of variation in sniffing behaviour between subjects and replicates. Therefore, any psychophysical testing made the single assumption that each panellist would receive an identical quantity of volatile laden air, which they would subsequently inhale at different rates and velocities.

In conclusion, three methods of controlling or predicting volatile concentrations in the gas phase were designed and tested for their efficacies. Each method has its own distinct advantages and disadvantages, which appears to be a common feature of olfaction research: compromise. If accuracy is improved instrumentally, then psychophysically the method may become impractical, and vice versa.

2.2 OLFACTOMETRY - METHOD OF PRODUCING VOLATILE PULSES

2.2.1 Design

The main constraint in designing a pulsing olfactometer was the mechanism of the valves. If a system is used with high air flow such 10 L min^{-1} (recommended olfactometer output flow rate (Laing, 1985b)), dilution between the pulses may prevent the emission of "square" peaks. Furthermore, at higher flows the valves can be cumbersome and require extensive plumbing as they are generally powered pneumatically. Another design constraint was the method of obtaining stable volatile concentrations in the gas output for long periods without needing to replenish the reservoir.

The design in this study made compromises due to limitations on the equipment available and utilised low gas flow rates through low diameter tubing to minimise pulse diffusion problems. The low gas flows meant that switching valves could be used under electronic control rather than being governed by pneumatics. Figure 2.9 shows this design.

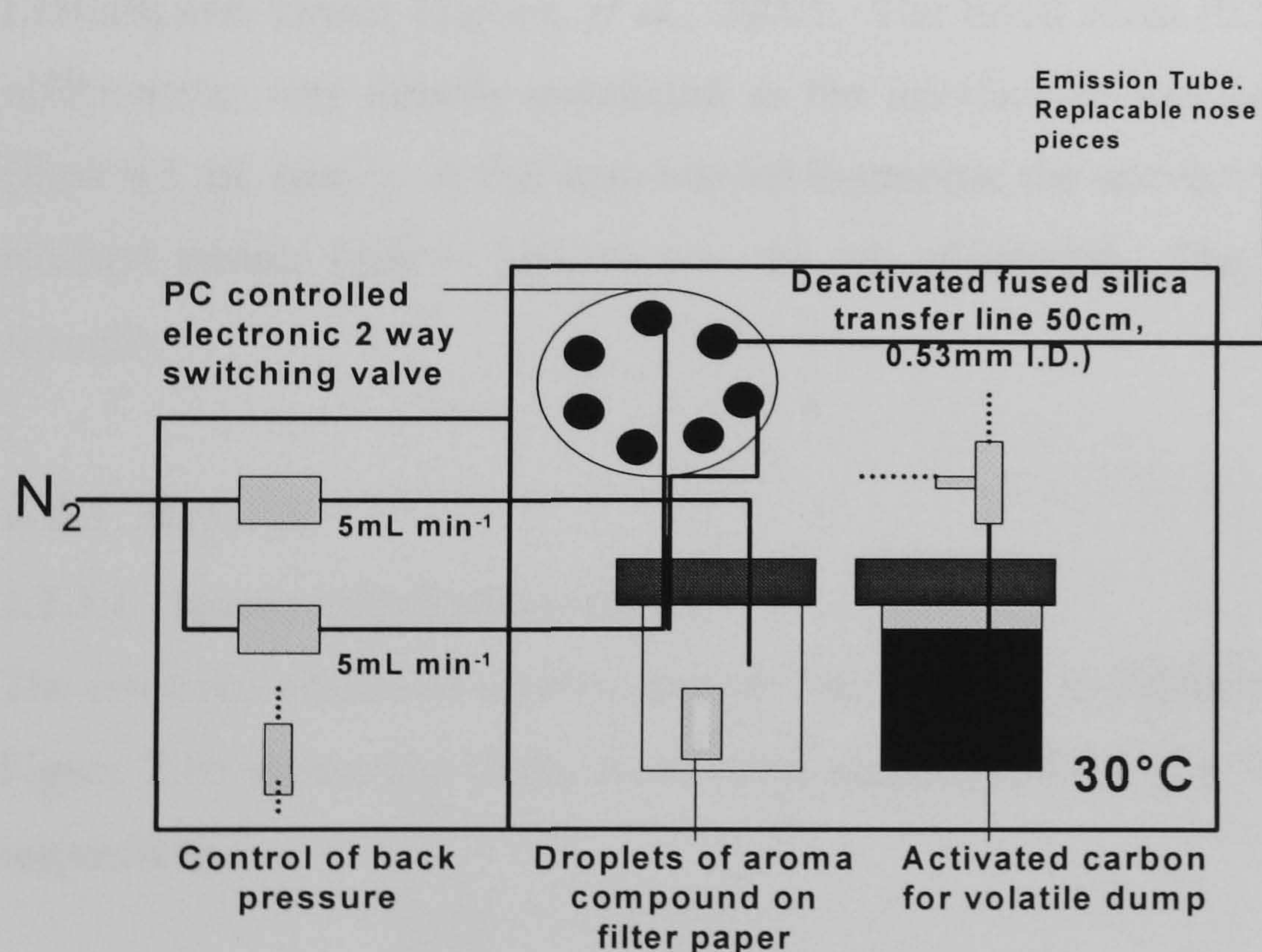


Figure 2.9. Pulsing olfactometer design using modified Liquid Chromatograph (Shimadzu, Tokyo, Japan).

The volatile reservoir consisted of filter paper soaked in aroma compound inside a 5 mL glass bottle with a cap containing 5x 2 mm apertures for diffusion. This was located inside a 100 mL Schott bottle, through which nitrogen gas was constantly blown. The volatile saturated gas was then either switched through deactivated fused silica and to the emission tube or to an activated carbon dump. In turn, the emission tube either produced volatile laden gas or just pure nitrogen, both at 5 mL min⁻¹. A back pressure regulator controlled changes in flow that occurred with each valve position. Due to this problem of back pressure, the olfactometer flow was restricted to 5 mL min⁻¹, which meant that the emission concentration could not be altered. The fastest rate at which the valves could be programmed to switch was 0.6 seconds.

2.2.2 Validation method - Ion Trap Mass Spectrometry (ITMS)

Unless indicated, each of the following pulsing validation experiments were monitored under identical conditions using a Thermo Finnigan LCQ Deca Xp Ion Trap Mass Spectrometer (ITMS) in conjunction with an APCI gas phase interface. The working principle of this source are similar to the APCI source developed by Linforth and Taylor (Taylor, *et al.*, 2000). The fused silica (0.53 mm ID) from the olfactometer was directly connected to the interface to simulate real time pulsing (flow = 5 mL min⁻¹). A full scan was set to monitor the relevant ions, in these studies isoamyl acetate ($m/z = 130.19$) was the ion of interest. The scan time was 0.02 seconds.

2.2.3 Results

2.2.3.1 Square edged pulse testing

The ion trace profile of isoamyl acetate being pulsed at different rates was assessed. Figure 2.10 shows ion traces from pulse durations of: 7.5, 6, 1.2 and 0.6 seconds, respectively.

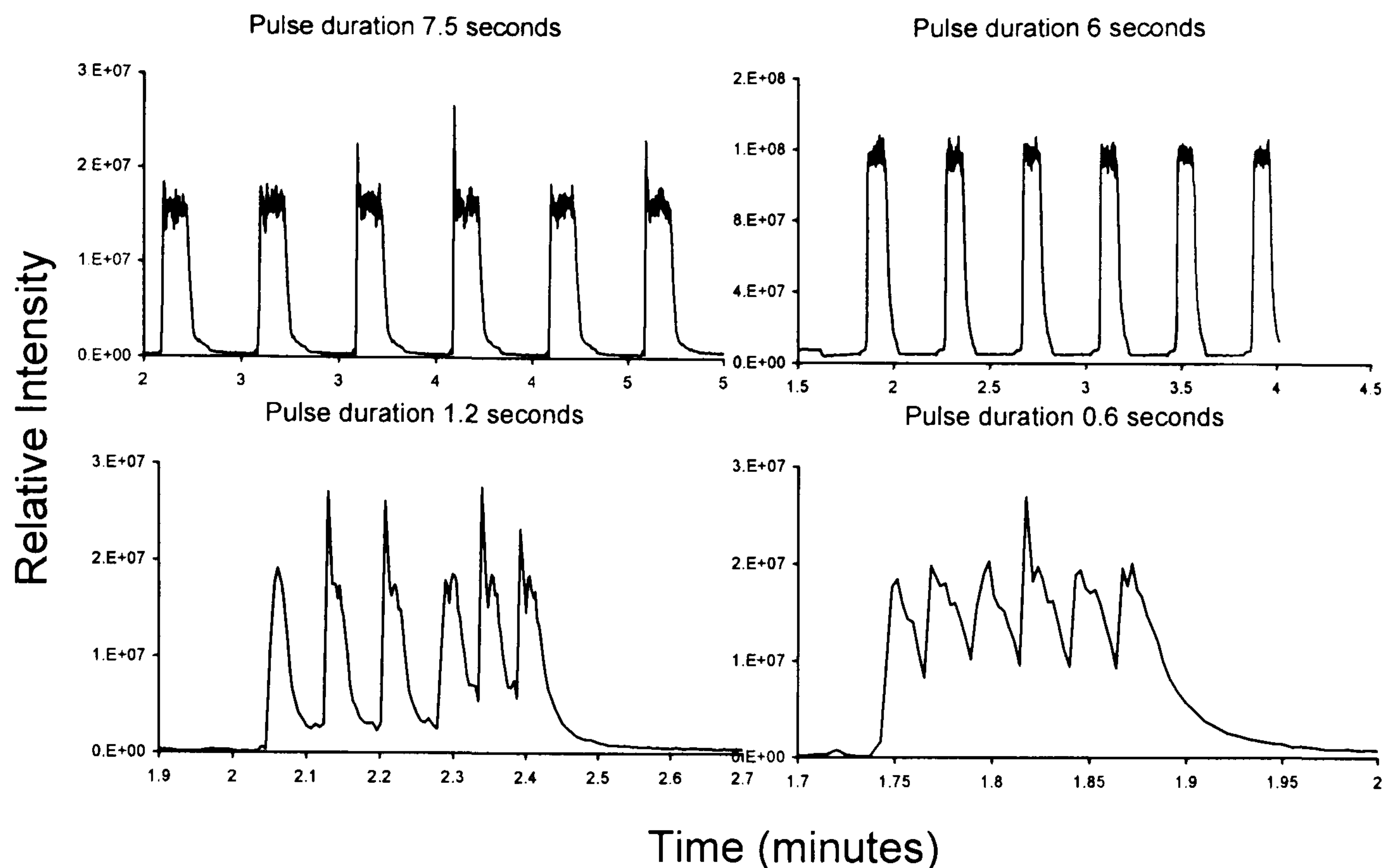


Figure 2.10 ITMS ion traces of pulsed isoamyl acetate at different pulse durations and frequencies.

It can be seen that at the lower frequencies (6 s and 7.5 s), the pulses produced were square edged, however, as the frequency increased there was less peak and trough resolution. It was unclear at this stage whether this was due to pulse diffusion issues in the fused silica or simply limitations on the speed of ionisation in the IT-mass spectrometer.

2.2.3.2 Pulse time accuracy

It was then necessary to assess the accuracy of the valve switching times in case communication between the PC and the switching valves incurred delays. This was achieved by extracting the ion trap times at which either a trough or peak occurred. These points were extracted from the 0.6 s pulse duration graph (pulse duration 0.01 min) in Figure 2.10. Figure 2.11 shows this graph, where the gradient of both peaks and troughs is 0.02x, thereby demonstrating the timing accuracy of the olfactometer pulses. The gradient of 0.02x indicates that in each line, either a peak or a trough occurred every 0.02 minutes (1.2 seconds) because the olfactometer switched every 0.01 minutes (0.06 secs).

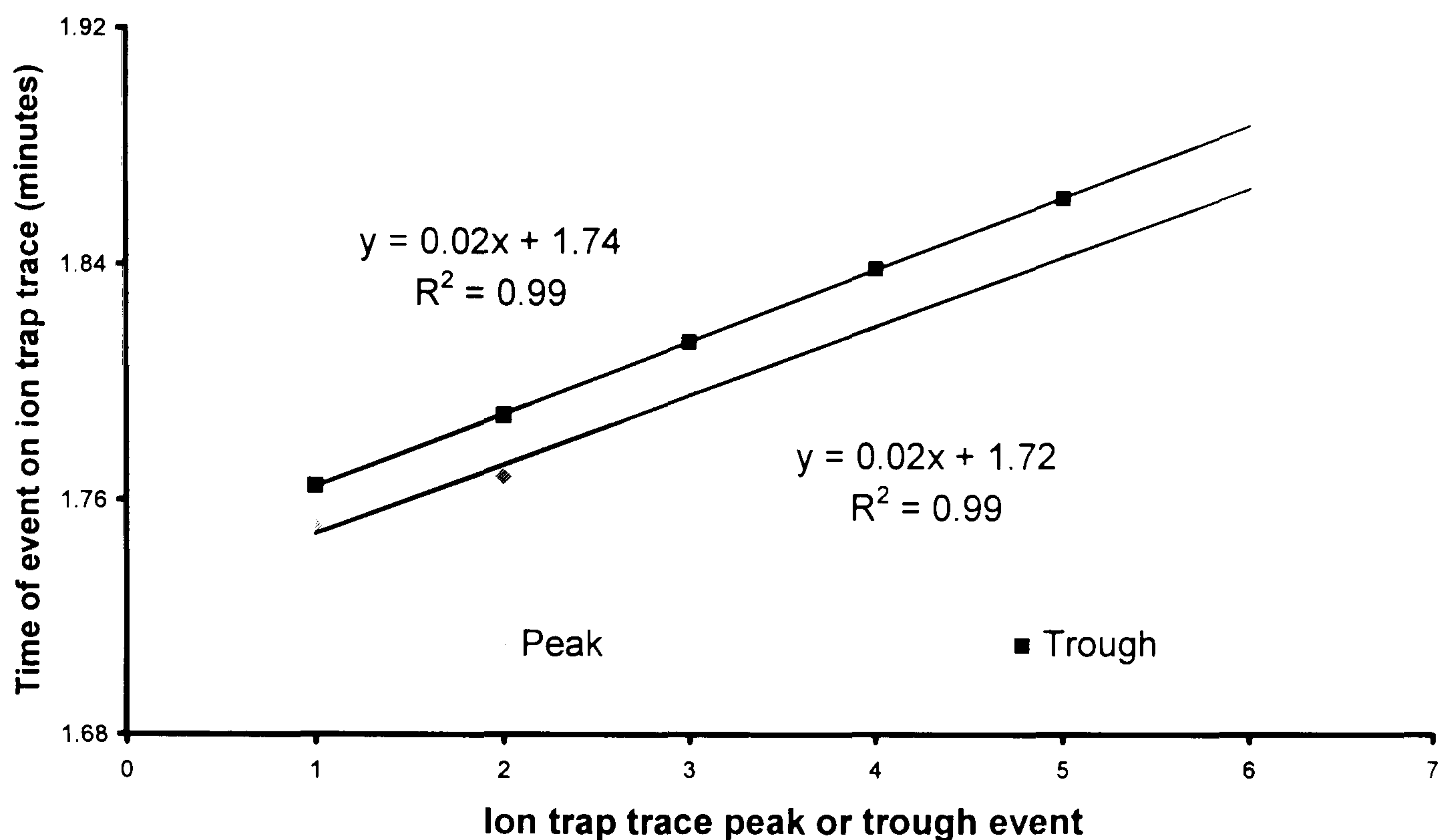


Figure 2.11 Peak and trough events versus time of occurrence on ion trap trace, gradient means that either a peak or trough occurred every 0.02 mins (1.2 seconds).

2.2.3.3 The effect of olfactometer oven temperature on pulse shape and height

A programme of pulses every 12 seconds was measured at 30°C, 40°C and 50°C to assess whether there was an optimum temperature for peak resolution or whether it changed the emission concentration. The three ion traces are shown in Figure 2.12 and demonstrate that changing the oven temperature had little or no effect on either pulse shape or emission concentration.

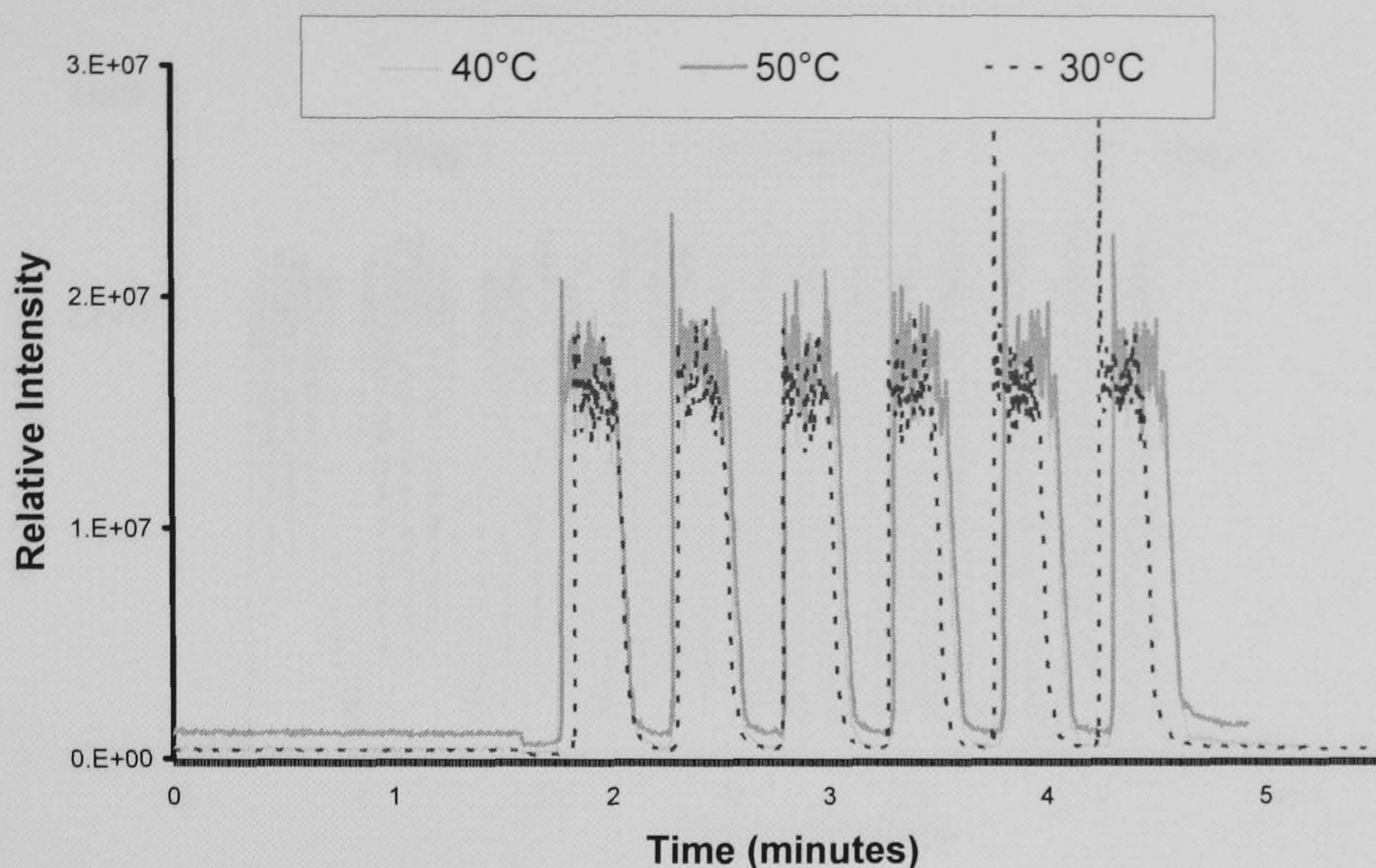


Figure 2.12 ITMS traces of 12 second pulse frequency at 3 different oven temperatures.

2.2.3.4 Repeatability of pulse programmes

The repeatability of the pulse programmes was a highly important aspect to assess in order that it could be completely omitted from the potential sources of variation in future sensory testing. Three repetitions of a pulse programme with increasing trough durations were run once again using the ion trap mass spectrometer to monitor the real time olfactometer output of isoamyl acetate. Figure 2.13 shows the three ion traces superimposed on the same time axis, and Figure 2.14 shows a plot of rep 1 versus rep 2 with a R^2 value of 0.92 and a gradient of 1.

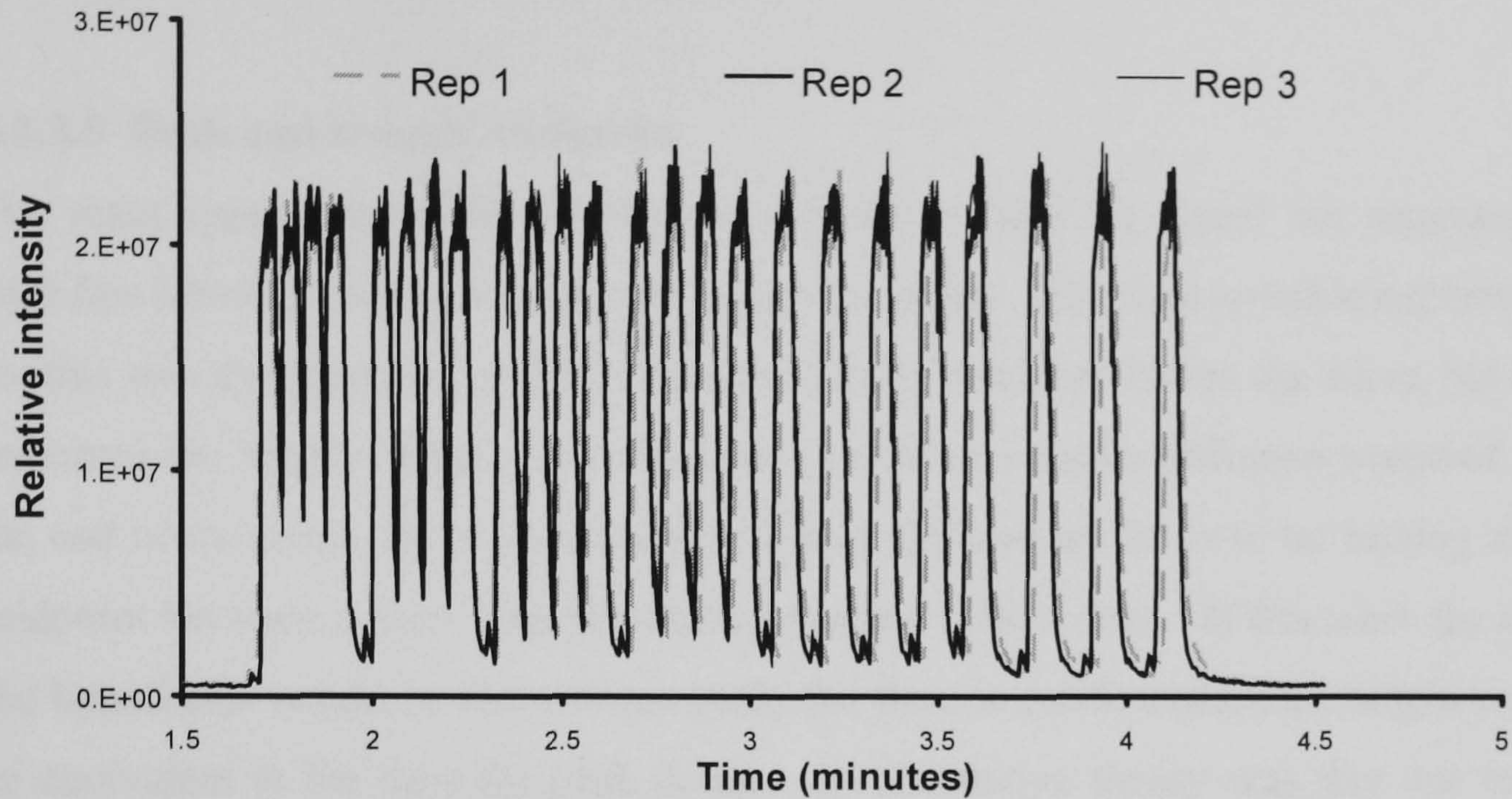


Figure 2.13 Consistency of isoamyl acetate emission ITMS trace from olfactometer with increasing trough time programme.

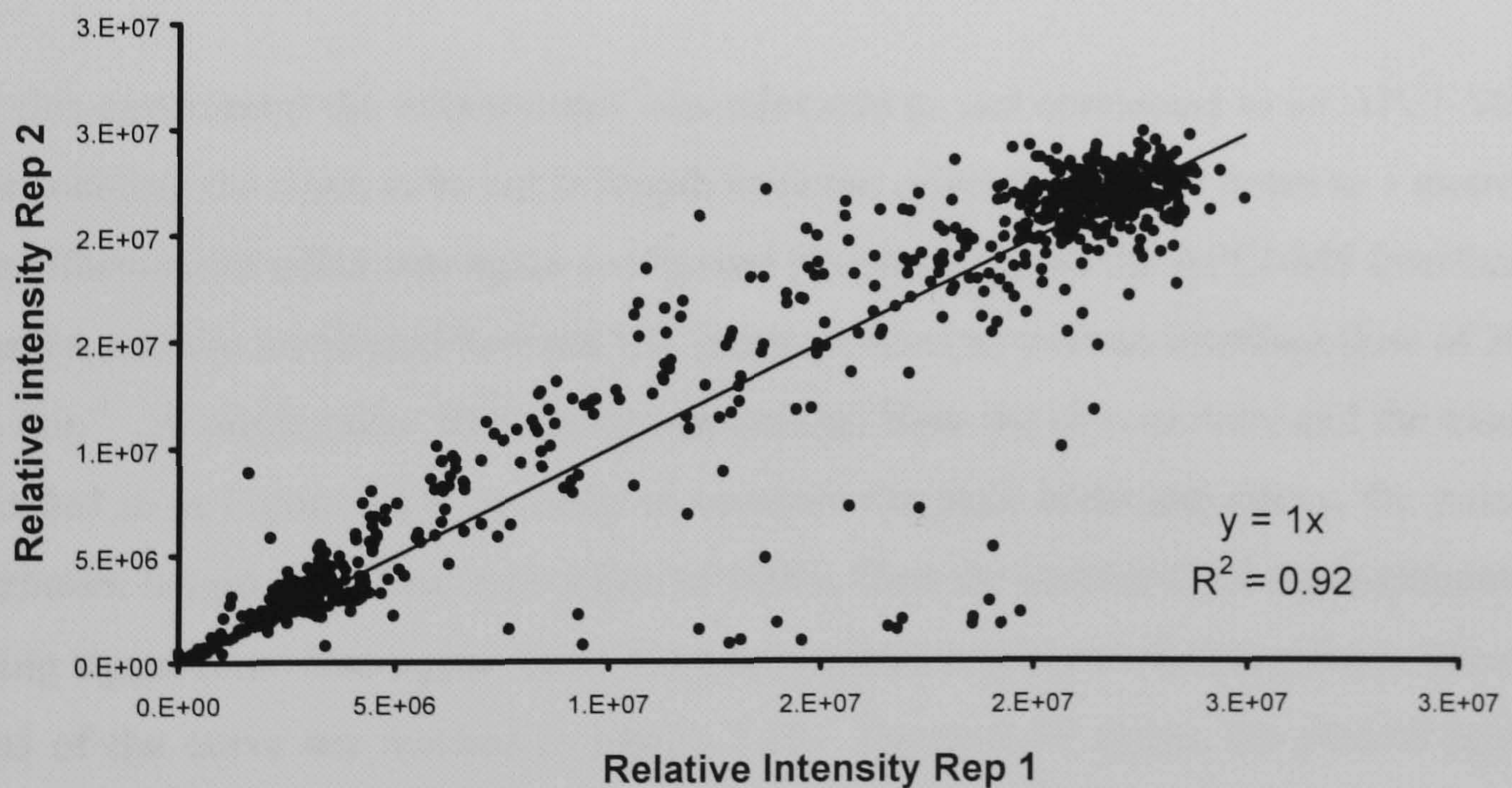


Figure 2.14 Plot of peak heights. Data points from replicates 1 and 2.

These results show that the repeatability of the olfactometer pulsing was sufficiently high to use in sensory testing. However, it can still be noted that the base line

increased with decreasing trough time. This was further investigated in section 2.2.3.5.

2.2.3.5 Peak and trough resolution

The mass spectrometer traces indicated a problem with the signal not returning to base line between pulses with shorter trough durations. The first possible explanation for this was that a square edged pulse was initially produced inside the silica, but as it traversed the length of the silica a certain amount of volatile diffusion occurred. At the end of the silica, the molecules would have diffused so far as to be mixing at the midpoint between pulses, thus preventing a return to base line. If this were the case, the hypothesis would be that on any peak, the time to reach maximum height would be equivalent to the time for peak decay. An alternative theory was that due to the relatively high concentration of volatiles entering the mass spectrometer, their removal from the source and thus the available voltage was the limiting factor in returning to base line. If this was the case, then time for peak onset and time for peak decay would be significantly different.

For this experiment the olfactometer was relocated to and connected to an APCI-MS thus enabling the silica to be cut in length from the original 3 metres down to 1 metre. The olfactometer silica was again configured in order to reach the APCI-MS interface directly, and the monitored ion was 131 (isoamyl acetate) with an interface flow of 20 mL min⁻¹. A single pulse 10.5 s long was emitted from the olfactometer and the trace recorded as in Figure 2.15. In order to compare the peak onset and decay, the pulse maximum height at plateau was taken as 100%, then the instrumental measurements during signal onset and signal decay were normalised relative to the maximum. These areas of the curve are marked in Figure 2.15. Figure 2.16 shows the plot of peak onset and decay in respect to the same time scales.

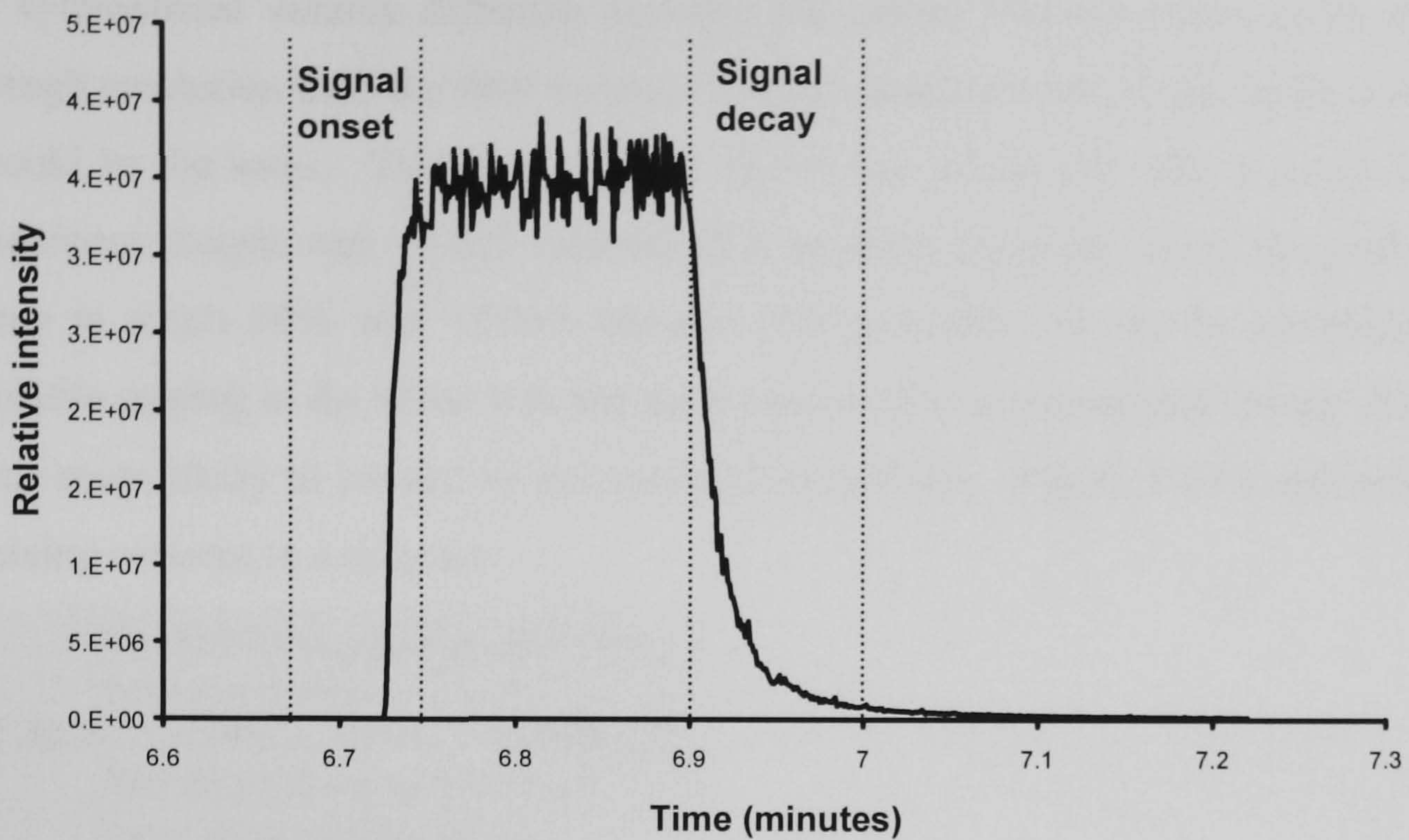


Figure 2.15. APCI-MS trace of IAA from a single 10.5 second emitted pulse. Lines showing decay and onset times are taken from the olfactometer program.

The lag time from signal to onset was caused by the volatile traversing the 1 metre length of the silica at only 5 mL/min, which took ~4 seconds.

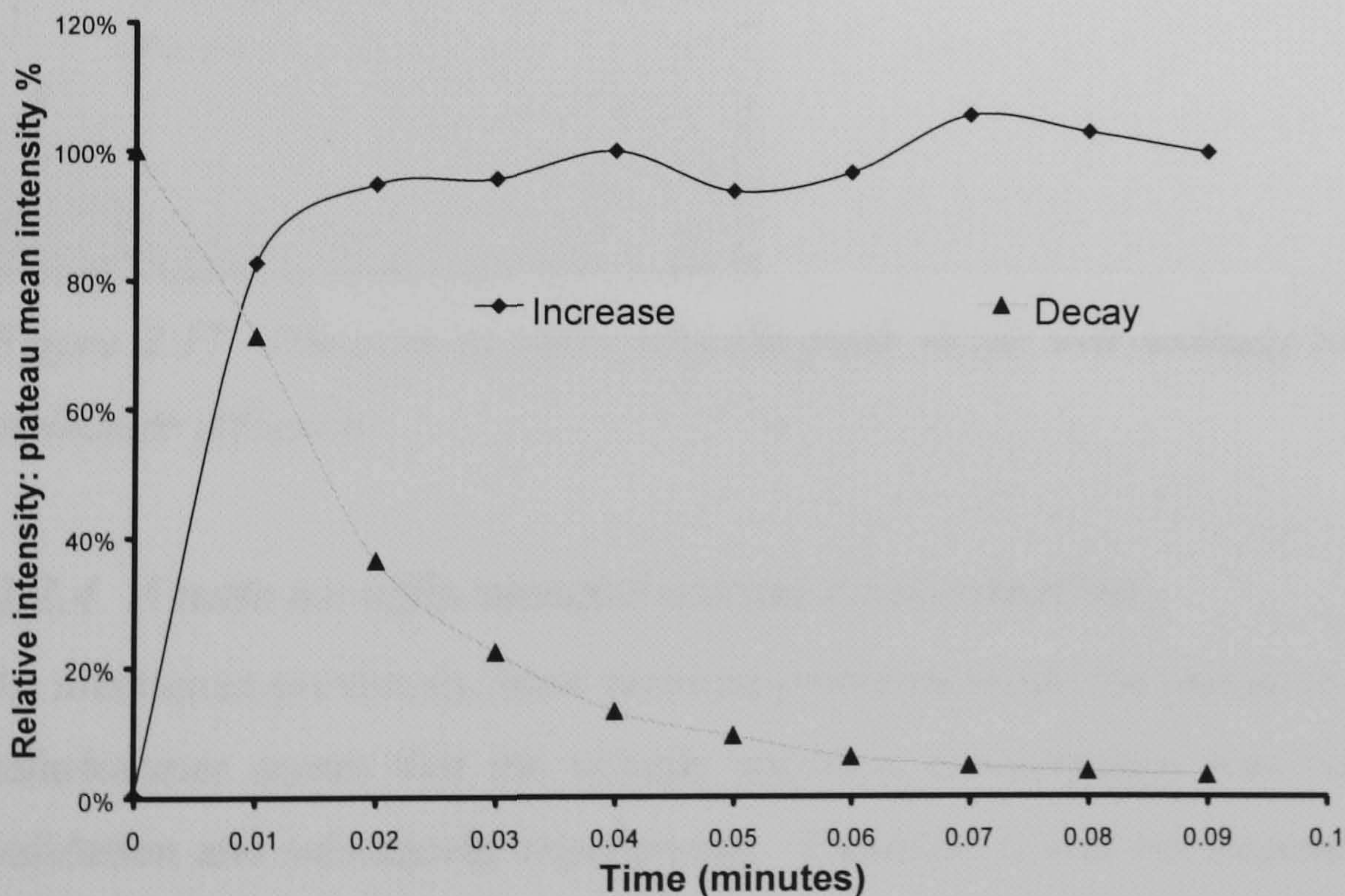


Figure 2.16. Signal onset and decay on the same time axis. Values are percentages of the maximum height of the pulse at plateau.

If symmetrical volatile diffusion between the pulses was the direct cause of poor trough resolution then the time to reach 50% of maximum height for onset and decay would be the same. This graph clearly shows that whilst the time to reach 50% of maximum height was ~ 0.005 minutes (0.3 seconds) for peak onset, for peak decay time to reach 50% was ~ 0.015 minutes (0.9 seconds). It can be concluded that volatile mixing in the silica was **not** the reason for the asymmetrical trough shape but was more likely to related to instrumental limitations. Figure 2.17 summarises the mixing concept in a diagram.

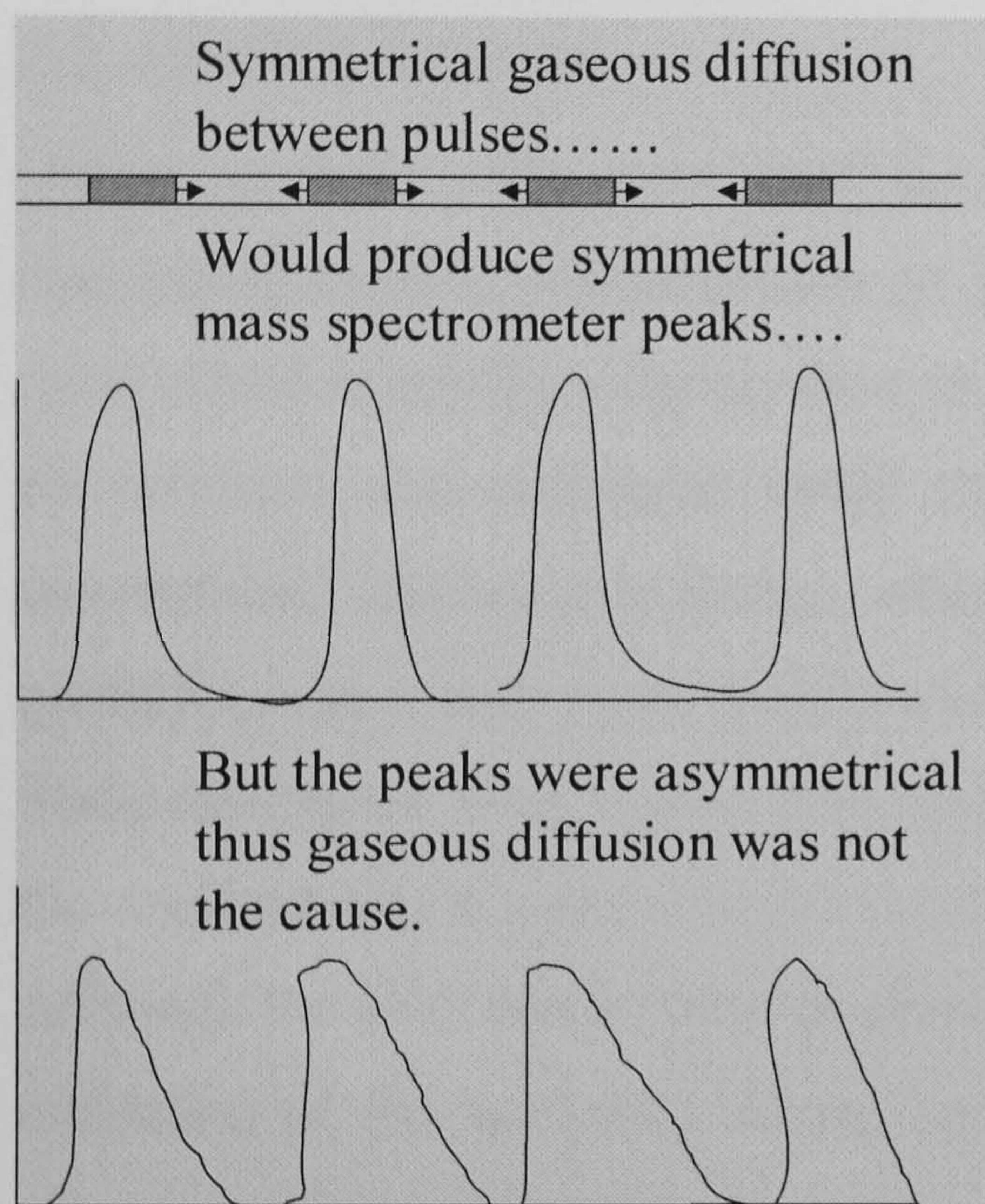


Figure 2.17 Diagram to show why the peak shape was unlikely to be caused by molecular diffusion.

2.2.4 A note on olfactometer output concentration

As mentioned previously, back pressure problems inside the pneumatic system of the olfactometer meant that the volatile emission concentration was static throughout validation and subsequent experiments. Therefore it was not necessary to calibrate the olfactometer for every experiment, as the arbitrary values (relative intensity) recorded by the APCI-MS (and ITMS) were proportional to the actual concentration (see section 2.1.1.4). Nevertheless, before any sensory testing was conducted it was appropriate to measure the actual concentration of isoamyl acetate in the raw

emission from the olfactometer. This concentration was assumed to be constant throughout the remainder of the testing and was based on the neat volatile reservoir being refreshed every 7 days of testing.

The calibration procedure involved calculation of the mean height of an olfactometer output for 1 minute, which was then compared to the mean height obtained from an injection of a known concentration of isoamyl acetate in hexane solution. The output concentration was found to be 270 mg m^{-3} (~50 ppmv).

2.3 CONCLUSION

Online methods have been used to assess the efficacies of four techniques for controlling volatile concentrations in the gas phase. The major advantage in this study was that designs ranging from the more complex, to the relatively simple could be validated and compared under the same conditions. This is in contrast to conventional olfactometer design, which would be more concerned with engineered prediction rather than actual measurement of the volatile emission.

Static headspace sniff bottles superseded the controlled volatilisation techniques in the compromise between concentration accuracy and psychophysical appropriateness. Although the sniff bottles may be prone to concentration disruption due to a subject's inhalation of the headspace during sensory testing, the method requires no organic solvent to disperse the volatiles. Whereas, the olfactometer would be less prone to subject disruption, yet requires a solvent that has a potentially odour/trigeminal activity.

An instrumental method has been successfully designed and tested in producing square edged aroma pulses, and has been shown to be most effective with low gas flow and plumbing with small internal diameter to minimise mixing.

3 RETRONASAL AND ORTHONASAL THRESHOLDS: CORRELATING PANEL VARIATION WITH *IN VIVO* ANALYSIS

The psychophysical study of olfactory thresholds is continuously evolving in order to increase the accuracy of the test and subsequently lessen the associated variance in group and individual measurement. Researchers have shown distinct correlations between olfactory sensitivity, olfactory processing and degenerative disorders such as Alzheimer's, HIV, Schizophrenia and Parkinson's. Consequently, threshold determination and odour recognition methods may be used to monitor such illnesses non-invasively (Bacon, *et al.*, 1998; Murphy, *et al.*, 1998; Lehrner, *et al.*, 1997; Hirsch and Trannel, 1996; Stevens and Cruz, 1995; Stevens and Dadarwala, 1993; Stevens, *et al.*, 1998; Elsner, 2001; Hornung, *et al.*, 1998; Griep, *et al.*, 1995). Many researchers have exposed the limitations and sources of variation between different existing test methods and why results can differ over time for the same method (Doty, *et al.*, 1995b). One source of variation is considered to be the method of sample presentation - as often the concentration of an odorant being presented to the assessor cannot be instrumentally confirmed, and thus is usually estimated (Vuilleumier, *et al.*, 2000). In particular, methods such as Teflon squeeze bottles produce highly variable odorant concentrations due to the inability of many volatiles to reach headspace equilibrium after each squeeze.

Psychophysical methods also vary from the most simplistic methods of constant stimuli, to the more complex methods that attempt to eliminate a subject's decision based criteria error e.g. R-index (Cliff, *et al.*, 2000). The threshold results obtained are often a function of the test used (Doty, *et al.*, 1995b; Doty, *et al.*, 1995a; Fortier, *et al.*, 1991; Kaernbach, 2001; Lawless, *et al.*, 1995; Stevens, *et al.*, 1988). This test specific variation increases the necessity to eliminate other potential sources of psychophysical variation, such as the physiological differences, in order to assess the methods more conclusively.

In vivo measurement of flavour release has been made possible through the use of venturi interfaced mass spectrometry, where volatiles in expired breath from the nose are monitored in real time (Taylor, *et al.*, 2000). However, large variations between individuals are often observed in this type of breath by breath analysis, whereby certain individuals consistently release more volatile into the nosespace than others despite consuming identical food samples (Baek, *et al.*, 1999; Brauss, *et al.*, 1999; Linforth, *et al.*, 1999; Linforth, *et al.*, 2002). Regardless of the large differences, it is often assumed that the height of the first exhalation breath peak on the APCI-MS trace is the most sensorially significant because it is consistently the highest concentration entering the nosespace when a foodstuff is consumed (Brauss, *et al.*, 1999). This may have implications in the differences observed in retronasal thresholds, in that the sensitivities of two individuals may differ greatly simply due to the differences in their in-nose concentration when they consume the same sample. Figure 3.1 depicts this theory as a schematic.

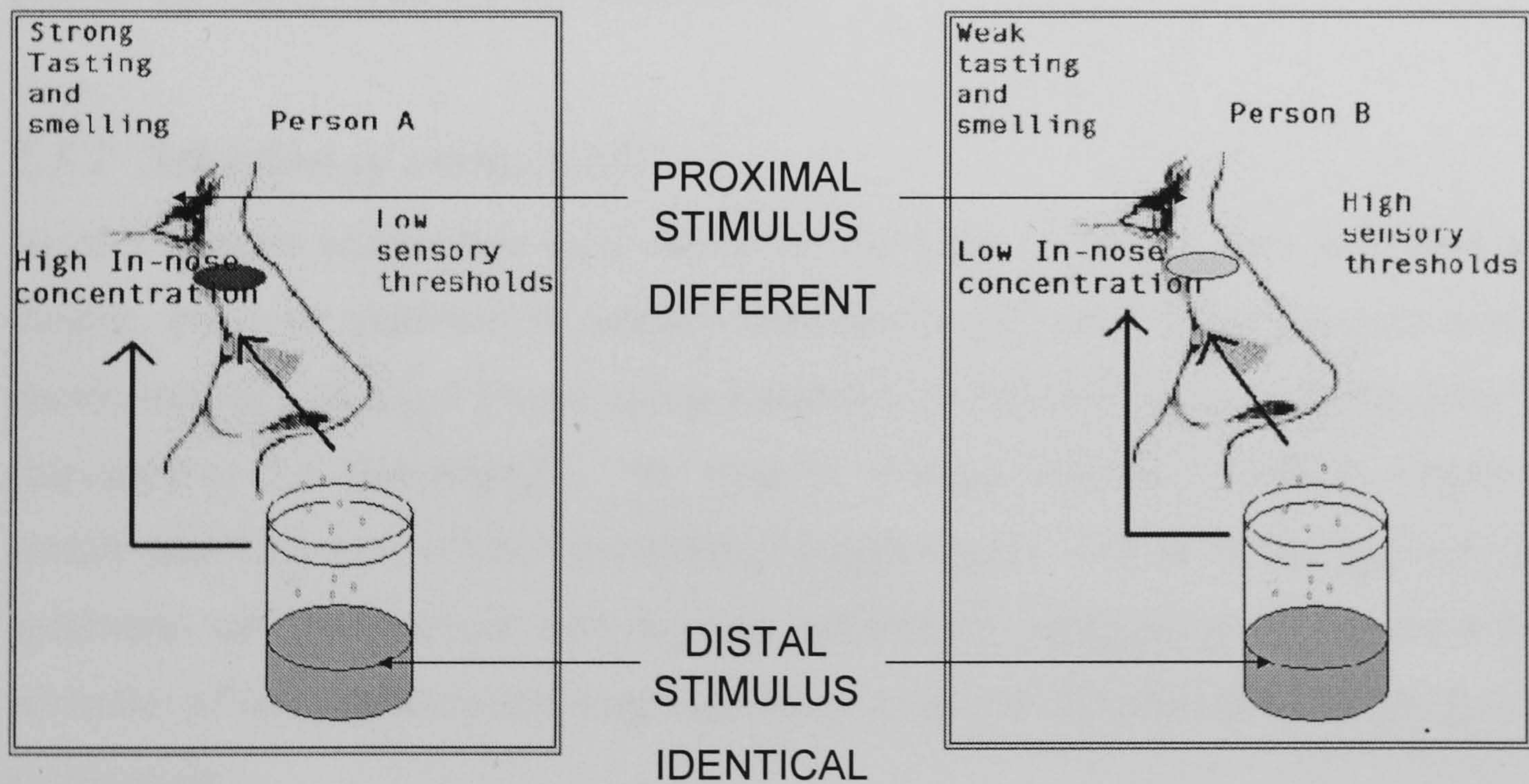


Figure 3.1 Schematic of the hypothesis.

It is generally assumed, and in some cases proven, that retronasal and orthonasal thresholds are related to one another as a function of the volatile concentration in the nose space (Pierce and Halpern, 1996; Aubry, *et al.*, 1999; Sakai, *et al.*, 2001; Heilmann, *et al.*, 2002; Voirol and Daget, 1986). However the transport of these molecules to their associated sites differs greatly between the two processes.

Orthonasal is the transport of molecules through the anterior nasal phares (nostrils) up into the nasal cavity. Retronasal is the transport of molecules from the mouth through the posterior nasal phares in the upper palate.

This study relates retronasal and orthonasal thresholds by eliminating the error associated with odorant presentation using on line monitoring of volatiles, it also assesses whether the variations in psychophysical determination are associated with the breath by breath variation.

3.1 MATERIALS AND METHODS

3.1.1 Subjects

Thirteen adult subjects participated in the study (10 female, 3 male; Age range = 37 – 64 years). Panellists had been trained in objective sensory assessment for four years prior to this study using mainly model foods.

3.1.2 Selection of compounds

Volatile organic compounds were chosen on the basis of criteria such as impact of flavour, previous exposure in sensory assessment and range of physico-chemical parameters such as log P (water-octanol partition coefficient) values ranging from 1 (carvone) to 3.6 (limonene)². The volatiles chosen: carvone, anethole, isoamyl acetate and limonene (Fisher Scientific, Loughborough, UK) have the flavours of spearmint, anis, banana/pear drop and lime, respectively, and had been regularly used in studies of time intensity and magnitude estimation over a period of four years prior to this study.

3.1.3 Sample preparation

One litre of 10 ppm (mg/kg) stock solution was prepared with mineral water purchased from a local supermarket, and was shaken for one hour. This was used to

² MOE chemical imaging database

make two concentration ranges of solutions which spanned the proposed threshold^{aq} of the volatile in water (Devos, *et al.*, 1990). Range 1 consisted of an increasing interval dilution with the estimated threshold^{aq} as the mid range, and the second range was more specific to the individual and was thus dependent on their threshold^{aq} calculated from Range 1. Table 1 shows the typical concentrations that were presented to the panellists, and thereafter the concentrations that were presented depending on the result of the first test range result. These ranges were suitable for all 4 volatiles. *Superscripts: aq = aqueous phase; gas = gas phase.*

Table 3.1 Volatile concentration ranges presented to assessors.

Aqueous Threshold First Range Limits $\mu\text{g}/\text{kg}$	RANGE 1	1-5	5-10	10-20	20-40	40-60	60-100	100-150	150-200	200-300	300-400	400-500
Individual Solutions within first range $\mu\text{g}/\text{kg}$	RANGE 2	0.5, 1, 2, 3, 4, 5, 7.5	3, 5, 7.5, 10, 12	7.5, 10, 15, 20, 25, 30, 35, 40, 50	15, 20, 25, 30, 35, 40, 50, 70	50, 60, 70, 80, 90, 100, 125	80, 100, 125, 150, 175	125, 150, 175, 200, 225	150, 200, 250, 300, 350	250, 300, 350, 400, 450	350, 400, 450, 500, 550	

All the solutions of Range 1 were given to all assessors. Once the approximate threshold had been found by Range 1, Range 2 solutions were given to each assessor that fell within the concentration ranges diagnosed in Range 1. Therefore, the assessor with the approximate Range 1 threshold of 60-100, would not receive Range 2 solutions from e.g. 1-5, or 400-500.

3.1.4 Calibration of the sniff bottles

The procedure and the subsequent results for this are described in section 2.1.2.

3.1.5 Nosespace concentration

The subjects were instructed to inhale, place 5 mL of the test solution in their mouths, swallow, and then exhale and inhale normally while their breath was sampled into the APCI-MS. Breath was sampled at a flow rate of 35 ± 5 mL/min under the same conditions as the headspace calibration, but the dwell time decreased to 0.01 s. Breath peaks were converted to concentration in the nosespace by the calibration curve. The maximum concentration of the first breath peak was considered the most sensorially significant, and was used in all instrumental measurements. Figures 3.2 and 3.3 show examples of typical breath by breath data for anethole and limonene, respectively. On both plots, Y1 is indicated to be the height of the first breath peak.

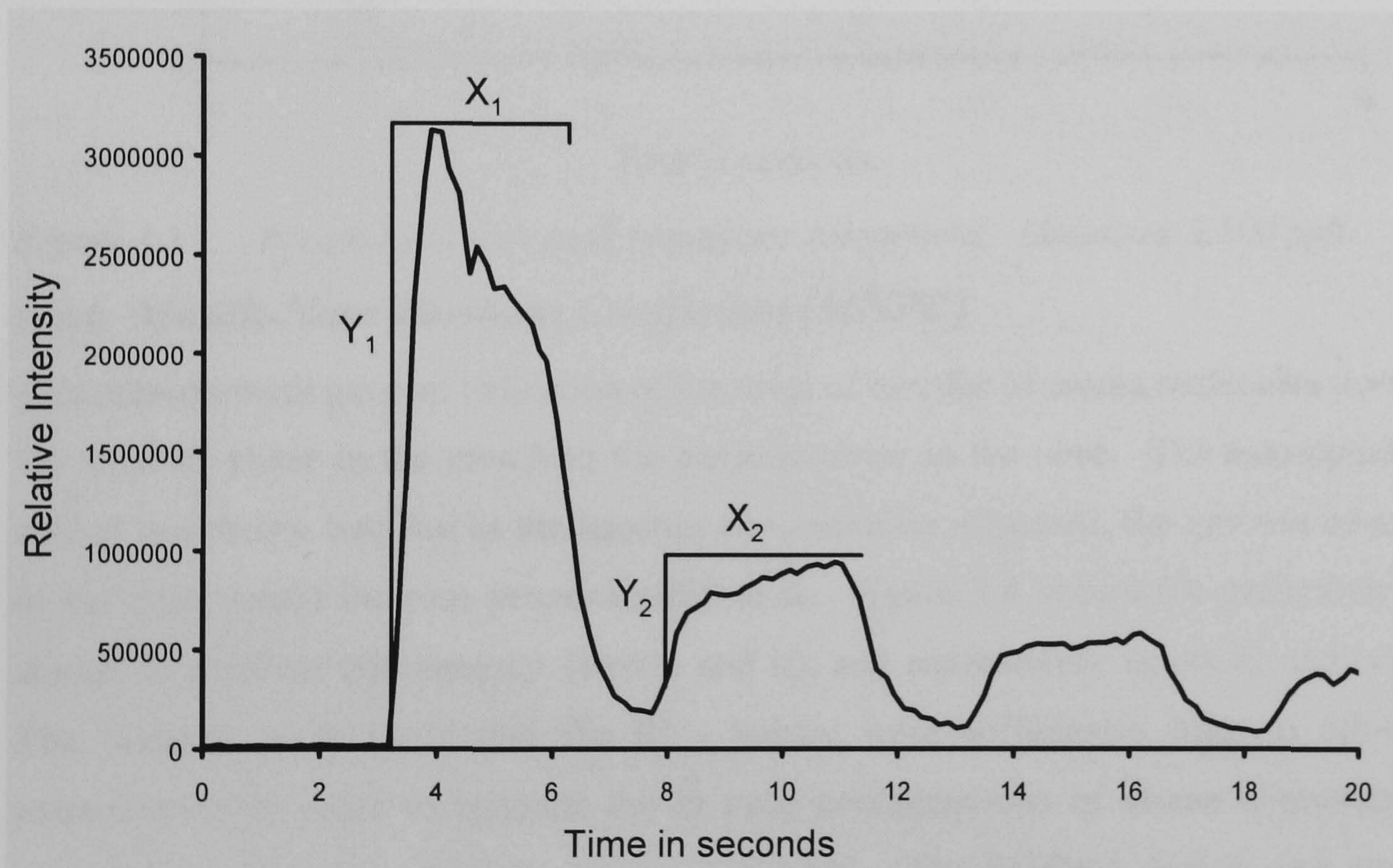


Figure 3.2 Breath by breath peak parameter extractions. Anethole 10 ppm.

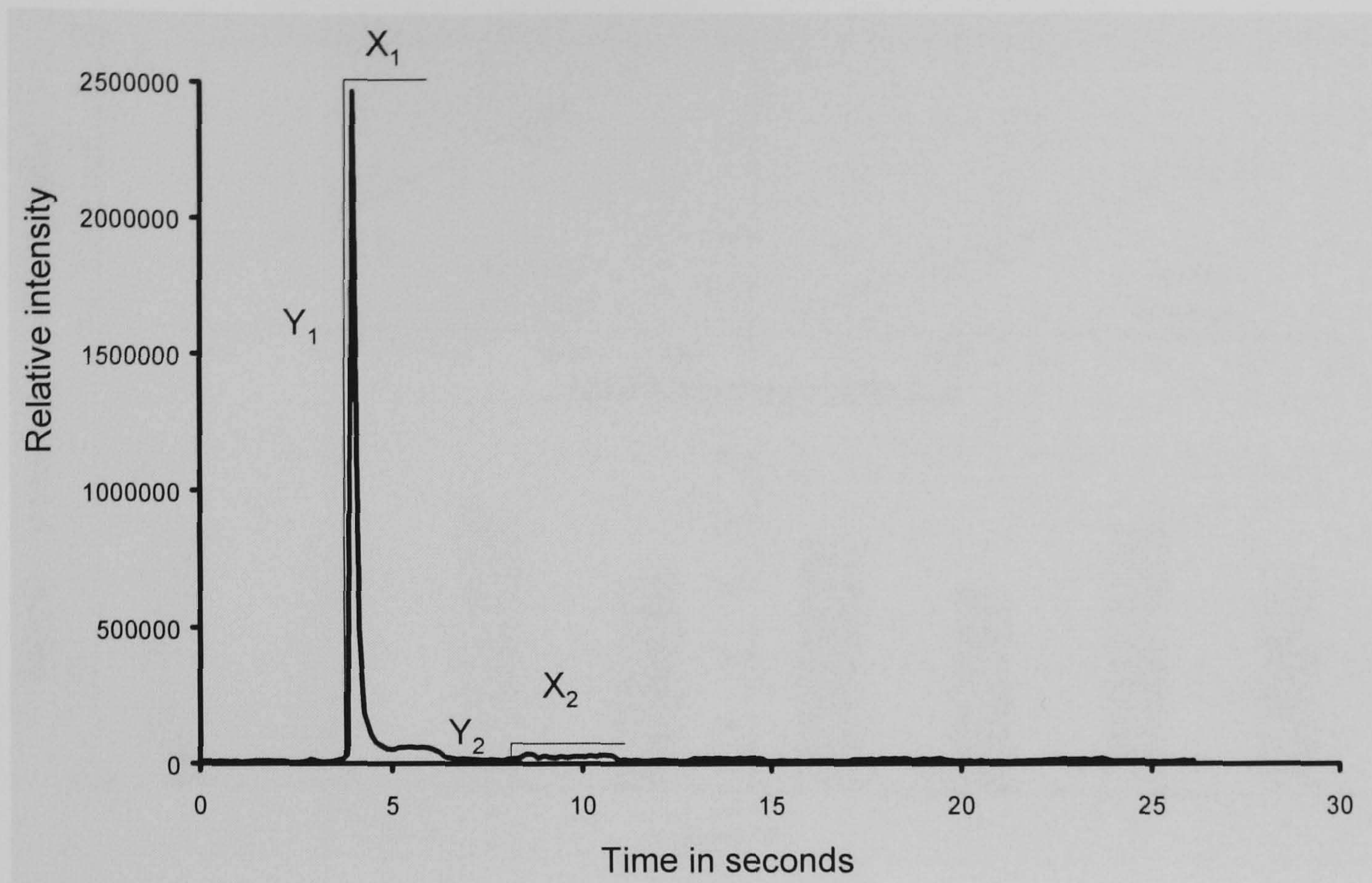


Figure 3.3 Breath by breath peak parameter extractions. Limonene 2,500 ppb.

3.1.6 Mouth-Nose Partition Coefficient (MNPC)

This measurement gave an indication of the level of transfer of aroma molecules from the aqueous phase in the mouth to the gaseous phase in the nose. The assumption behind this theory was that as the aqueous concentration increased, the gaseous conc. in the nose would increase proportionally to it. Figure 3.4 shows the preliminary studies to confirm this linearity (plots i and ii), and repeatability (plots iii and iv). The linearity plots show that the R^2 - values were sufficiently high to allow extrapolation in order to estimate the in nose concentrations of aroma compound below the analytical capabilities of the APCI-MS. The graphs i. and ii. can also demonstrate how the MNPC was used to calculate a $\text{threshold}^{\text{aq}}$ from a $\text{threshold}^{\text{gas}}$. An aqueous threshold concentration can be read along the X axis, which will then correspond to a gas phase concentration in the nose according to the gradient of the line (i.e. MNPC). Therefore in the case of graph i, if the individual's $\text{threshold}^{\text{aq}}$ was 1000 ppb, then their $\text{threshold}^{\text{gas}}$ would be circa 20 ppbv.

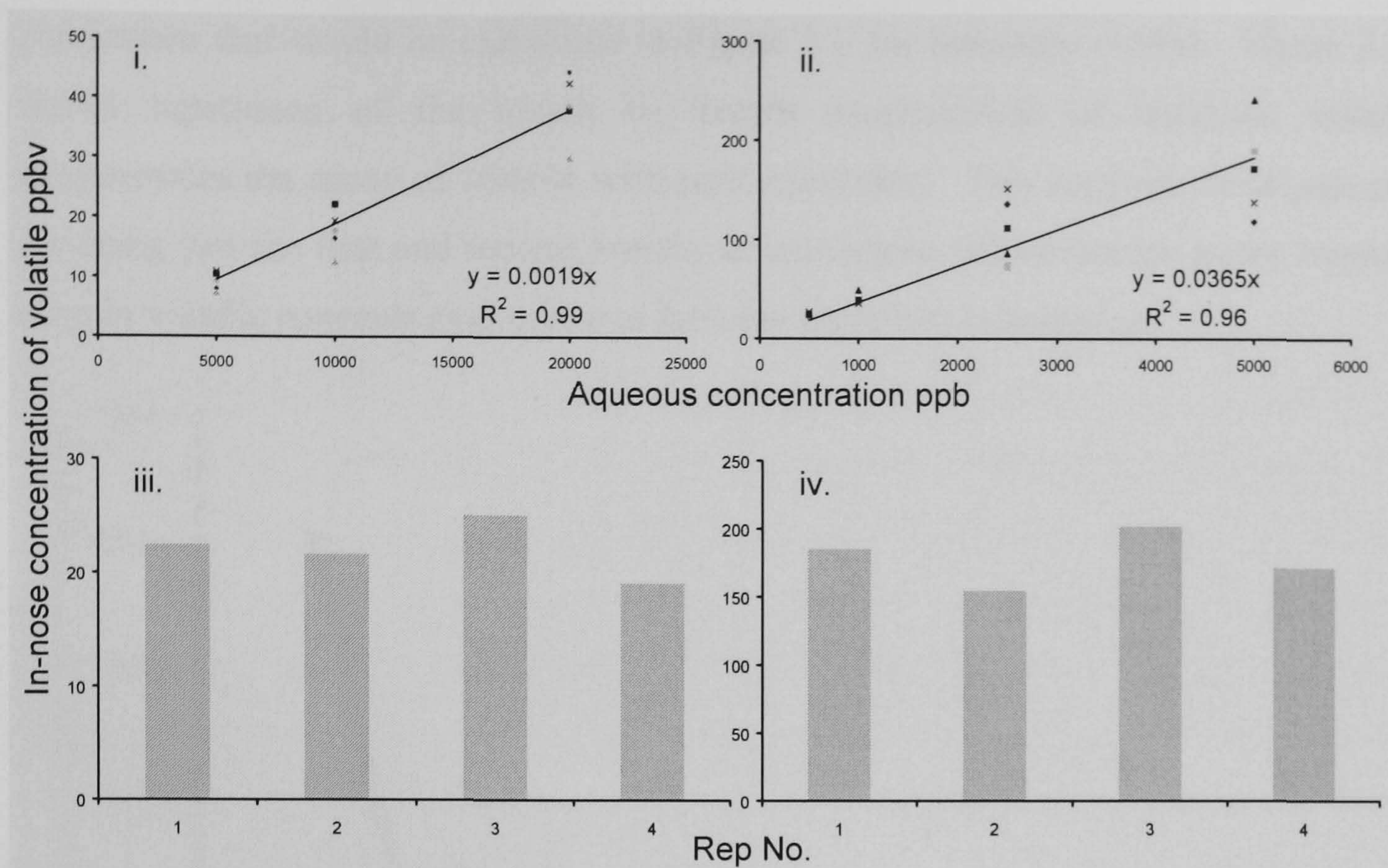


Figure 3.4 In-nose volatile concentration calibration curves: i. carvone ii. isoamyl acetate. Bar charts to show repeatability: iii. limonene 2500 ppb. iv. anethole 10 ppm.

Panellists (13) consumed aliquots (6 replicates) of solution as described above. The mean peak height of the first breath was converted into a concentration (nL of volatile per L of air; ppbv) as in the headspace procedure. The MNPC (ppbv/ppb) was then calculated using this formula:

$$MNPC = \frac{\chi \text{conc}^{\text{gas}}}{\text{conc}^{\text{aq}}}$$

where; $\chi \text{conc}^{\text{gas}}$ = mean volatile conc. of the Y1 first breath peak (ppbv), and, conc^{aq} = volatile conc. in the aqueous phase (ppb). This MNPC value was then multiplied by the individual threshold^{aq} to give the retronasal threshold^{gas}.

3.1.7 Volatile persistence in the breath

This was calculated by expressing the second breath peak height as a percentage of the height of the first breath peak (Linforth and Taylor, 2000). In regards to Figure 3.2, this would be the value of Y2 as a percentage of Y1. This shows a relatively high persistence value (~30%) for anethole in contrast to the small level of

persistence that would be calculated in Figure 3.3 for limonene (<5%). Figure 3.5 shows repetitions of the breath by breath measurement of anethole, which demonstrates the decay of volatile with each exhalation. This supports the argument for using just the first and second breaths as indications of persistence as the largest drop in volatile concentration occurred between these two exhalations.

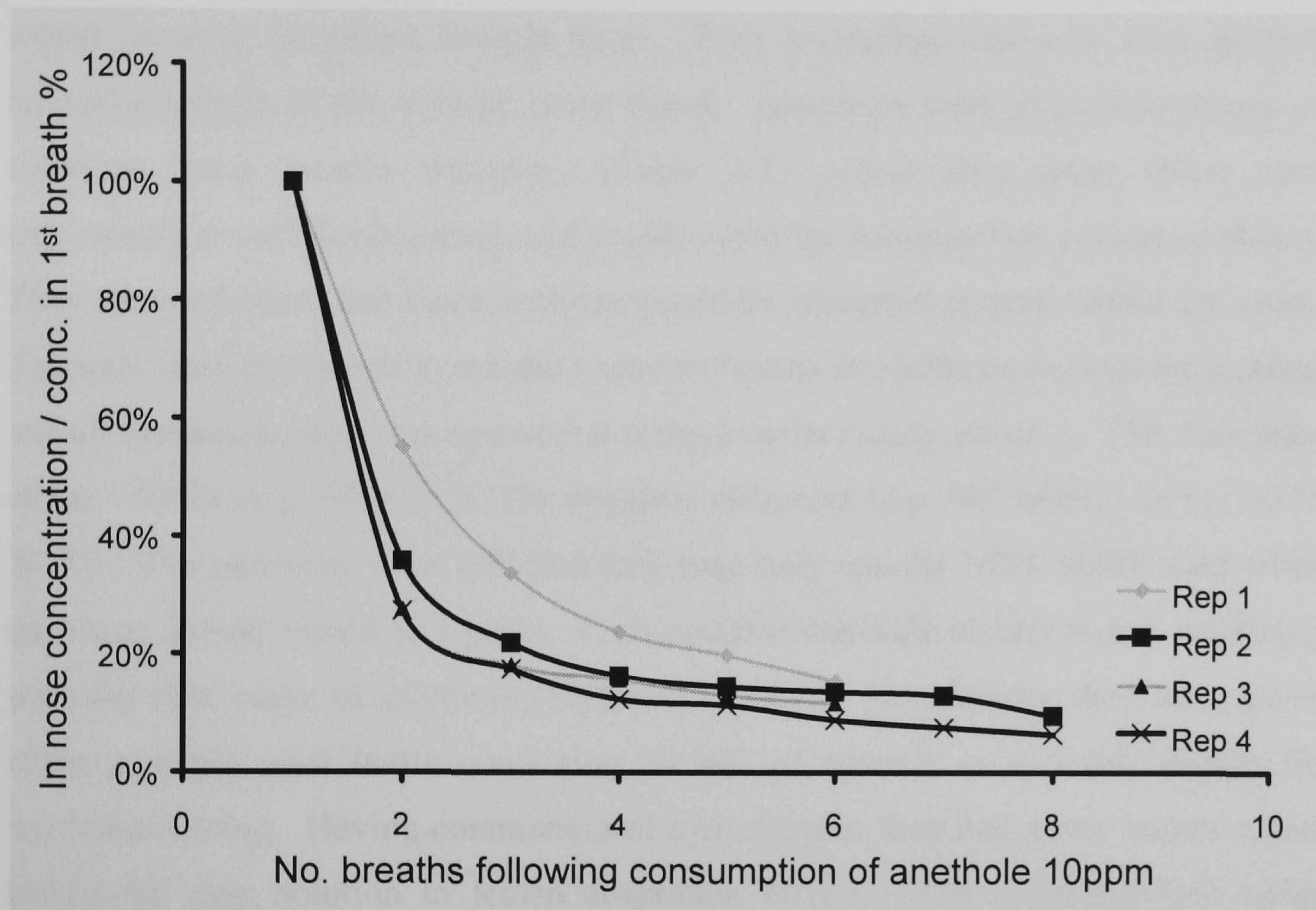


Figure 3.5 Volatile persistence in the breath. Anethole (aq) 10 ppm.

3.1.8 Peak height: area ratio

This was calculated by dividing the first breath peak height by the peak area or $Y1/X1$ in Figure 3.2. This measurement was used as an indication of the mass transfer of volatile from the mouth to the nasal cavity. For example, a large peak height:area ratio would indicate that in the first breath after swallowing, the time for complete release of volatile to the nosespace was a faster event than the overall exhalation time. This would be in contrast to a low peak height:area ratio, where the mass transfer of molecules to the nosespace continues for the entire duration of the exhalation.

3.1.9 Threshold determination methods

A descending staircase method was used (Cornsweet, 1965). This is in contrast to the more commonly used ascending method to compensate for the effects of adaptation. However, in preliminary tests the assessors showed a tendency to increase the number of false hits due to panic guessing when they assumed they would perceive the odour straight away. Prior to testing, assessors were given a reference sample of the volatile being tested. Assessors were given two ranges of solutions, each sample separately (Table 3.1), which they must either taste (retronasal) or sniff (orthonasal), and decide when the stimulus was present or absent. They were informed that blank samples would be randomly present within the series. The tests were performed in standard sensory booths in Northern hemisphere lighting and all communication was non-verbal using 3 cards stating either: i. The descriptor of the volatile (e.g. MINT), ii. The negative statement (e.g. NO MINT) or iii. NOT SURE. The assessors were told that they may only use the NOT SURE card when any other answer would be a guess. Each assessor was individually tested, beginning with the first range of dilutions (Table 3.1, RANGE 1), whereby they were given either a single sniff bottle containing 50 mL of odorant or a 5 mL aliquot for retronasal testing. Having communicated their answer, they had a one minute pause before the next solution to lessen adaptation effects. The estimated first range spanning their threshold was determined as the highest concentration where 3 or more negative answers were given to a stimulus, up to the lowest concentration where 3 or more positive answers were given. On average, assessors were given 7-10 blank samples per test set, and it was expected that 80% negative answers to these blank samples would be provided in one test set. After a 30 minute break, subjects repeated the test with the second range of solutions that were specifically designed, dependent on their predicted threshold from range 1 (Table 3.1, RANGE 2). The individual threshold^{gas} was finally calculated as per the geometric mean formula:

$$OdourThreshold = \sqrt{Y^{stimabsent} \times Y^{stimpresent}}$$

Where; $Y^{stimabsent}$ = highest presented volatile concentration^{gas} to which three or more negative answers were given, and;

$Y^{\text{stimpresent}}$ = lowest presented volatile concentration^{gas} to which three or more positive answers were given. The method was assessed for repeatability of isoamyl acetate 8 months post testing.

3.1.10 Data analysis

Orthonasal and retronasal thresholds were subjected to regression analysis. All instrumental variables were assessed for correlations versus the psychophysical data points also using regression analysis. The software used was Design Expert © 2002.

3.2 RESULTS

3.2.1 Repeatability of the psychophysical and instrumental method

Figure 3.6 shows the repeatability of the psychophysical method 8 months post testing of isoamyl acetate, achieving R-squared values of 0.91 and 0.74, for orthonasal and retronasal thresholds, respectively. It is important to mention this first to show that the threshold determination procedure could provide repeatable results and thus variation between panellists was not a function of the test used.

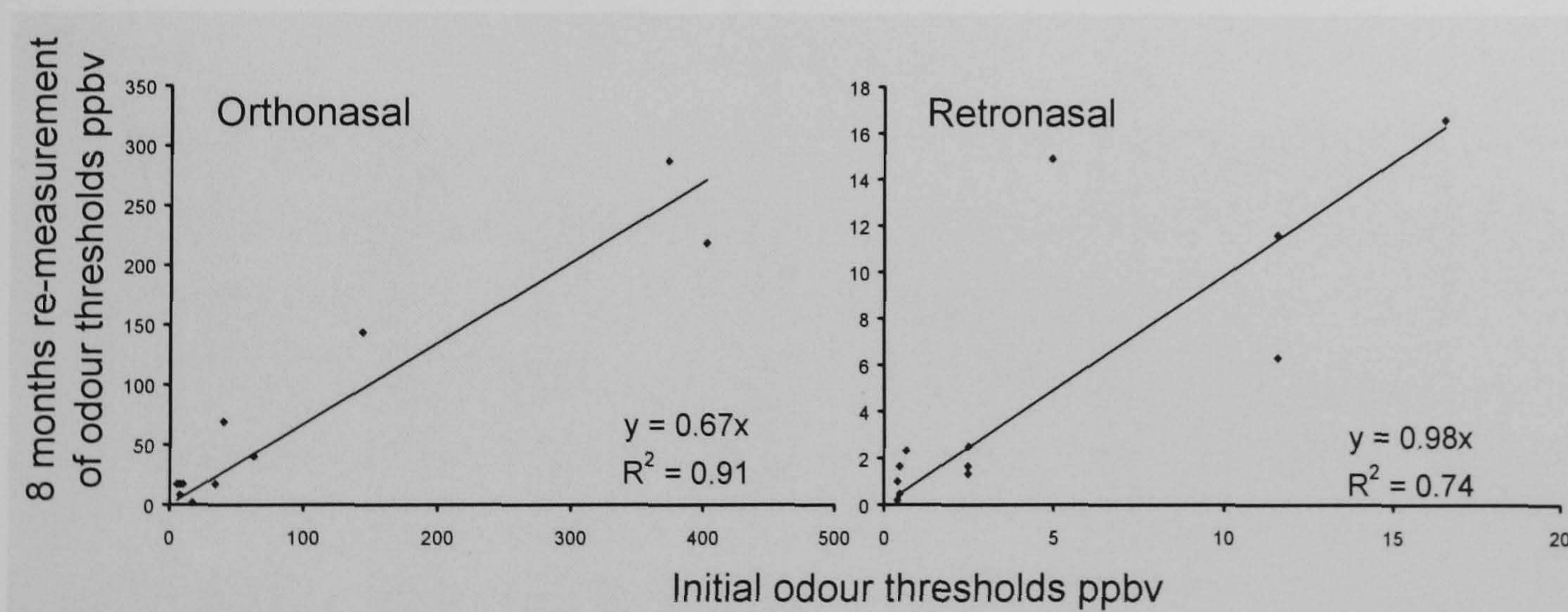


Figure 3.6 Repeatability of threshold determination of isoamyl acetate with 8 month difference. Significance at the 95% confidence level.

3.2.2 Psychophysical results analysis

Table 2 shows a summary of the individual orthonasal and retronasal thresholds for each of the volatiles. As a comparison, orthonasal odour threshold^{gas} values are given as reported by Devos et al (1990). The group thresholds were calculated as a geometric mean under the assumption that the data set would follow a normal sigmoidal distribution as seen in typical data from normal group detection of constant stimuli (Coren, *et al.*, 1999). However, due to the individuality of the test used in this study, it was not appropriate to calculate the group threshold as the value yielding the 50th percentile.

Table 3.2 Orthonasal and Retronasal Thresholds in mL of volatile per L of air (ppbv).

Assessor	Anethole Orthonasal Threshold	Anethole Retronasal Threshold	Carvone Orthonasal Threshold	Carvone Retronasal threshold
Carole	3	0.61	1.98	0.273
Clare	1.5	0.21	0.49	0.013
Diane	0.48	0.07	0.16	0.021
Gill	6.72	0.17	1.98	0.033
John	3	0.19	1.92	0.027
Joyce	1.5	0.06	0.86	0.071
Kay	21.2	1.73	3.13	0.122
Maureen	3	0.66	1.21	0.108
Mike	3	1.85	5.86	0.019
Nina	11.6	0.49	2.42	0.154
Sally	1.34	0.16	1.98	0.008
Stuart	11.6	0.95	4.14	0.048
Sue	6.72	0.05	0.1	0.005
GEO MEAN	3.57	0.3	1.27	0.038
Minimum	0.48	0.05	0.1	0.005
Maximum	21.2	1.85	5.86	0.273
Reported Orthonasal ^a	22.39		7	
Assessor	Isoamyl acetate Orthonasal Threshold	Isoamyl acetate Retronasal Threshold	Limonene Orthonasal Threshold	Limonene Retronasal threshold
Carole	62.9*	20.93*	727.46	20.42
Clare	7.27	0.7	93.91*	35.13*
Diane	10.29	0.25	93.91	9.31
Gill	5.14	0.31	42	0.41
John	140.8	1.84	1188	20.39
Joyce	7.27	0.32	514	9.07
Kay	51.4	1.88	297	3.53
Maureen	36.3*	21.00*	594*	65.76*
Mike	398.3	11.37	594	13
Nina	545.4	3.19	297	11.56
Sally	25.71	1.6	93.91	14.25
Stuart	363.6	15.09	1188	15.49
Sue	16.26	0.37	297	1.56
GEO MEAN	42.63	2.01	301.4	9.63
Minimum	5.14	0.25	42	0.41
Maximum	545.5	21	1188	65.76
Reported Orthonasal ^a	22.39		436	

Orthonasal thresholds calculated by converting the threshold concentration in the aqueous phase to the concentration in the gas phase using the air-water partition coefficients. Retronasal thresholds calculated by converting the aqueous phase concentrations of each threshold to the estimated concentration in the nasal phase concentrations using each assessors individual MNPC. Reported orthonasal values from Devos et al.(1990). Data points indicated as outliers (as per regression analysis) are marked with *.

3.2.3 Relationship between orthonasal and retronasal thresholds

Figures 3.7 and 3.8 indicate the expected positive linear relationship between orthonasal and retronasal thresholds. Figure 3.8 shows the mean threshold over the four volatiles for each assessor, and demonstrates that the linearity is not simply a function of the large differences in overall threshold between the different volatiles. When these data sets were subjected to regression analysis, the model suggested the presence of eight data points that did not support the linearity of the data set (*data not shown*). These points were removed from all subsequent calculations, and originated from three assessors, who suffered intermittently from minor ailments.

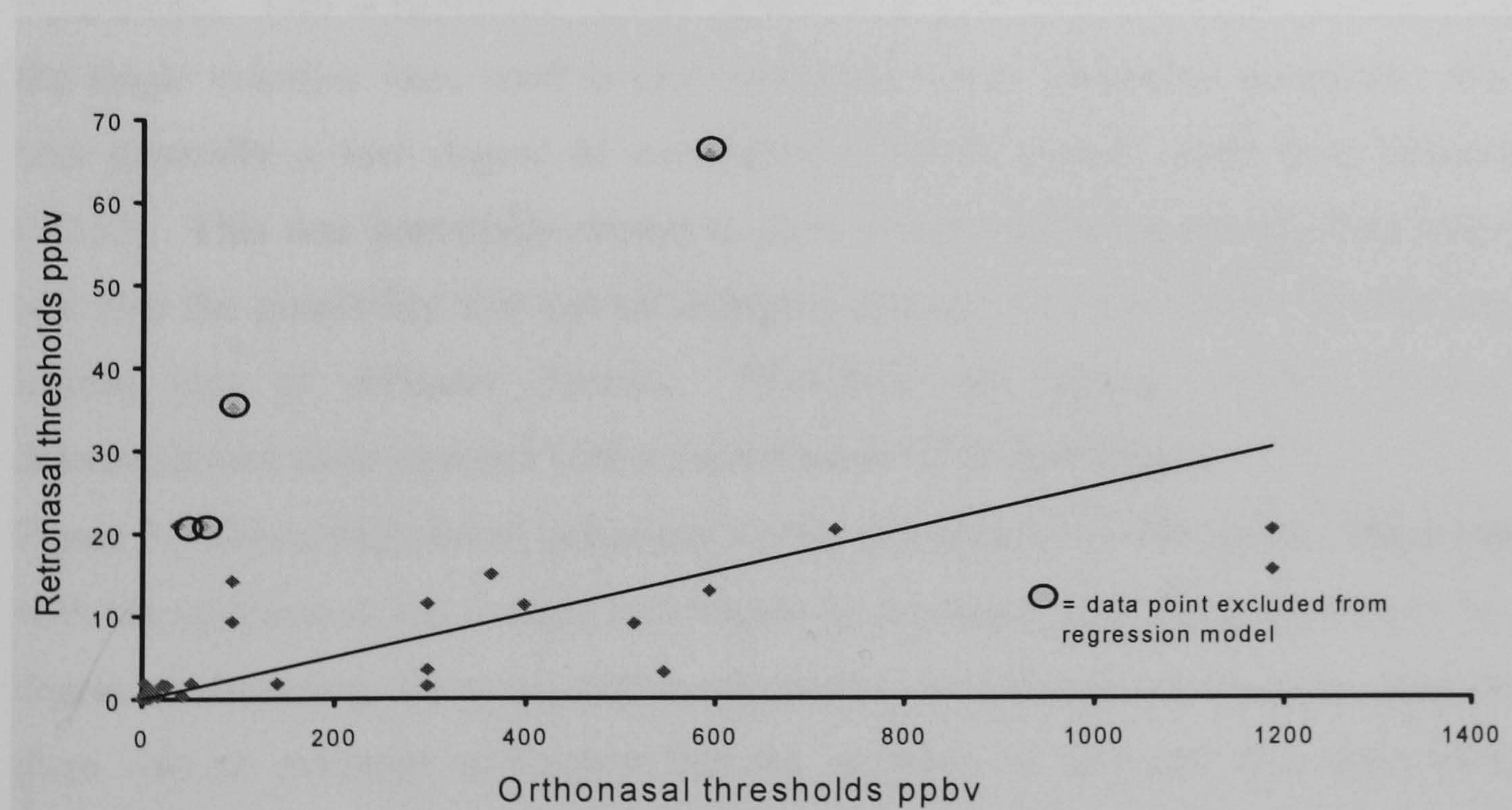


Figure 3.7 Orthonasal versus retronasal thresholds in the gas phase (ppbv). Data points taken from all 4 aroma compounds.

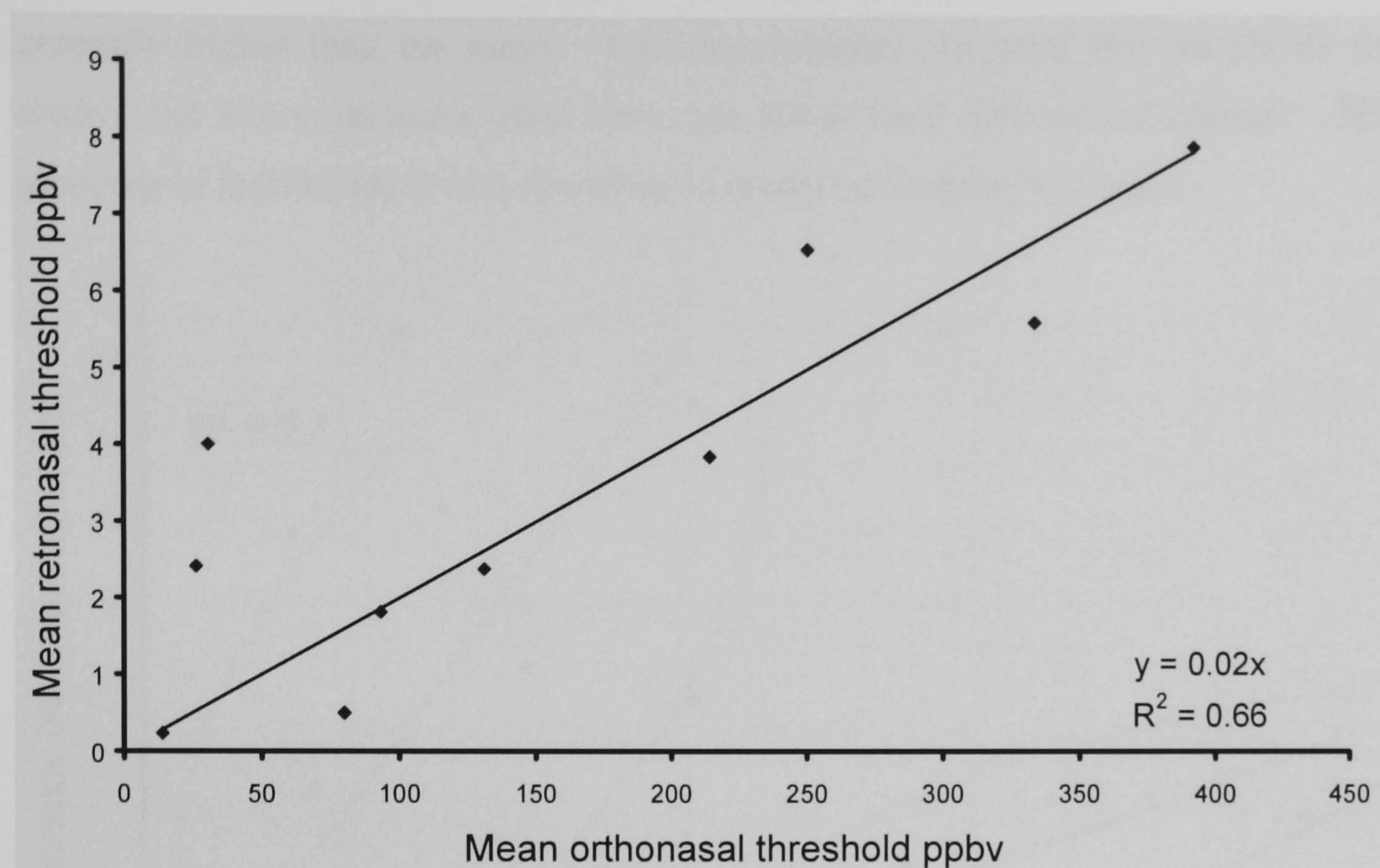


Figure 3.8 Mean orthonasal versus retronasal thresholds (ppbv). Significant at 95% confidence.

These graphs indicate that although the two types of threshold in this study were related, the retronasal thresholds were on average 50 times lower than their orthonasal equivalents. This is explained further in section 3.3. It was also observed that when the single volatiles were used to plot orthonasal versus retronasal thresholds, there was generally a low degree of correlation (<0.6 R^2 value) apart from anethole (>0.65). This was potentially related to there simply not being enough data points, and also the possibility that not all assessors obeyed the same linear law but were instead part of different clusters. Therefore, the isoamyl acetate threshold determinations were repeated with a trained panel of 20 individuals.

Figure 3.9 shows this plot of orthonasal versus retronasal IAA thresholds, where even with the addition of just 7 more individuals in the experiment, there appears to be a degree of clustering, albeit not statistically tested. It is also important to mention that there was no evidence to suggest that the presence of new and less experienced assessors was the cause of the clustering as they were spread into both groups. The smaller group indicated a relationship whereby their retronasal thresholds were relatively low compared to the average, whereas their orthonasal thresholds were

generally higher than the mean. This demonstrates the point that people do not always fall into continuous trend lines, but rather form behavioural clusters. This grouping of individuals is also described in results of chapters 4, 5 and 6.

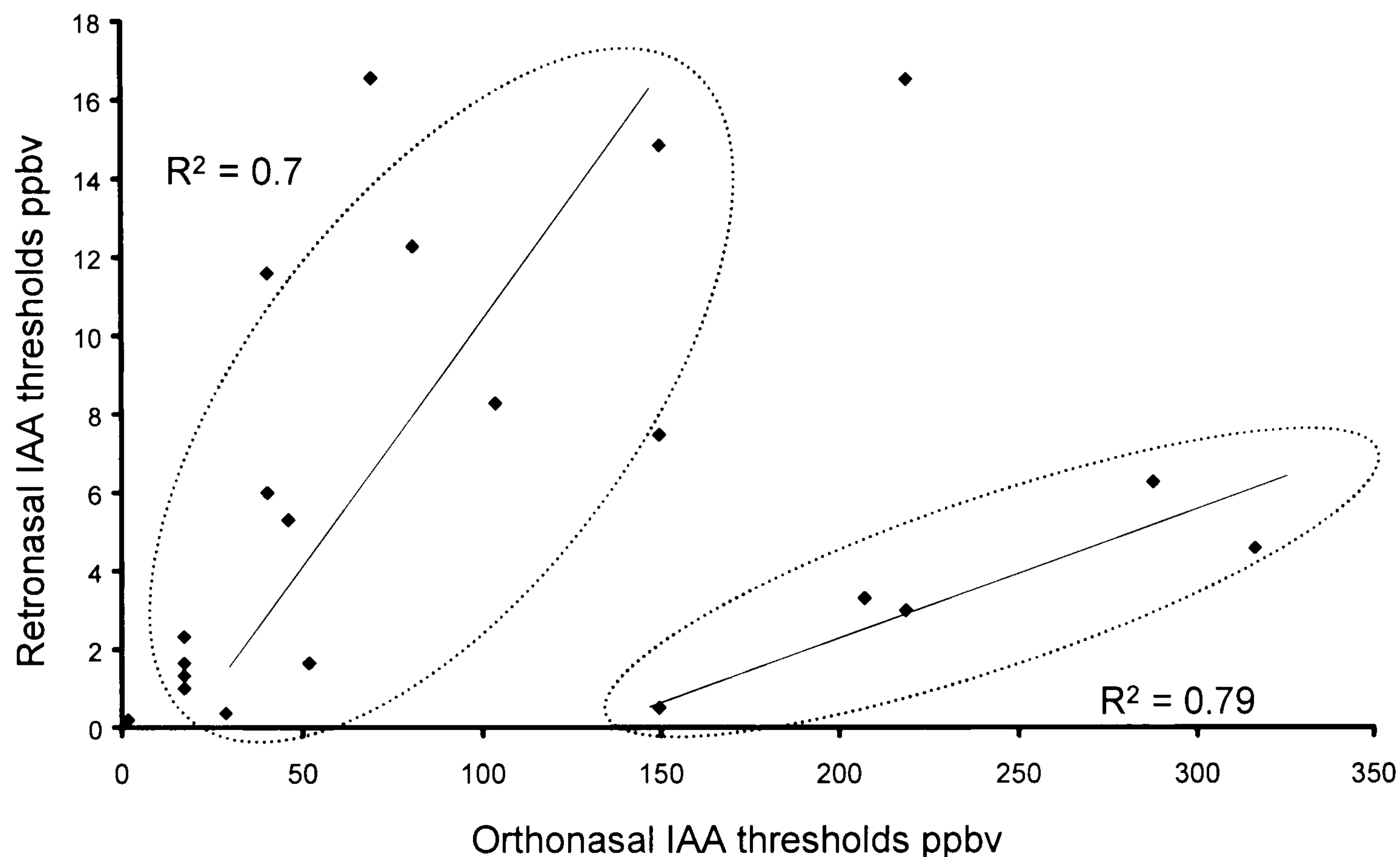


Figure 3.9 IAA retronasal versus orthonasal thresholds with 20 assessors. The dotted lines suggest the data falls into clusters of behaviour rather than a continuous single trend line.

3.2.4 Regression analysis of instrumental measurements versus psychophysical

Regression analysis was used to model relationships between the individual thresholds and the instrumental values. It is important to note that for these analyses, the aforementioned outlying data points were also excluded. Due to the fact that the retronasal thresholds were calculated upon calibration curves, whose gradients then formed the MNPCs, it was therefore illogical to subsequently regress these two variables. In order to assess whether the in-nose concentration of a volatile was related to threshold, any significant correlations between the different volatile

MNPCs were compared to any significant correlations between the volatile retronasal^{gas} thresholds. Table 3.3 shows that there was a significant relationship between the MNPC value of anethole and that of carvone ($R^2=0.76$), however, there is no correlation between the respective retronasal^{gas} thresholds ($R^2= <0.05$). This indicates that in this data set, there was no relationship between retronasal^{gas} threshold and the individual in-nose concentration. Furthermore, volatile persistence and peak height:area ratio were not shown to have a significant effect on the psychophysical data. In other words, the variation in thresholds between these individuals could not be explained by the differences in measured parameters in this study.

Table 3.3 Correlation matrix (R^2 values) of volatile mouth-nose partition coefficients (MNPC) and retronasal^{gas} thresholds. R^2 values of high significance are in bold.

MNPC R^2	Anethole	Carvone	Isoamyl acetate	Limonene
Anethole				
Carvone	0.76			
Isoamyl acetate	0.06	0.09		
Limonene	0.2	0.2	0.06	
Retronasal ^{gas} R^2	Anethole	Carvone	Isoamyl acetate	Limonene
Anethole				
Carvone	0.05			
Isoamyl acetate	0.4	0		
Limonene	0.002	0.1	0.2	

3.2.5 Relationships between instrumental and psychophysical measurements: variation between the volatiles.

The means of the different measurements were calculated (geometric mean for the thresholds) for each of the four volatiles. This analysis investigated the differences between the volatiles in terms of their psychophysical measurements, physico-

chemical properties and their APCI-MS results. Figure 3.10 shows all these measurements on one plot.

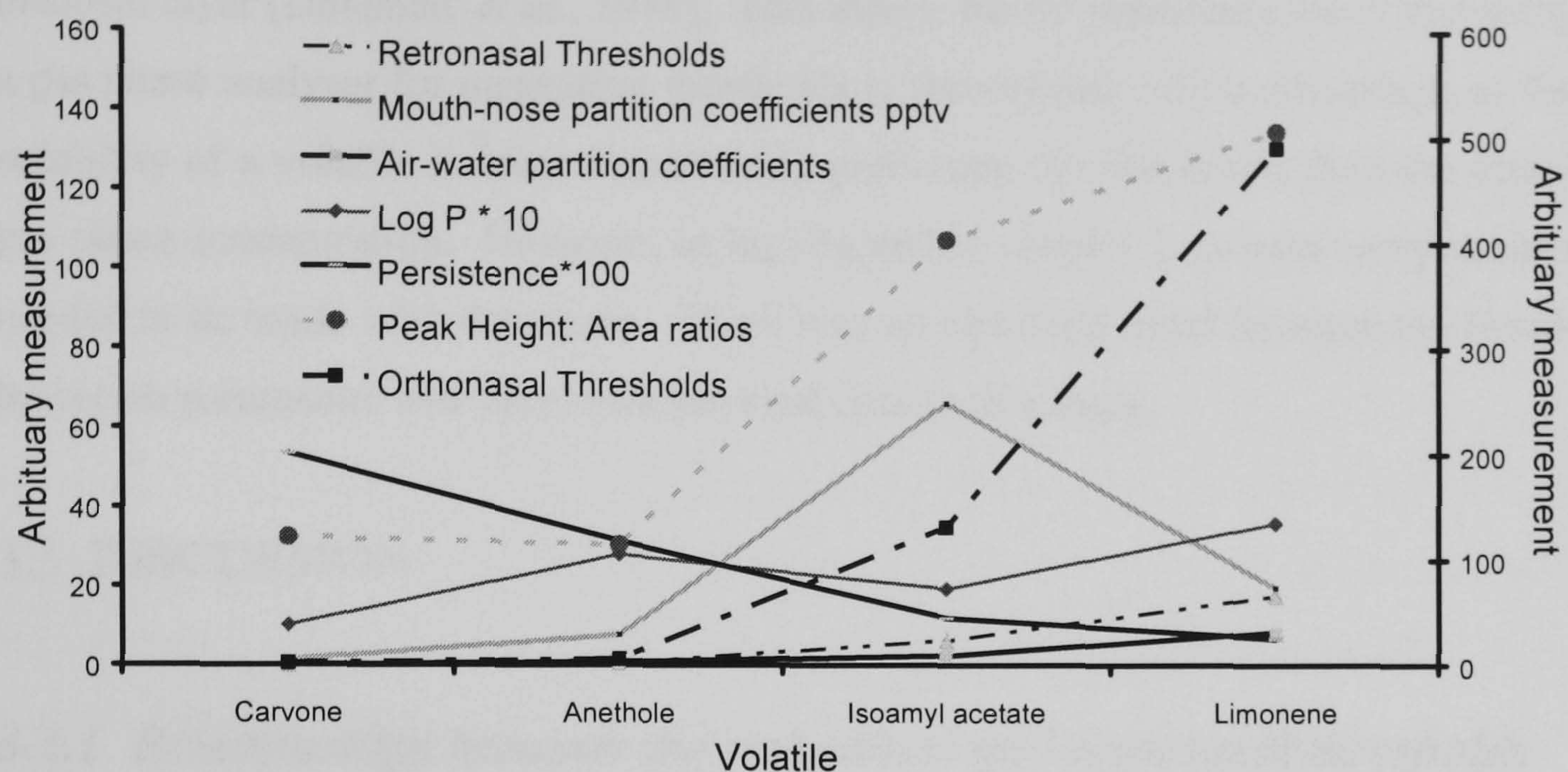


Figure 3.10 Physico-chemical and psychophysical differences between the four volatiles. Orthonasal thresholds, retronasal thresholds, Log P and the air-water partition coefficient all correlate above an R^2 of 0.9, with a significance of 5%. Regression analysis not shown. MNPC, Peak height:area ratio and persistence do not significantly regress to any other variables.

Based on differences between just four volatiles, the relationships between the different measurements might appear more favourable than if more volatiles had been investigated rather than this small sample. Nevertheless, the Figure 3.10 does indicate trends between the thresholds, hydrophobicity (Log P) and the air-water partition coefficient. This would indicate that as the rate of evaporation of a given volatile from an aqueous solution increases with hydrophobicity, the corresponding threshold^{aq} increases proportionally. However, this does not take into account the sorption into the mucosal layer because this would be too difficult to measure, and is therefore not strictly an indication of the concentration at the receptor site. Furthermore, olfaction is not greatly influenced by volatility, as the evaporation process is the inverse of the solubility if the activity coefficients of the odorants are similar for the aqueous phase and the mucosal phase (Doleman, *et al.*, 1998; Gopel, 2000). This would indicate that for the different volatiles, the actual number of

molecules reaching the receptors would be closer to one another than is observed in the air of the nosespace alone, due to their differential partitioning through the mucosal layer (Doleman, *et al.*, 1998). This theory would potentially show that using a gas phase analyser for measuring thresholds is theoretically of no advantage, as the solubility of a volatile is more important in predicting the thresholds than the actual gas phase concentration. However, as highlighted in chapter 2, certain compromises needed to be made with this study. There was no observed trend between the breath by breath parameters and the psychophysical data in this study.

3.3 DISCUSSION

3.3.1 Relationships between the orthonasal and retronasal thresholds

It has been previously stated that retronasal and orthonasal thresholds are probably determined via the same mechanism but differ by the route of odorant transport. However, this has been a conclusion that has frequently been based on assumptions rather than actual measurements of the real volatile concentration in the nasal cavity (Heilmann, *et al.*, 2002; Aubry, *et al.*, 1999; Voirol and Daget, 1986). The advantage of this study was that the measurements were based on real volatile concentration estimations; *in situ* (orthonasal) and *in vivo* (retronasal). The results showed and confirmed the findings of other authors (Vuilleumier, *et al.*, 2000), that the two different routes of odour perception are related, although other research has indicated differential processing of olfactory stimuli when retronasally presented. This has been shown by assessing differences in the olfactory event related potentials between the two types of olfaction, where retronasal stimulation resulted in smaller amplitudes than orthonasal perception (Heilmann and Hummel, 2004). Differences in central processing between the two mechanisms were also observed using functional magnetic resonance imaging, whereby activation of some brain parts appeared to be specific to the presentation of aroma compounds in aqueous solution on the tongue (Cerf-Ducastel and Murphy, 2001). These brain areas (dorsal insula) would normally have been associated with tastant perception and lingual somatic stimulation rather than olfactory stimulation.

In this study, retronasal thresholds were ~50 times lower than their orthonasal counterparts, and this finding was likely to be related to the slight differences in the time points at which the volatile concentrations were estimated. The orthonasal thresholds^{gas} were calculated using the air-water partition coefficients, and thus were the concentration of volatile in the gas phase **before** the event of olfactory perception. The retronasal thresholds^{gas} were calculated using the average concentration of a volatile in the first exhalation, and were therefore the concentration **after** the event of olfactory perception. Thus, any absorptive effects of the volatiles in the mucosal layer are not included in the nosespace concentration estimations, which may be the reason why the retronasal thresholds^{gas} are a factor lower than their orthonasal^{gas} counterparts. The mucosa has been shown to be almost 'chromatographic-like' in its differential absorption of odourants, and is therefore a factor that cannot be ignored in this study (Hornung and Mozell, 1977; Hornung, *et al.*, 1984; Hornung, *et al.*, 1987). Additionally, the difference could simply be related to differences in the level of trigeminal stimulation between the two types of odorant transport (Voirol and Daget, 1986). Other researchers have studied the phenomenon of sub-threshold additivity of different modes. In one example, orthonasal sub-threshold concentrations of benzaldehyde could be detected in the presence of sub threshold levels of sucrose (Dalton, *et al.*, 2000).

3.3.2 Regression of psychophysical and instrumental data

The original hypothesis behind this experiment was to assess whether a measurement of the first exhalation breath peak after consumption of a volatile in water from the APCI-MS was an adequate predictor of the individual's threshold. This in turn would provide an indication of whether the variations in instrumental and psychophysical data were in some way related. In this data set, the only significant relationship existed between the mouth-nose partition coefficients (MNPC) of carvone and anethole, whereby, if one increases then the other will also linearly increase. If retronasal sensitivity to a given odorant was similar for the individuals in this study, and the actual variation was due to differences in individual MNPC alone, then a

significant correlation would have also been expected between the retronasal^{gas} thresholds of carvone and anethole. This was not the case and suggests that in this group of individuals, other factors account for the differences in their retronasal thresholds.

Volatile persistence and peak height:area ratio were also shown to be unrelated to the individual psychophysical data. However, it is important to mention that these instrumental parameters may also be related to the individual breathing rates and volumes as well as the volatile release kinetics and volatile/mucosa interactions (Hodgson, *et al.*, 2003; Linforth and Taylor, 2000). Breathing measurements were not taken in this study but should be included in further experiments of this kind.

In conclusion, this study has shown that a method has been reliably employed in the assessment of individual odour thresholds. Furthermore, it has shown that thresholds can be measured as a function of the volatiles in the gas phase rather than an estimation based on the aqueous concentration alone. This may be useful in panel monitoring, particularly panels that are involved with quality control of product taints, where the gas phase threshold may be more relevant than the aqueous. It has also supported the hypothesis and previous research, that orthonasal and retronasal thresholds are related to one another, but with retronasal thresholds being a factor lower than other. Whether this a true difference, or purely a difference associated with the methods of measuring aroma molecules in the gas phase remains to be seen. The study shows that person to person variation in odour threshold is unlikely to be solely accounted for by the variation in APCI-MS breath by breath data.

4 PERCEPTION OF PULSED AROMA: DISCRIMINATION TESTING

This set of experiments utilised aroma pulsing techniques to answer a wide range of questions pertaining to the relationships between breath by breath measurements and the corresponding percepts. The first and original question was concerned with the famous phenomenon of flicker fusion from Gestalt psychology. Primarily acknowledged in vision studies, flicker fusion frequency - the rate at which two separate flashes of light are perceived as a single flash, is the theory behind the enjoyment of cinema. Static pictures in quick succession become motion in the brain (Gomez, *et al.*, 1999; Davy, 1952; Coile, *et al.*, 1989). As mentioned in Section 2.2, this theory may transfer to flavour perception, whereby the oscillatory movement of volatiles into the nose with tidal breathing becomes perceptually smoothed. This process may be the product of a fusion threshold between temporal stimuli, below which they become one stimulus, and above which they are perceived as separate pulses. A measure of this threshold has been successful in aquatic animals, where odour plumes can be easily controlled (Gomez, *et al.*, 1999). This is however far more complicated to simulate in the gas phase. If a subject perceives this effect of retronasal pulsing as a continuum, does the same effect occur orthonasally? This conclusion would potentially separate aroma concentration as a time dependent stimulus (APCI-MS height) from total aroma delivery (area under the curve). In other words, if an aroma stream with frequent aroma pulses is sensed as being constant, is it then perceived as being the same as an aroma stream that is not pulsed? If yes, then the height is more important than the integrated total aroma delivery.

This chapter highlights the problems encountered in answering this complex problem, such as the unpredictable disruption of aroma stimulus presented to an assessor.

However, the use of simultaneous breath by breath techniques was serendipitous in answering a set of questions related to the original hypothesis.

4.1 PRELIMINARY STUDIES

4.1.1 Discrimination testing

In this preliminary study, the aim was to investigate the most effective sensory procedure to use in further experiments by comparing type of test, breathing technique and data interpretation methods.

4.1.1.1 Method

4.1.1.1.1 Olfactometer programme

Olfactometer programmes were designed to compare constant concentration aroma streams (**stream A**) versus pulsed (**stream C**). The frequency in stream C was chosen on the basis that it was higher than the mean breathing rate of a person (4 secs for 1 breath cycle) but was slow enough to provide square edged pulses. At this stage the silica was 3 metres long due to configuration issues and thus the pulse rate was limited, but was later cut to 1 metre and the pulse frequency was then increased to 0.6 seconds (refer to 2.2.3.5 for more detail). The olfactometer described in section 2.2 was used for this experiment with the volatile isoamyl acetate (Fischer scientific, Loughborough, UK), output flow 5 mL min⁻¹. Figure 4.1 shows the APCI-MS measured olfactometer output of A versus C. There were also three other programmes: A versus A, C versus A and C versus C.

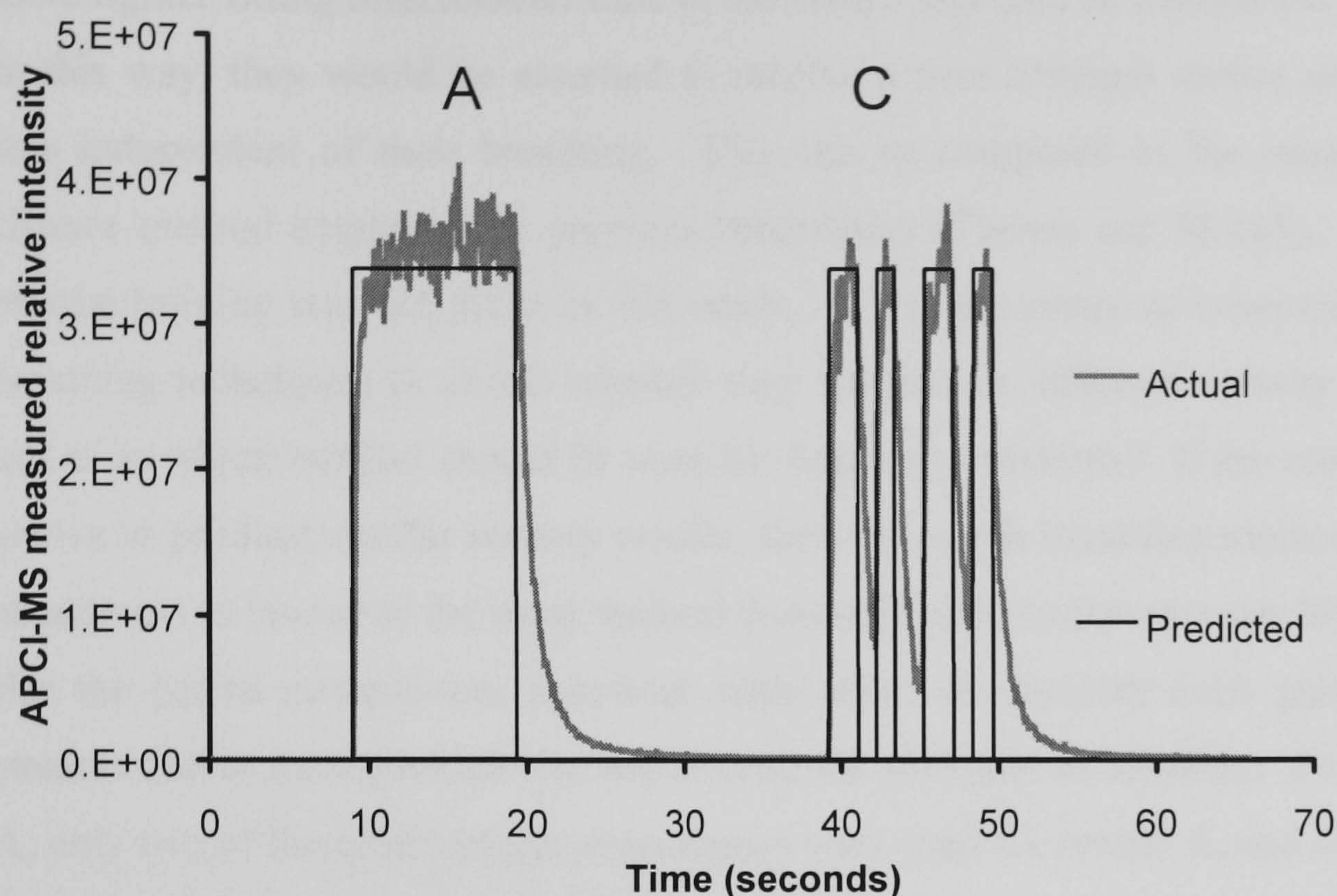


Figure 4.1 APCI-MS ion trace of pulse programme A versus C. A = 10.2 seconds of constant concentration of isoamyl acetate. C = 10.2 seconds of 4 pulses of isoamyl acetate.

4.1.1.1.2 Sensory procedure

Sixteen to twenty untrained assessors, frequently used for internal sensory testing, were asked to complete a series of paired comparison and A not A tests (6 per person) in two different sessions. Each time they were each given a 5 second example of the aroma as a reference at the start. Soundproofing headphones were worn throughout the test. White noise was played in the headphones in synchrony with each aroma stream onset and ceased as the stream ended. This served two purposes: to block the olfactometer switching sounds, and to communicate to the assessor that an otherwise invisible stimulus was being presented to them to aid their attention. Assessors were also asked to inhale the stimulus using two different methods. The first was to breathe at a slow and constant rate through the nose with the mouth shut and the olfactometer emission tube held just inside a nostril entrance. This method represented how potential *in vivo* studies would be conducted, where the exhalations of volatiles would be monitored from the other nostril. The second was to hold a

more tighter fitting olfactometer tube in the nostril and then to breathe out the mouth. In this way, they would be assumed to receive a near constant aroma stimulus that was independent of their breathing. This can be compared to the velopharyngeal closure method employed by previous researchers (Thesen and Murphy, 2001), but intense training was not given in this study. It was necessary to compare these two breathing techniques to assess whether they resulted in different sensory responses, and if so which method should be used for future experiments? If the methods were shown to produce similar sensory results, then the mouth breathing method would be abandoned in favour of the nasal method that could also incorporate the APCI-MS.

For the paired comparison, assessors were asked to compare each pair of aroma streams and to record which one was overall the strongest in intensity. For the A not A, only two of the olfactometer programmes were used (A versus A, and A versus C), where assessors were told that the first stream of each pair was always called A, and they were asked to decide whether the second stream was the same as A or different from A. If perceived as different, they were asked whether it appeared stronger or weaker than A.

4.1.1.1.3 Choice of aroma

Isoamyl acetate was used in all experiments of this type. The main reason for its choice was that it had been previously used in many studies with both the external and internal panel. In particular, the trained panel had previously worked with the aroma in a variety of food matrices. The disadvantage was that this compound has been shown to have a trigeminal effect, which must be acknowledged in any experimentation (Wang, *et al.*, 2002).

4.1.1.2 Results

Table 4.1 Paired comparison results: constant IAA stimulus (A) versus slow frequency pulsed IAA (C). Binomial probability distribution.

		No. of assessors perceiving the stream as the stronger		
A versus A	First A	Second A		
Mouth breathing	11	5	NS >0.05	
Nose breathing	9	7		

A versus C	A stream	C stream		
Mouth breathing	10	6	NS >0.05	
Nose breathing	10	6		

C versus A	C stream	A stream		
Mouth breathing	9	7	NS >0.05	
Nose breathing	6	10		

C versus C	First C	Second C		
Mouth breathing	9	7	NS >0.05	
Nose breathing	9	7		

The results in Table 4.1 show that there were no perceived significant differences between constant (A) and pulsed (C) stimuli and no differences between identical stimuli irrespective of presentation order. Furthermore, there was no significant difference between the results of the mouth breathing and the nose breathing. This result showed that different breathing methods were unlikely to affect the choice of the more intense aroma stream. Therefore, the mouth breathing method was discontinued in favour of the nasal breathing, which was more conducive to future simultaneous instrumental analysis. The results of the same stream discrimination tests meant that a response bias towards a stream due to its order in the test was not significant. In other words, any effect of adaptation was unlikely to influence an assessors decision in selecting the stronger stream.

Table 4.2 shows the results from the A not A test of the same stimuli. This also showed that assessors were unable to distinguish stream A from C.

Table 4.2 Results of A not A test including direction of stream (mouth or nose) Chi squared distribution of observed versus expected results showed no significant differences. See explanation of table below.

A versus A rep 1 mouth breathing			A versus A rep 1 nose breathing		
Sensory decision	No. of assessors	%	Sensory decision	No. of assessors	%
No difference	8 (A)	40%	No difference	9	45%
2 nd stronger	5 +	25%	2 nd stronger	3	15%
2 nd weaker	7 = 12 (B)	35%	2 nd weaker	8	40%
Total	20 (G)		total	20	

A versus A rep 2 mouth breathing			A versus A rep 2 nose breathing		
No difference	8	40%	No difference	5	25%
2 nd stronger	3	15%	2 nd stronger	9	45%
2 nd weaker	9	45%	2 nd weaker	6	30%
Total	20		total	20	

A versus C rep 1 mouth breathing			A versus C rep 1 nose breathing		
No difference	3 (C)	15%	No difference	5	25%
2 nd stronger	5 +	25%	2 nd stronger	1	5%
2 nd weaker	12 = 17 (D)	60%	2 nd weaker	14	70%
Total	20 (H)		total	20	

A versus C rep 2 mouth breathing			A versus C rep 2 nose breathing		
No difference	9	45%	No difference	8	40%
2 nd stronger	3	15%	2 nd stronger	3	15%
2 nd weaker	8	40%	2 nd weaker	9	45%
Total	20		total	20	

This table was not configured to a conventional A not A table with observed and expected results, in order to show the direction when a difference was perceived. Nevertheless, the Chi squared calculations were performed using the standard

method. This was calculated by the hypothesis; the expected results would be that all assessors should perceive A = A, and C ≠ A. In my results, each statistic was rendered non-significant due to many assessors not recognising A = A. An example of this calculation comes from the table results that are indicated with a letter. See table 4.3 for an example of expected versus observed results.

Table 4.3 Example of Chi square results for A not A test-mouth breathing rep 1.

Letters as indicated in Table 4.2	No. of assessors perceiving it to be A	No. of assessors perceiving it NOT to be A	Total
Stream A	A 8	B 12	G 20
Stream C	C 3	D 17	H 20
Total	E 11	F 29	N 40
Calculation	$\text{Chi} = \frac{N(AD-BC)^2}{E_x F_x G_x N}$ $\text{Chi} = 400,000 / 127,600$ $\text{Chi} = 3.13 (>\text{Crit. Value } P=0.05; 9.34), \text{ therefore not significant}$ This calculation was applied to the other repetitions and the nose breathing tests. All were >0.05. Calculations not shown.		

Although no significant differences were observed, most panellists were very certain about each of their decisions of which stream appeared the most intense. This suggested that each of the sensory tests were potentially being experienced in very different ways between panellists and repetitions. It was therefore necessary to use simultaneous breath by breath techniques to observe these differences.

4.1.2 Simultaneous breath measurement and discrimination test

4.1.2.1 Method

4.1.2.1.1 Procedure

The A versus C olfactometer output programme and the paired comparison procedure was used with a trained panel of 22 individuals. The stream order was the same for all assessors under the assumption that, although the olfactometer output was identical before entering a subject's nose, each assessor would then disrupt the stimuli in a different way. As before, headphones were worn and the white noise onset

indicated to the assessors that an odour stream was being presented. They were also asked to breathe at a slow and steady rate, and not be tempted to actively sniff. The experiment took place in standard booths using FIZZ software to record their choice of stronger stimulus. Only the nose breathing method was used.

4.1.2.1.2 Simultaneous instrumental measurement

Figure 4.2 shows how the APCI-MS interface was configured to the assessors' breathing.

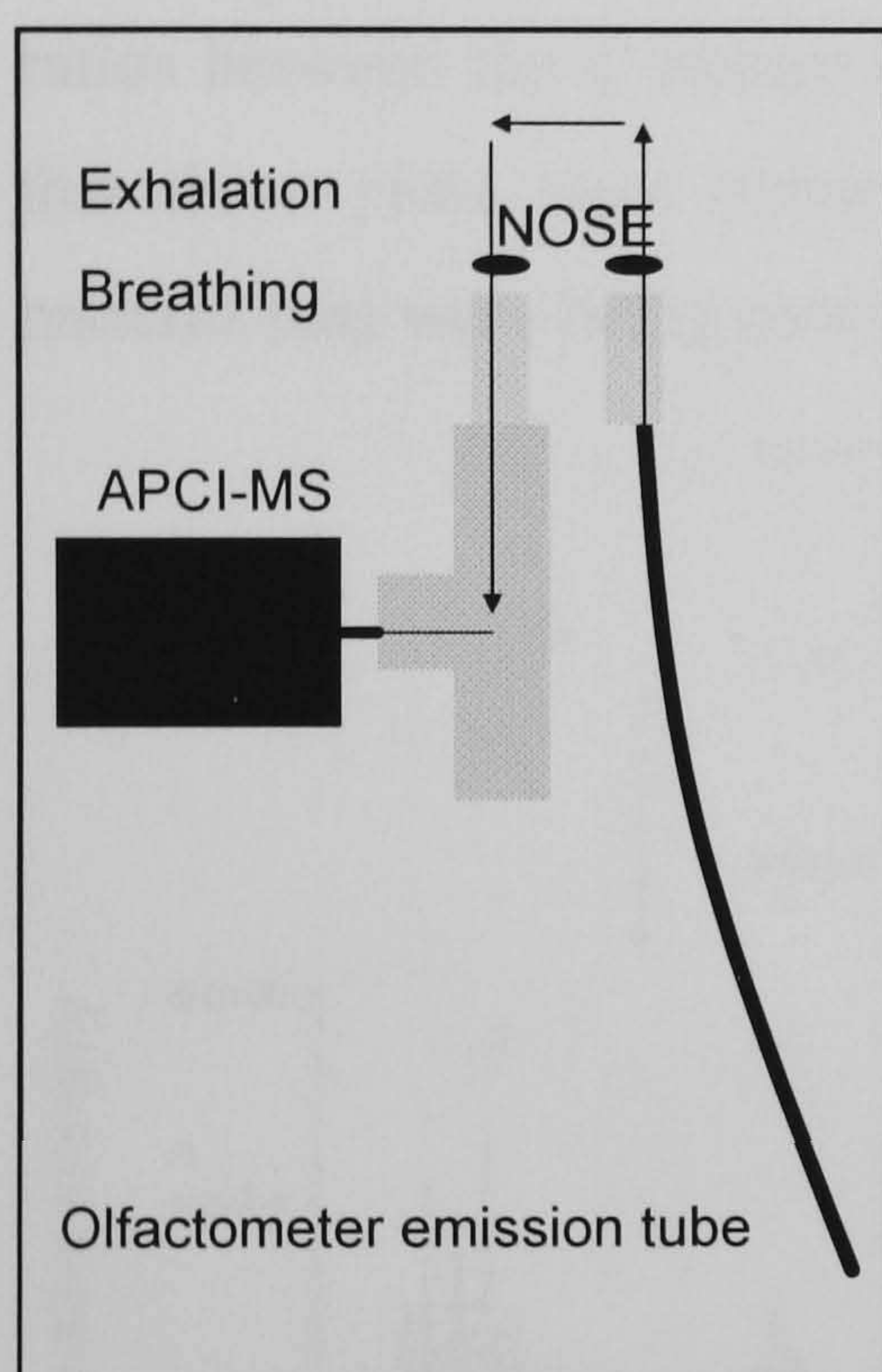


Figure 4.2 Exhalation breath by breath APCI-MS configuration. Olfactometer emission tube was placed in one nostril, whilst other nostril was placed over the tube directed to the mass spectrometer.

The APCI-MS was operated under the same conditions described in Chapters 2 and 3.

4.1.2.1.3 Instrumental data extraction method

As mentioned in the introduction of this chapter, APCI-MS breath by breath data is tidal as it is driven by respiration, which means that raw instrumental data is impossible to interpret unless specifically processed into certain parameters in order to make comparisons under the same treatment. Recently however, there has been some debate about how data points should be extracted from breath by breath data. One author reports using a normalisation technique, whereby ratios between areas of

the flavour and the acetone peaks are calculated (Normand, *et al.*, 2004). This method is mainly designed for retronasal breath by breath studies of flavour release from a beverage, and would not have been appropriate in this study.

Figure 4.3 shows how the raw olfactometer emission in Figure 4.1 became typically disrupted by an individual's breathing. The individual's breathing was clearly seen by monitoring acetone, which is expired in the breath, thus an exhalation is seen as the peak onset. Figures 4.4a-d show how certain instrumental parameters were interpreted from this disruption. This processed data was used to calculate parameter ratios between the C stream data as a percentage of the A stream data. It is stressed that these plots were obtained from APCI-MS data where it was the exhalation patterns that were being monitored and not the inhalation patterns.

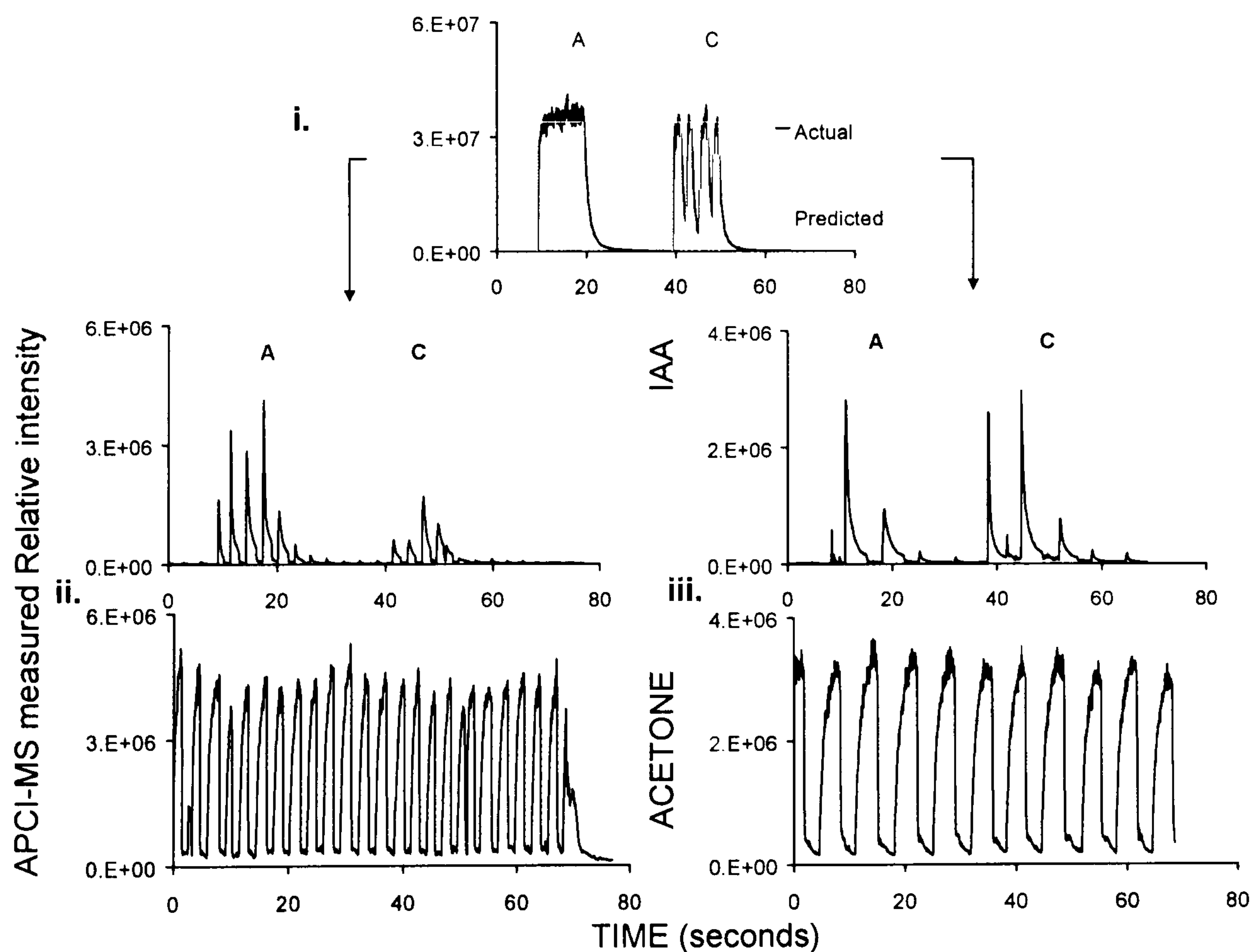


Figure 4.3 APCI-MS traces to show subject dependent disruption of the (i) original olfactometer output by two different assessors. ii. plots show breathing as shown by monitoring acetone peaks that coincide with the aroma peaks. Isoamyl acetate peaks are disrupted to the extent that peaks associated with C become much smaller than A. iii. Contrastingly this assessor breathes slower and thus produces less IAA peaks, and both A and C reach similar intensities.

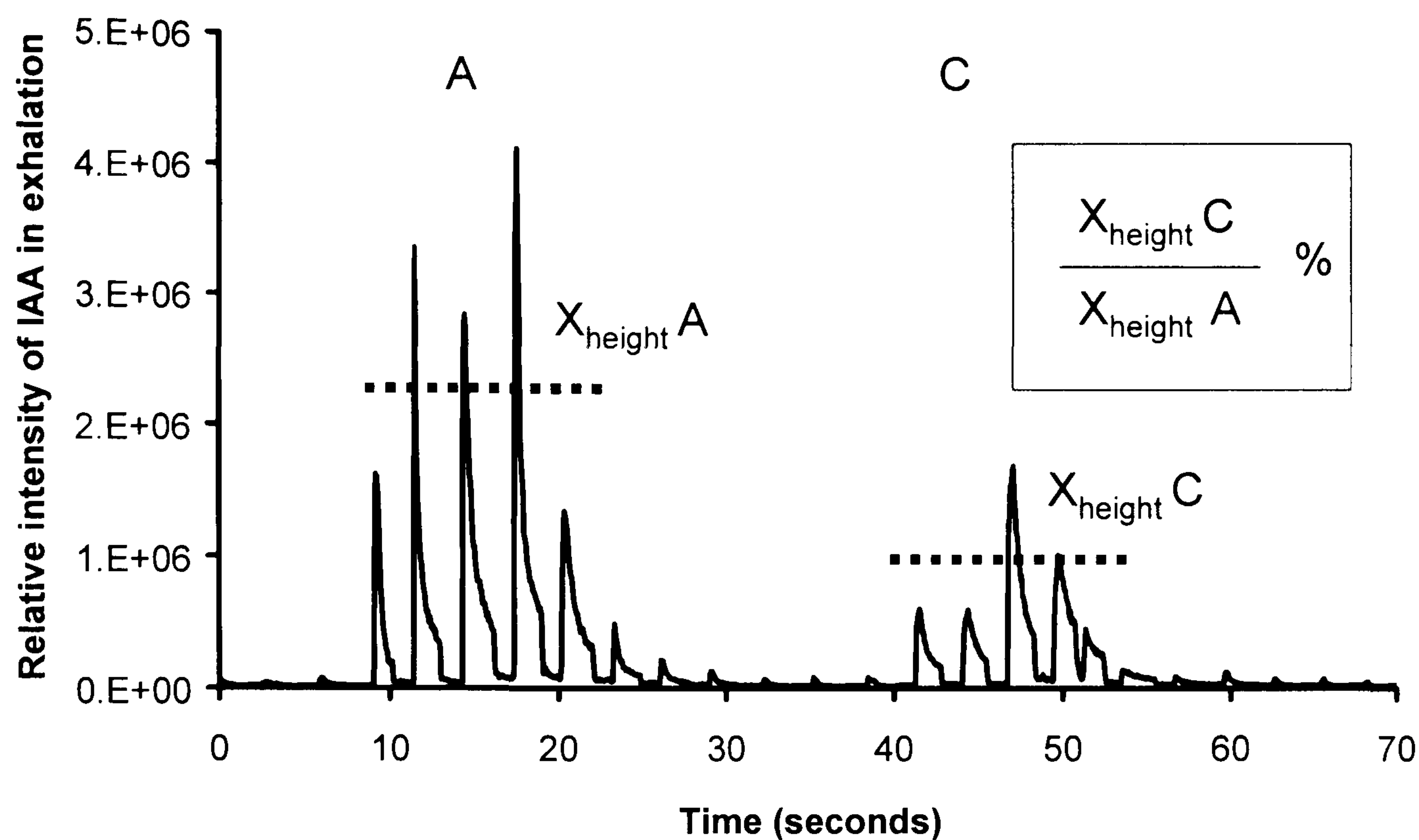


Figure 4.4a Instrumental parameters from breath by breath exhalation data. Mean heights.

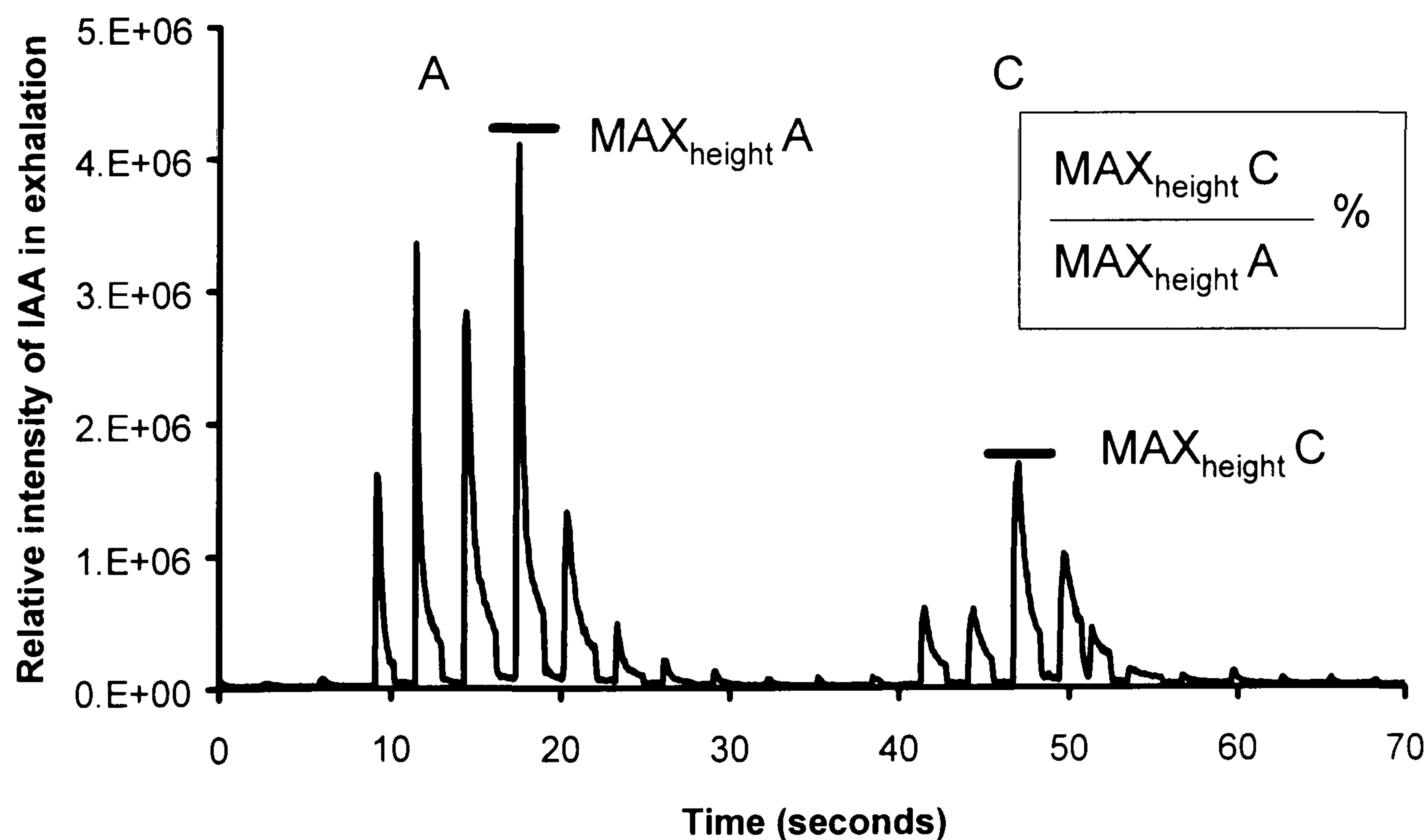


Figure 4.4b Instrumental parameters from breath by breath exhalation. Maximum height.

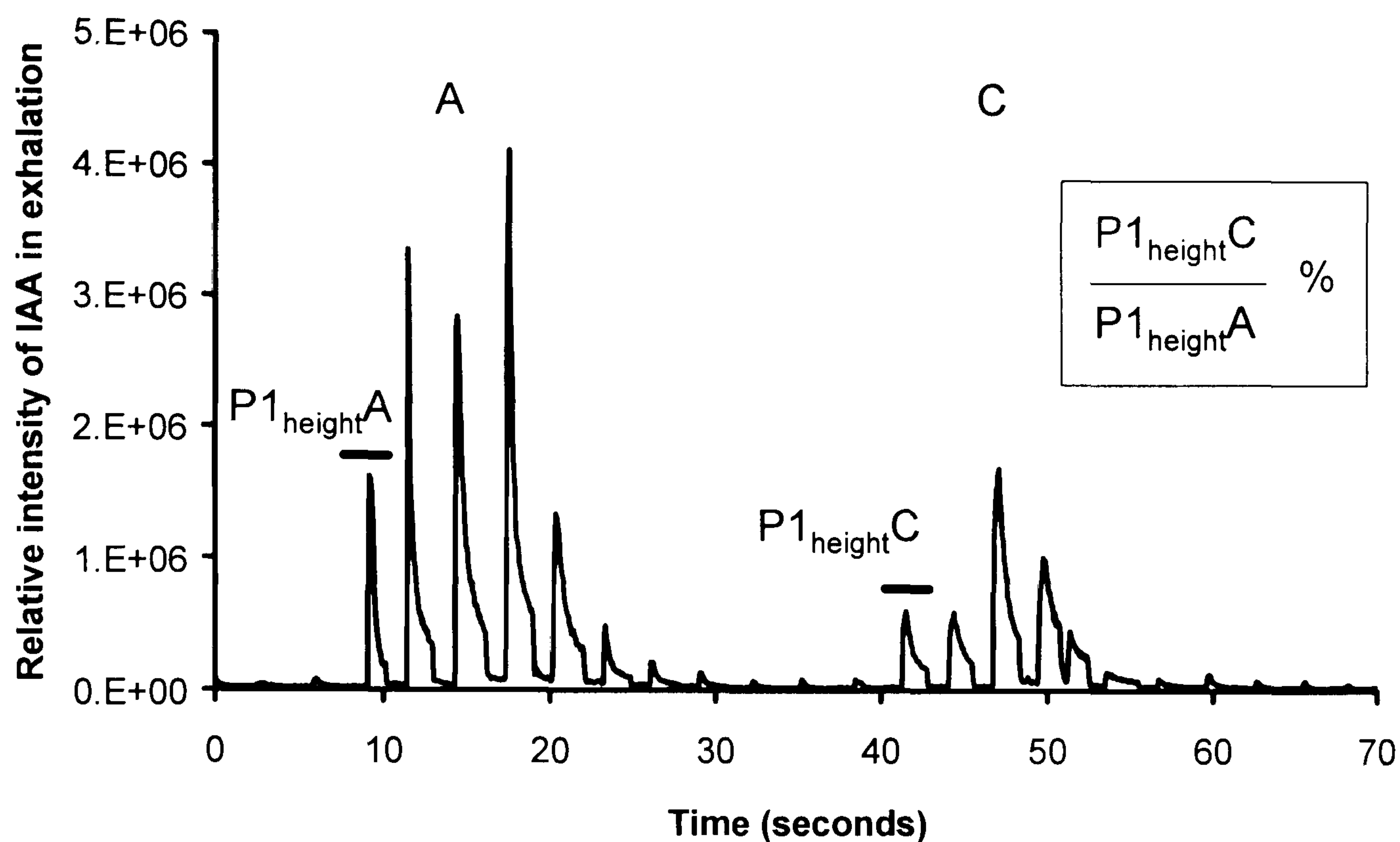


Figure 4.4c Instrumental parameters from breath by breath exhalation. First breath peak.

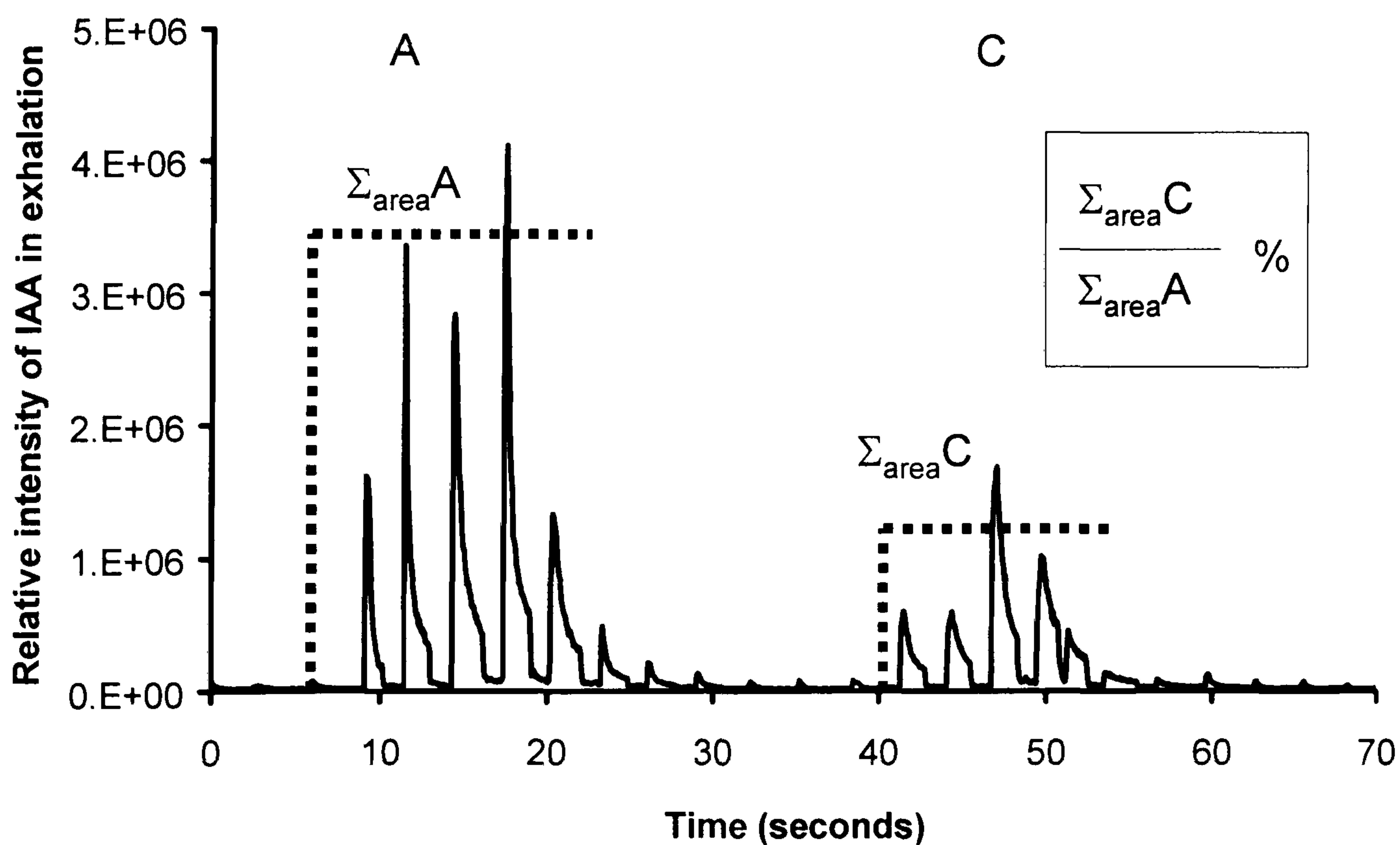


Figure 4.4d Instrumental parameters from breath by breath exhalation data. Total sum of breath peak areas.

4.1.2.2 Results

In this experiment, assessors received either stream A or C and were asked to compare them while the in-nose IAA concentration was monitored simultaneously.

4.1.2.2.1 Sensory result

Table 4.4 shows the sensory result of the paired comparison, where there was no significant difference perceived between stream A and stream C. This supported the result of the same test performed by the untrained panel in section 4.1.1.2.

Table 4.4 Sensory result of A versus C paired comparison test.

No. of Assessors perceiving A as the stronger aroma	No. of Assessors perceiving C as the stronger aroma	
15	7	0.2 Not significant (binomial probability). >0.05

4.1.2.2.2 Relationship between sensory choice and instrumental parameters

As depicted in Figures 4.4a-d, percentages of C parameters over A were calculated for each individual test. Therefore, if the ratio was <100%, it meant that the associated measured A parameter was greater than that of C. If >100%, then the opposite was true. This led to the hypothesis where, the number of observed A>C sensory results could be significantly tested against those observed instrumentally. This would follow a Chi-squared distribution as depicted in Figure 4.5.

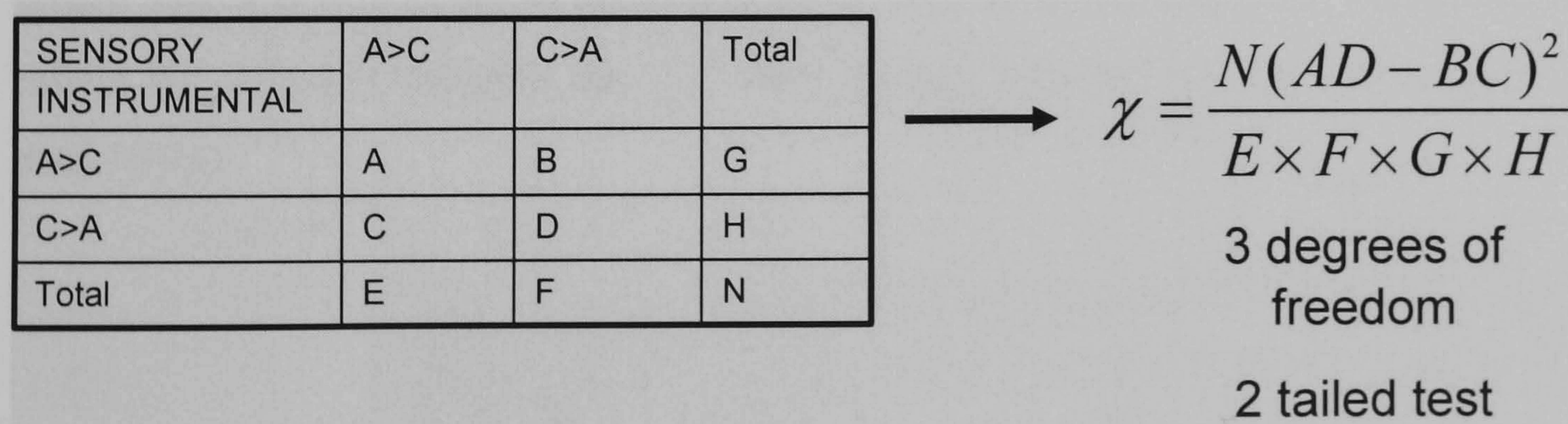


Figure 4.5 Chi-squared formula of expected versus observed results. The test has 3 degrees of freedom and is two tailed because there are 4 independent outcomes where the direction is not presumed. Critical Chi value is 9.35, P=0.05.

This formula was then applied to the results in Table 4.5 and the calculations showed that none of the instrumental parameters were likely to support the direction of the sensory choices.

Table 4.5 Observed and expected sensory and instrumental results.

Sensory	Assessors perceiving A>C			Assessors perceiving C>A			Chi Square calculation from formula in Figure 4.5. Probability: Instrumental A>C, therefore assessor perceives A>C. Crit val. 9.35 (0.05)
	A	B	G	C	D	H	
Instrumental parameters	A>C	C>A	TOTAL	A>C	C>A	TOTAL	
Average Breath Peak Heights	15	0	15	5	2	7	Chi= 19,800/4,200 = 4.71 (NS)
Maximum breath peak Height	15	0	15	7	0	7	Chi= 19,800/2,310 = 8.6 (NS)
First breath peak Height	14	1	15	3	4	7	Chi= 33,462/8,925 = 3.75 (NS)
Total sum of breath peak areas	15	0	15	7	0	7	Chi= 19,800/2,310 = 8.6 (NS)

These results could also be plotted as the actual values of the instrumental ratios in respect to sensory choice. Figure 4.6 shows this bar chart where, although not significant, those assessors who chose C as the stronger aroma produced breath by breath data that was closer to 100% than those assessors who chose A. This does not reflect the assumed outcome that if C were chosen, then the ratios would be greater than 100%.

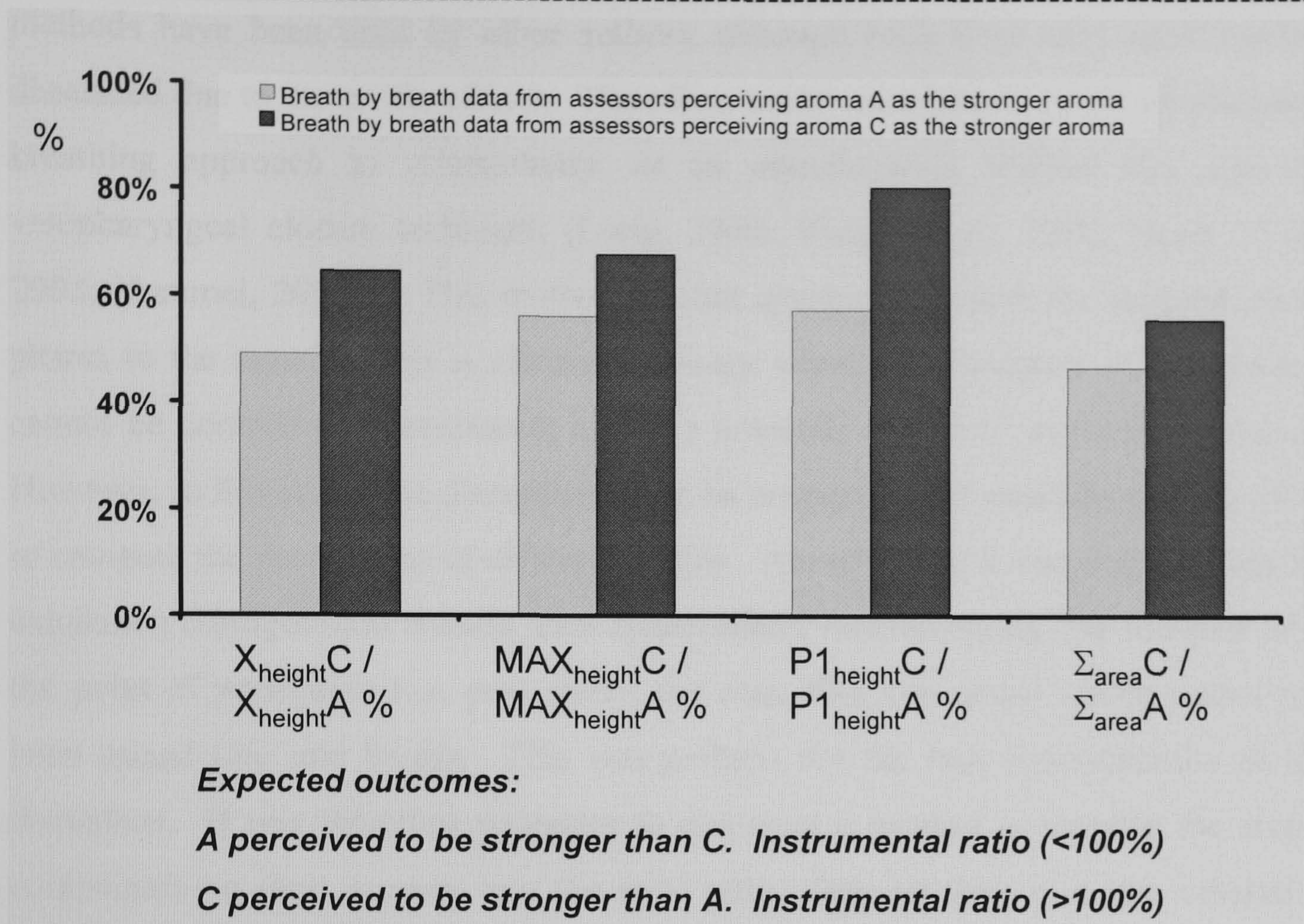


Figure 4.6 Chart of instrumental parameters as C/A % ratios plotted against the sensory choice of stronger perceived aroma stream.

The test used to calculate significant differences between ratio populations of different sensory choice was Mann-Whitney, which is a non-parametric equivalent T-test for two populations. A non-parametric test was used as APCI-MS breath by breath data can often be prone to a large number of points that would not generally produce a normal distribution.

4.1.2.3 Discussion

The results in these preliminary experiments have shown that comparing constant concentration and pulsed aroma becomes irrelevant once the stimuli are exposed to the panellist, who then disrupted the stimuli in their own way. Due to this fact, each panellist received a different test and their experience was sensorially quite different to one another. In this way, some panellists inhaled an aroma stream at a point where the concentration had dropped, and others experienced it at its maximum. The dramatic effect of this disruption could have been potentially minimised by high flow rates (>10 L/min) or synchronising a pulse with an inspiration. Both of these

methods have been used by other authors, although high flow rates have become dissuaded due to the tactile effects. Therefore, many researchers use the synchronous breathing approach to olfactometry, or an asynchronous method but with the velopharyngeal closure technique (Lorig, 2000; Wang, *et al.*, 2002; Jacob, *et al.*, 2003; Hummel, 2000b). This method teaches assessors to close the posterior nasal phares to the mouth. This is clearly necessary when the disruption of the stimulus cannot be controlled or measured, and is a potential source of unwanted variation. However, in this study, the disruption could be measured, and could be used as a tool to compare the perceptions of different people. Nevertheless, it was possible that the exhalation configuration created mass spectrometry measurements that occurred after the point of perception (i.e. post sniff), and also after any actual aroma pulses had been mixed into one breath. This was perhaps not the best representation of the disruption. It was therefore necessary to construct a method to monitor the aroma compounds on their journey into the nose rather than on their exit (i.e. inhalation configuration).

4.2 SIMULTANEOUS DISCRIMINATION TEST AND INHALATION BREATH BY BREATH STUDIES

It was necessary to use an instrumental configuration that monitored the aroma compounds as they were inhaled by a subject. This would provide data on how subjects disrupted the profile of the pulses and whether this influenced their sensory decision.

This section of results tests two main hypotheses:

- There are significant differences between the instrumental parameters of groups of assessors who differ by their choice of certainty in selecting the same sample.
- There are significant differences between the instrumental parameters of groups of assessors who differ by their choice of overall stronger aroma stream.

4.2.1 Method

4.2.1.1 Olfactometer programme

The original C stream was not pulsing at a fast enough frequency for individual pulses to be captured within 1 inhalation. Furthermore, on average assessors only exhaled 4-5 times during either stream, which captured insufficient instrumental data from the original A and C streams. Therefore, a new programme was constructed where both aroma streams were increased in duration to 20 seconds, and the pulse frequency of C increased to every 0.6 seconds. A raw olfactometer output trace of the altered programme (A2 versus C_{0.6}) as measured by the APCI-MS is shown in Figure 4.7.

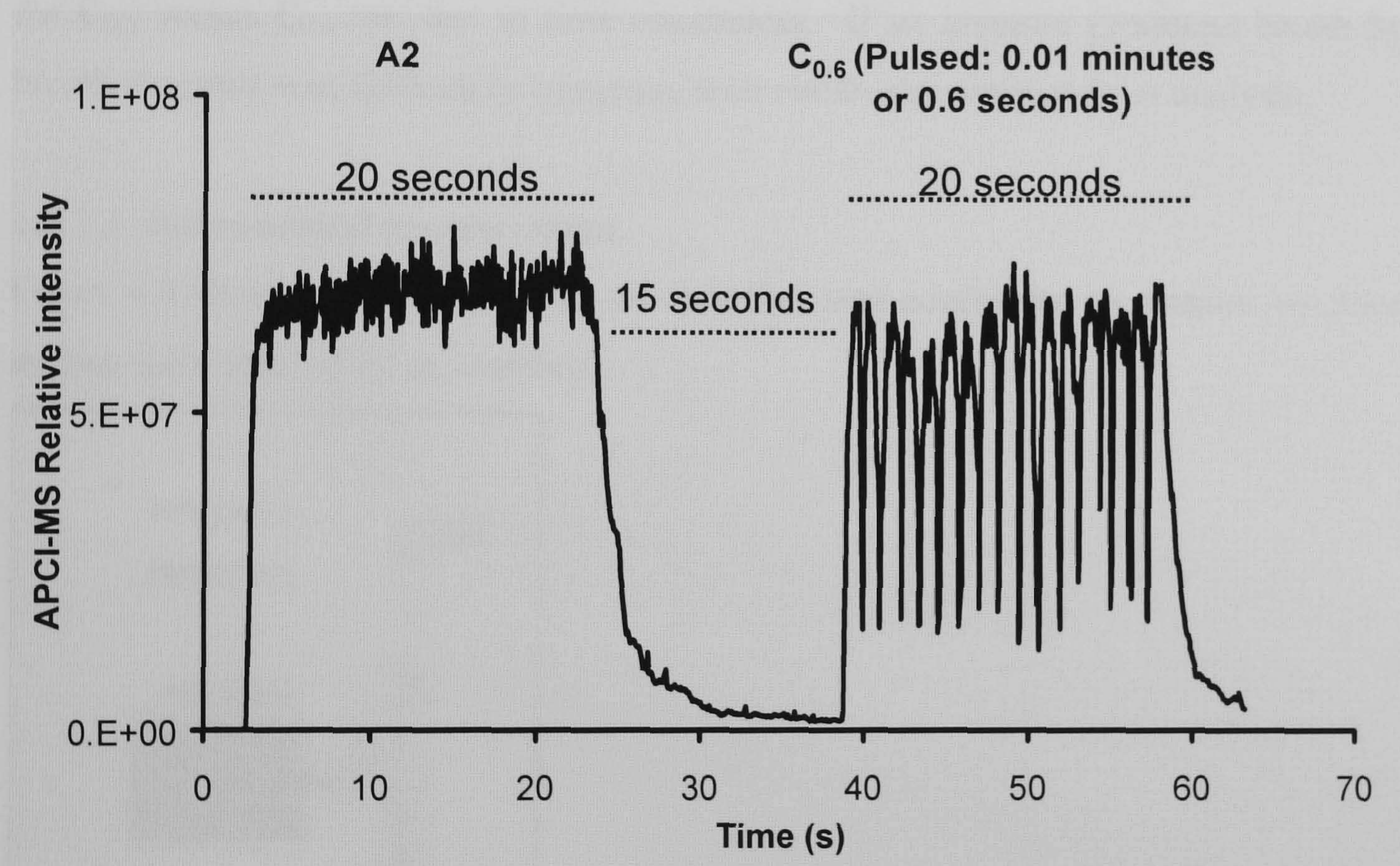


Figure 4.7 APCI-MS trace of olfactometer emission with an altered paired comparison programme. Both aroma streams have been increased in duration to 20 seconds, with a 15 second gap between them. Stream C pulse frequency increased to 0.6 seconds (0.01 minutes), now called C_{0.6}.

Other programmes used were A2 versus A2, C_{0.6} versus A2, and C_{0.6} versus C_{0.6}. Each assessor received all of the four tests with three replicates. However, under the assumption that no two tests produce the same instrumental profile, the replicates were not considered as true replicates. This also meant that test and sample order

became irrelevant, and therefore it was decided that each assessor would receive an identical presentation order.

4.2.1.2 Sensory procedure

The same method was used as described in 4.1.2.1. with 23 trained assessors. One difference was that assessors were asked to assign a certainty rating to their sensory choice. The certainty labels provided were; POSITIVE, QUITE SURE, NOT SURE and GUESSED. For analysis, these certainty ratings were categorized on the basis of their being either a positive decision (i.e. POSITIVE and QUITE SURE) or a negative one (i.e. NOT SURE and GUESSED). Only 14 assessors were asked to do the $C_{0.6}$ versus $C_{0.6}$ test due to time constraints. If an assessor produced breath data that was difficult to integrate, their result was omitted from analysis.

4.2.1.3 Instrumental measurement

Figure 4.8 shows a diagram of how the interface was configured to monitor volatiles as they were inhaled by an assessor.

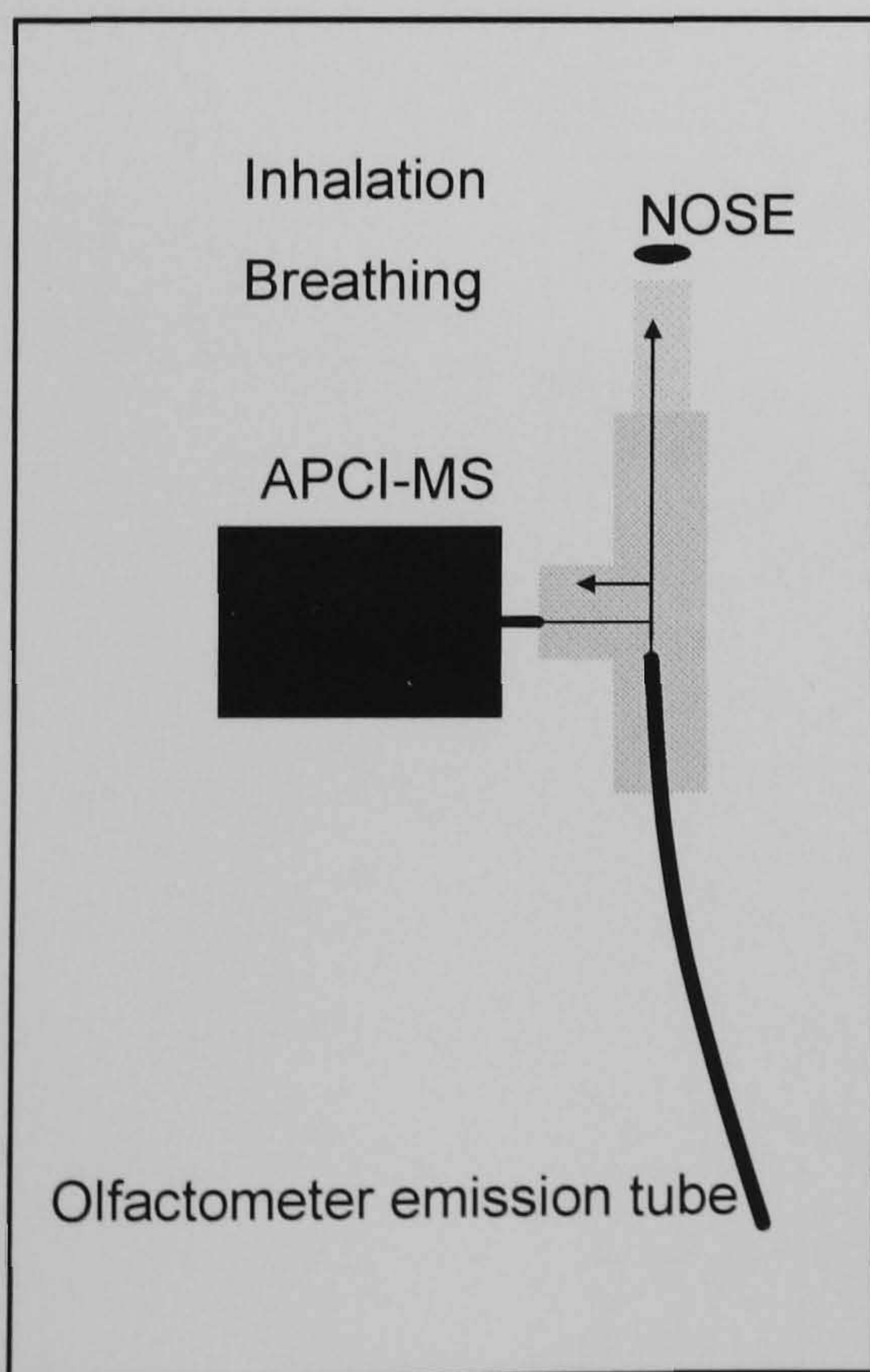


Figure 4.8 Inhalation breath by breath APCI-MS configuration. Olfactometer emission tube was placed in line with silica leading to APCI-MS, thus an inhalation transported volatiles past the probe, of which a proportion was sampled.

The APCI-MS was operated under the same conditions as described in section 4.1.2.1, but the signal strength was higher and therefore the sample flow was decreased to 15 mL min^{-1} to compensate for this.

4.2.1.4 Instrumental data interpretation method

The ion profiles produced by the inhalation configuration were far more complicated and noisy than the exhalation method. For most panellists each inhalation yielded more than one peak per 'sniff', which was made more intricate by the presence of isoamyl acetate peaks that were unlikely to have been inhaled by a panellist (i.e. peaks at the onset of exhalation). These peaks were assumed to be an amount of 'dead-space' volatile in the sample tubes being forced back past the silica as the assessor began to breathe out. It could not be confirmed whether they were volatiles present in the breath (i.e. as in the exhalation configuration) or simply residual compounds. Therefore, any peaks associated with an exhalation were ignored. Figure 4.9 shows a typical ion trace of an inhalation profile of A2 versus $C_{0.6}$, and shows an example of how parameters were interpreted from the data.

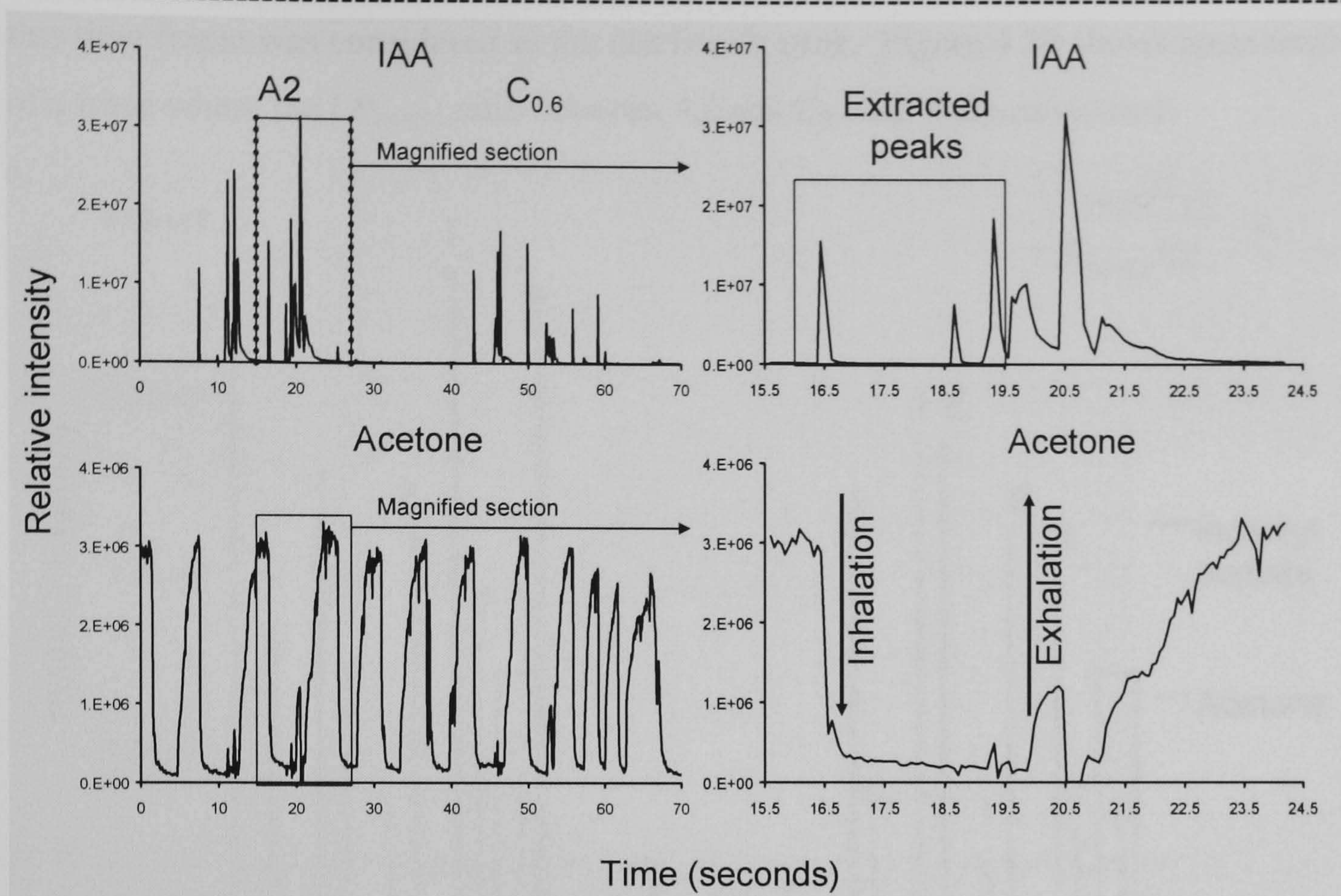


Figure 4.9 Example of how raw output signal of A2 versus $C_{0.6}$ from Figure 4.7 was typically disrupted by a panellist breathing in the inhalation configuration, and how the subsequent breath peaks were interpreted. Only volatile peaks corresponding to an inhalation were used, and these were confirmed by observing the acetone peaks. Acetone peaks indicate an exhalation on the onset, and an inhalation on the decay.

In the example given, one inhalation has been magnified and three separate isoamyl acetate peaks can be seen. For any measurement involving peak heights, the tallest peak was taken, but for any area measurement, the sum of all three was used. Once again, ratios were calculated between instrumental parameters of one stream versus another (as in Figure 4.4a-d). An additional instrumental parameter was used that was described as the last breath peak height of an aroma stream (i.e. LP_{height}). Its exact position on the APCI-MS trace per aroma stream was calculated by manually recording the time of first olfactometer valve switch. Twenty seconds of stream duration (pre-set olfactometer duration) plus a further 4.2 s to allow for the signal delay time, was added to the initial time (see section 2.2.3.5). The final peak within

this time frame was considered as the last breath peak. Figure 4.10 shows an example of a trace where the LP_{height} ratio between A2 and C_{0.6} has been calculated.

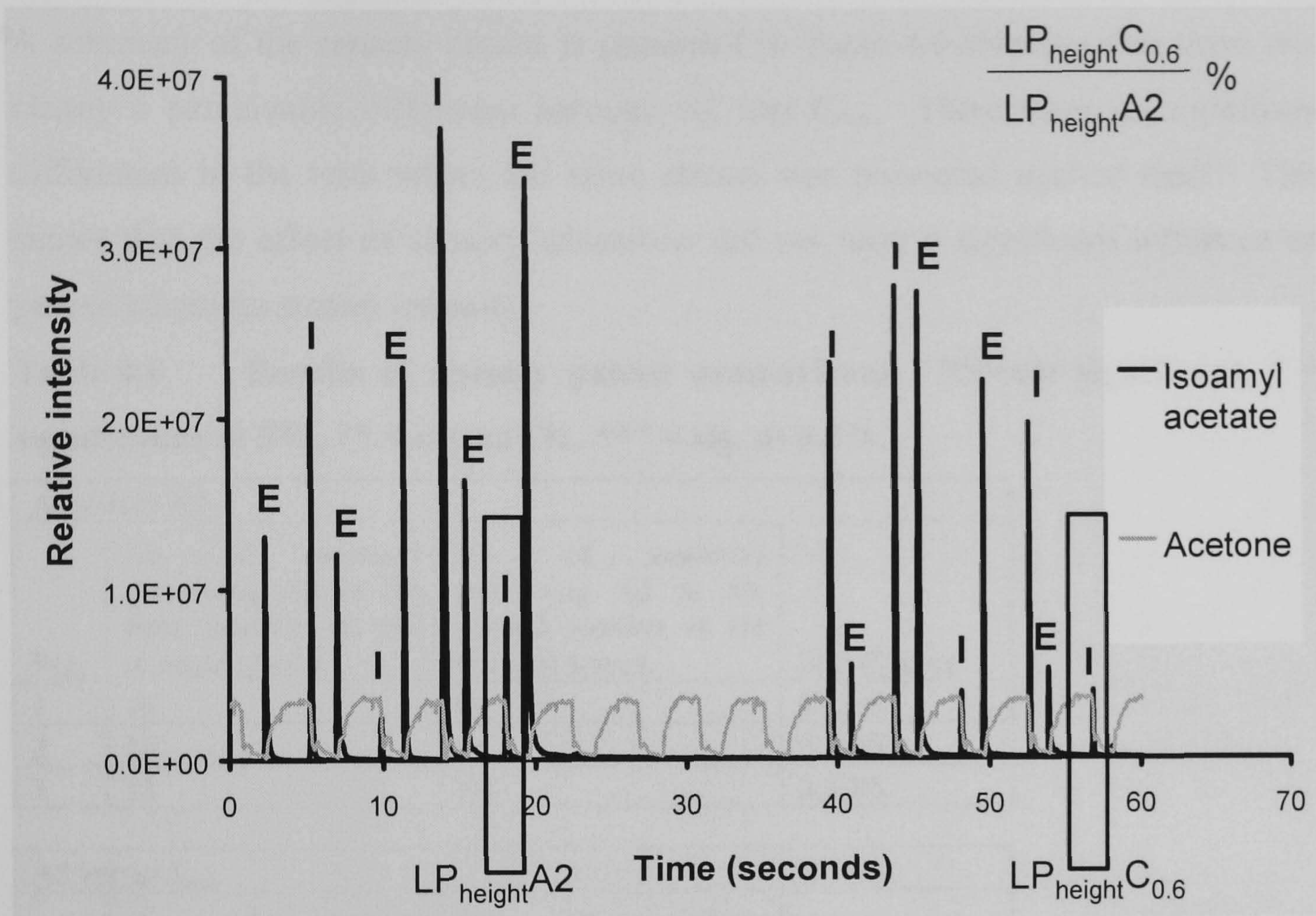


Figure 4.10 Example of a last peak height extraction (LP_{height}). I peaks are inhalation associated peaks, E peaks are the exhalation associated peaks, which were not used.

4.2.2 Results

4.2.2.1 Sensory results

A summary of the sensory results is presented in Table 4.6 showing that there was clearly a perceivable difference between A2 and C_{0.6}. There were no significant differences in the tests where the same stream was presented against itself. This shows that the effect of sensory adaptation did not have a significant influence on perceived aroma stream intensity.

Table 4.6 Results of sensory paired comparisons. NS=not significant, * = significance at 5%, ** = sig. at 1%, * = sig. at 0.1%.**

A2 versus A2			
Rep	No. of assessors perceiving A2 in the first position as the stronger stream	No. of assessors perceiving A2 in the second position as the stronger stream	Significance
1	14	9	0.4 NS
2	13	10	0.7 NS
3	13	10	0.7 NS

A2 versus C _{0.6}			
Rep	No. of assessors perceiving A2 as the stronger stream	No. of assessors perceiving C _{0.6} as the stronger stream	Significance
1	20	3	0.0005***
2	19	4	0.0026**
3	19	4	0.0026**

C _{0.6} versus A2			
Rep	No. of assessors perceiving C _{0.6} as the stronger stream	No. of assessors perceiving A2 as the stronger stream	Significance
1	6	17	0.0347*
2	6	17	0.0347*
3	8	15	0.21 NS

C _{0.6} versus C _{0.6}			
Rep	No. of assessors perceiving C _{0.6} in the first position as the stronger stream	No. of assessors perceiving C _{0.6} in the second position as the stronger stream	Significance
1	8	6	0.79 NS
2	9	5	0.42 NS
3	7	7	0.99 NS

4.2.2.2 Instrumental results - experimental control

It was necessary to first compare the instrumental parameters independently from the sensory choice for each test. This determined whether there were in fact any significant differences between A2 and C_{0.6}, and also between the same streams in different positions. Figure 4.11 shows a bar chart summarising this data. Significant differences were calculated between each population in each separate test using Mann-Whitney, assuming that all tests were independent of one another.

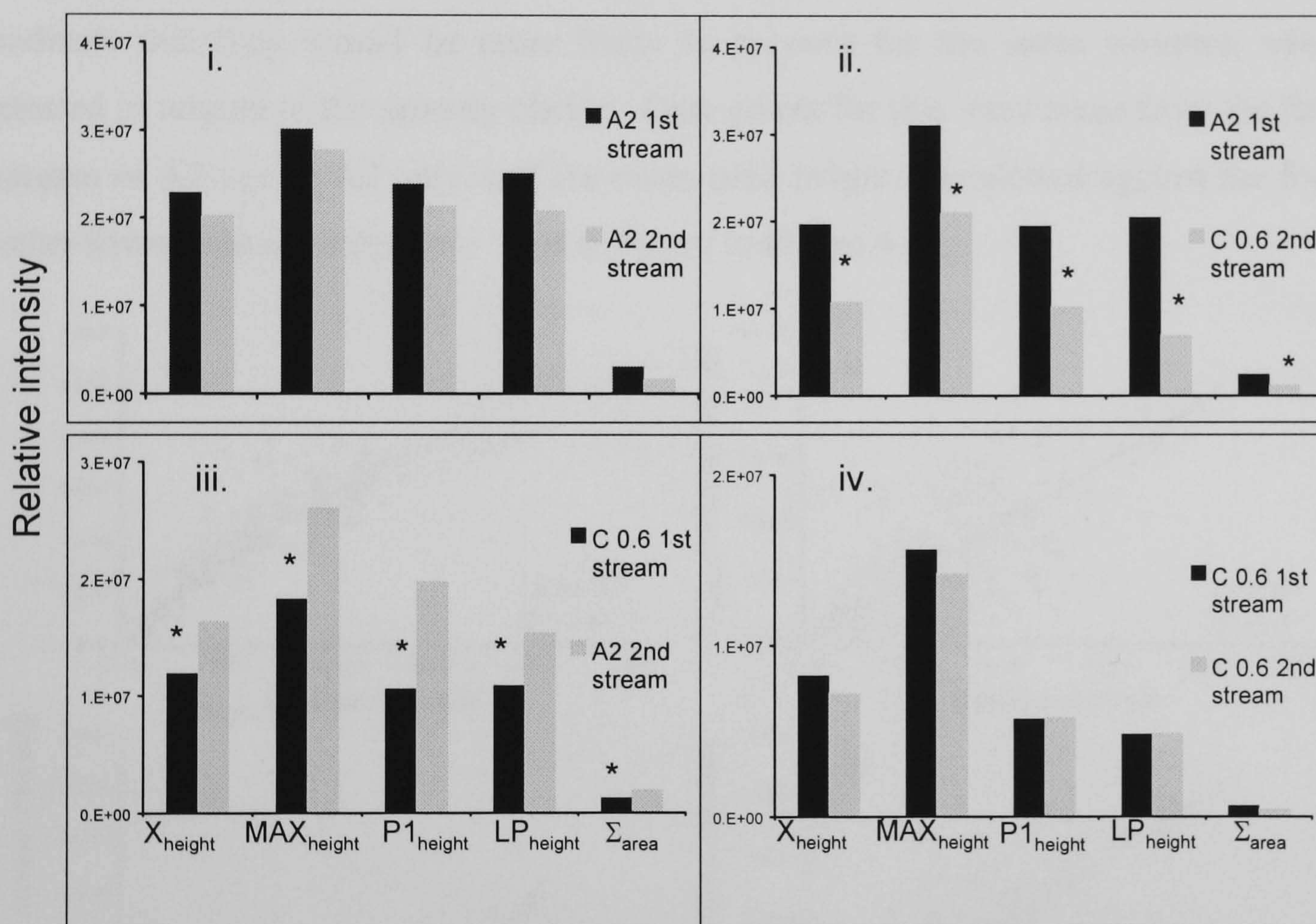


Figure 4.11 APCI-MS parameters from 4 programmes: i. A2 versus A2, ii. A2 versus C_{0.6}, C_{0.6} versus A2 and C_{0.6} versus C_{0.6}. * Represents stream with significantly lower parameter (at 5% level).

It is interesting to note that although there were no significant differences in instrumental parameters between streams in the A2 versus A2 test, the second stream produced slightly lower measurements. This effect was also observed to a lesser extent in the C_{0.6} versus C_{0.6} tests. The reason for this may have been that although assessors were asked to breathe slowly and steadily in their instructions, they may have slightly increased their breathing frequency as the test proceeded and as their attention focussed onto the aroma streams. Approximately 50% of the assessors

showed this effect (results not shown). A metronome to regulate breathing might have been a potential solution, however, it would have meant removal of the headphones, tailoring the frequency to each individual, and would probably have interfered with their attention.

It was necessary to assess the level of background noise between the different instrumental parameters, although there were obvious connections between instrumental heights and areas. High correlations between measurements would indicate that they would be more likely to account for the same variation when plotted in respect to the sensory choice. Data points for this were taken from the first stream of A2 versus A2 only, and the mean peak height was plotted against the four other instrumental parameters. This is shown in Figure 4.12.

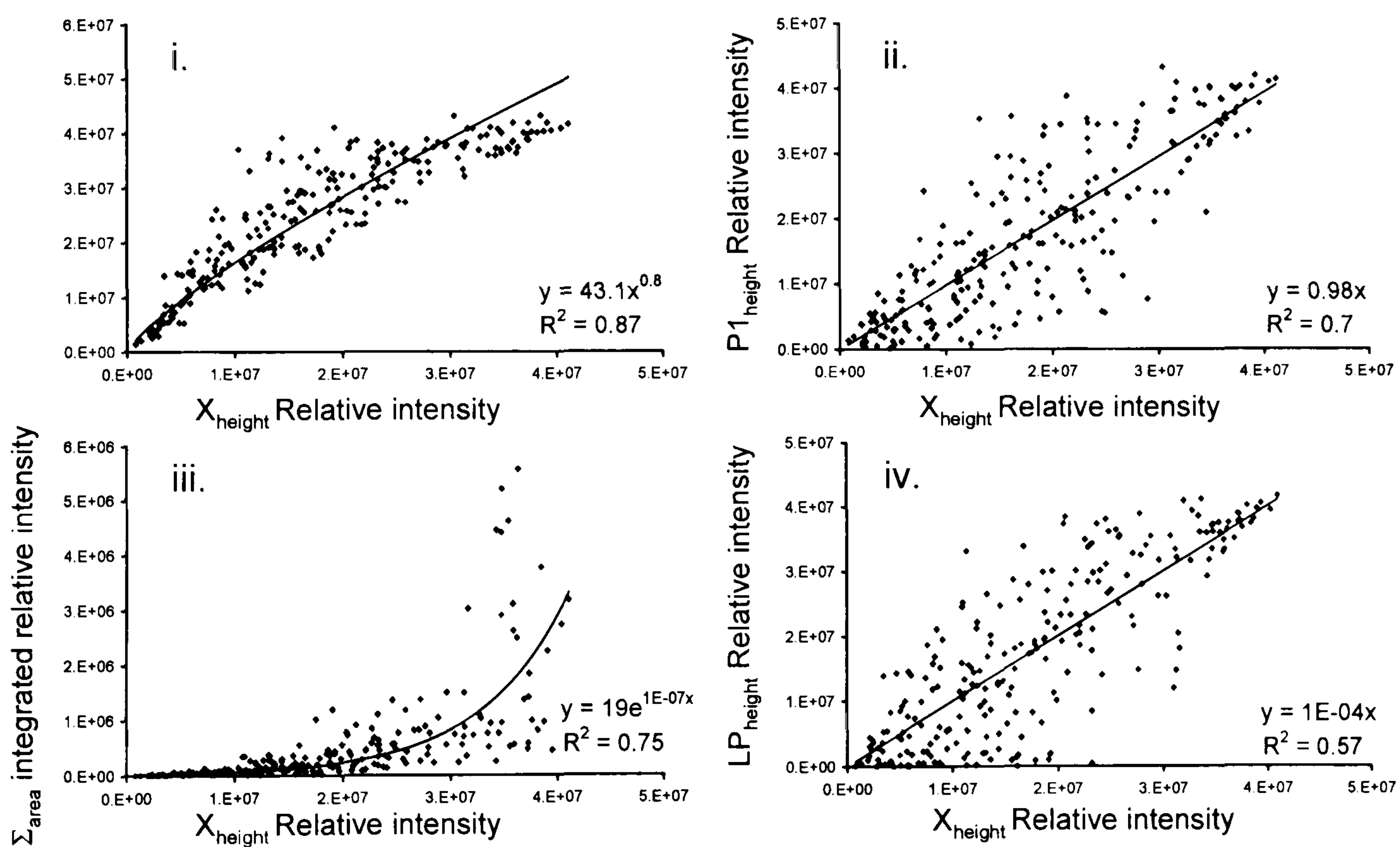


Figure 4.12 Relationships between instrumental parameters. X axis = X_{height} . Y axis: i. MAX_{height} , ii. $P1_{height}$, iii. Σ_{area} , iv. LP_{height} .

The graphs show that there were significant relationships between the mean peak heights and the other parameters, including the total sum of stream area. However, the correlations were relatively low with $P1_{height}$ and LP_{height} , (R squared values of 0.7 and 0.57 respectively). This demonstrates the inherent variability in assessors despite the controlled nature of the experiment.

4.2.2.3 Instrumental parameter ratios and their effect on certainty rating

The hypothesis that the certainty of a sensory decision could be attributed to differences in the corresponding in nose aroma behaviour was tested. This was analysed by studying each of the four paired comparisons individually. Assessors were divided into groups depending on whether they differed in their choice of certainty score (i.e. two assessors complete the same test and choose the same stream to be the strongest, yet one chooses a negative rating, the other positive). The in-nose ratios for these groups were then compared in all four tests, the ratios were calculated as the second stream parameters being a percentage of the first stream, regardless of whether the second stream was A2 or C_{0.6}. This ratio was based on positions rather than streams in order to also include the discrimination tests in which two identical streams were compared, and thus the only real difference was the position. The data analysis compared the means of all these ratios between 'certain' and 'uncertain' assessors. The hypothetical result of this was that uncertain assessors would have breath by breath ratios significantly closer to 100% than certain assessors, i.e. the same height etc. However, no significant differences were observed, which meant that if two assessors differed in their certainty, it should not be attributed to differences in the breath by breath patterns of aroma inhalation. Thus was more likely to be a cognitive factor.

4.2.2.4 Ratios of instrumental parameters with respect to sensory choice when the same stream was presented twice

This section of results was concerned with the paired comparisons: A2 versus A2, and C_{0.6} versus C_{0.6}. As expected, the sensory results in section 4.2.2.1 showed that there were no significant differences between aroma streams of identical nature. Consequently, this showed that the effect of adaptation within a sensory test was not biasing the sensory decision. Nevertheless, only 17 out of 64 tests (26%) were assigned a negative certainty by assessors and thus 74% were certain in their choice. It was therefore postulated that where the sensory choice was assigned a positive rating, the assessor must have disrupted the original identical aroma streams in a pair to such an extent that an instrumental difference influenced their decision.

The hypothesis was tested by first taking all of the instrumental parameters from the second stream of each identical pair and calculating it as a percentage of those parameters from the first stream. The values above 100% were counted as being the instrumental values where the second stream instrumental parameter was greater than the first, and vice versa. These numbers were then used in an observed and expected frequency table shown in Tables 4.7 and 4.8, which both used the function in Figure 4.5. It is important to note that all answers regardless of certainty were included in this analysis. This was because in section 4.2.2.3, the certainty score was not shown to be significantly linked with the instrumental parameters. The tables show that the weighting of the instrumental parameters could not predict the sensory choice when the test presented was the same sample twice.

Table 4.7 Observed and expected frequency table for A2 versus A2 tests. The values were also calculated using the function in Figure 4.5.

Sensory	No. perceiving First A2 > Second A2			No. perceiving Second A2 > First A2			Significance. 0.05 ($\chi > 9.35$)
	A	B	G	C	D	H	
Instrumental parameters	1 st A2 > 2 nd A2	2 nd A2 > 1 st A2	TOTAL no. of tests	1 st A2 > 2 nd A2	2 nd A2 > 1 st A2	TOTAL no. of tests	
Average Breath Peak Heights	22	14	36	15	9	24	0.01 NS
Maximum breath peak Height	26	10	36	15	9	24	0.6 NS
First breath peak Height	22	14	36	12	12	24	0.72 NS
Last breath peak Height	23	13	36	14	10	24	0.2 NS
Total sum of breath peak areas	23	13	36	14	10	24	0.2 NS

Table 4.8 Observed and expected frequencies table for C_{0.6} versus C_{0.6} tests.

Sensory	No. perceiving First C _{0.6} > Second C _{0.6}			No. perceiving Second C _{0.6} > First C _{0.6}			Significance . 0.05 ($\chi > 9.35$)
	A	B	G	C	D	H	
Instrumental parameters	1 st C _{0.6} > 2 nd C _{0.6}	2 nd C _{0.6} > 1 st C _{0.6}	TOTAL no. of tests	1 st C _{0.6} > 2 nd C _{0.6}	2 nd C _{0.6} > 1 st C _{0.6}	TOTAL no. of tests	
Average Breath Peak Heights	11	13	24	14	4	18	4.4 NS
Maximum breath peak Height	8	16	24	12	6	18	4.6 NS
First breath peak Height	10	14	24	8	10	18	0.03 NS
Last breath peak Height	10	14	24	12	6	18	2.3 NS
Total sum of breath peak areas	12	12	24	12	6	18	1.2 NS

4.2.2.5 Ratios of instrumental parameters with respect to sensory choice in tests where A2 was paired against C_{0.6} in either direction

It was observed in section 4.2.2.1, that there was in fact a significant sensory difference between aroma stream A2 and C_{0.6}. Instrumentally, there were also significant differences in the peak parameters (see section 4.2.2.2). The final question remains; are the instrumental results were weighted in the same direction as the sensory?

This was tested using two statistical procedures, both of which used percentages of C parameters over A parameters. This was in contrast to the previous section that calculated these percentages according to the position of the aroma stream in the test.

Table 4.9 shows the results as frequencies of values where A2 was greater than C_{0.6}, and vice versa (Chi squared formula in Figure 4.5). Figure 4.13 depicts this table as a bar chart based on the percentages with respect to the sensory choice. It is important to note that the total value of observations was higher as it included all tests from A2 versus C_{0.6} and C_{0.6} versus A2. Significant differences between populations of C_{0.6} over A2 ratios were calculated using Mann-Whitney test for two populations. Both

statistical tests suggested that there was a relationship between the weighting of the sensory decision and the difference in last peak height of A2 and C_{0.6}.

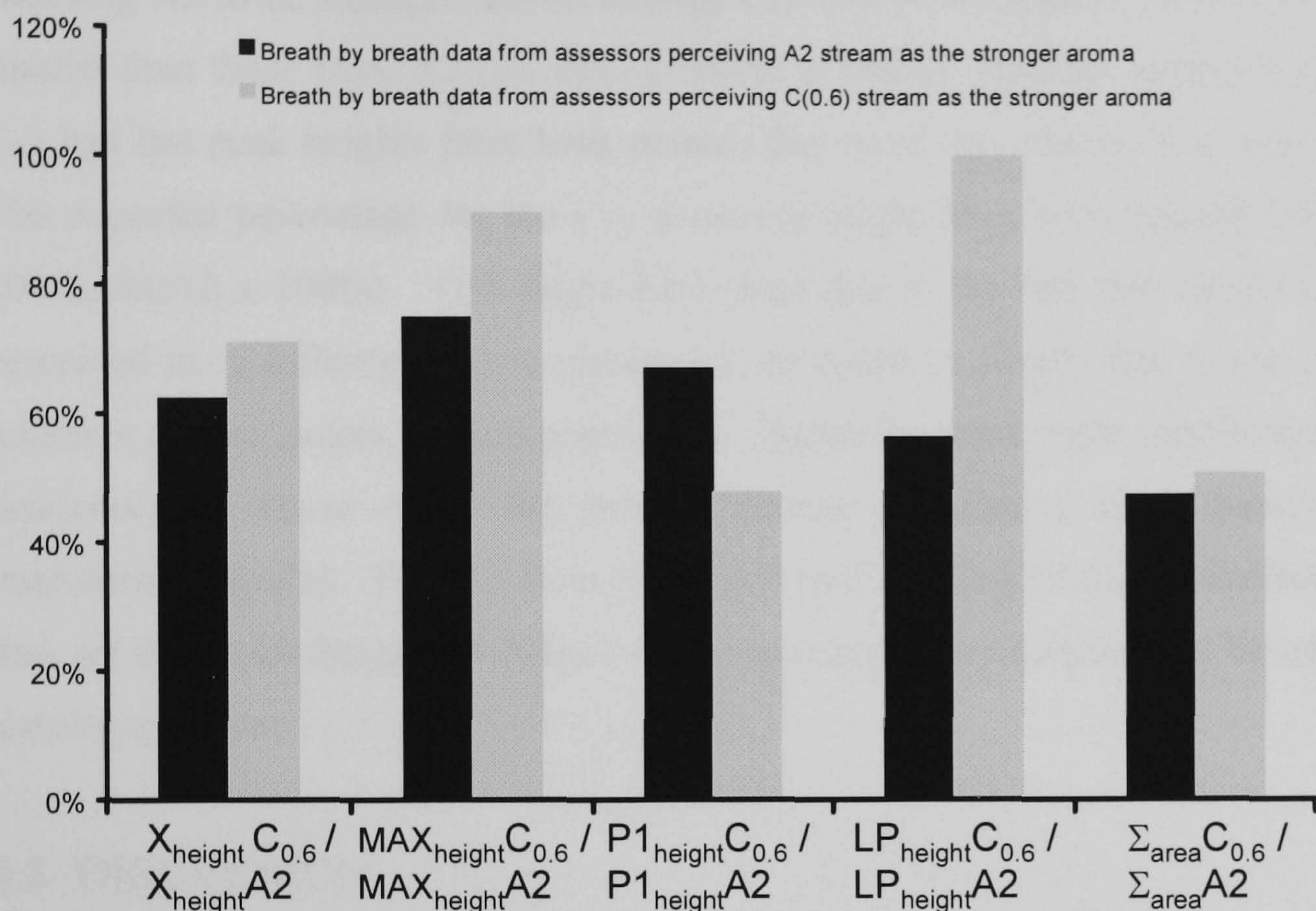


Figure 4.13 Stream A versus stream C_{0.6}. Instrumental parameter ratios in respect to sensory choice of stronger perceived aroma stream.

Table 4.9 Observed and expected frequency table of A2 versus C_{0.6}.

Sensory	No. perceiving A2 > C _{0.6}			No. perceiving C _{0.6} > A2			Significance ce. 0.05 ($\chi > 9.35$)
	A	B	G	C	D	H	
Instrumental parameters	A2 > C _{0.6}	C _{0.6} > A2	TOTAL no. of tests	A2 > C _{0.6}	C _{0.6} > A2	TOTAL no. of tests	
Average Breath Peak Heights	96	11	107	28	3	31	0.009 NS
Maximum breath peak Height	84	23	107	21	10	31	0.16 NS
First breath peak Height	82	25	107	23	8	31	0.08 NS
Last breath peak Height	79	28	107	13	18	31	Significance ce $\chi=11$ (P=0.05)
Total sum of breath peak areas	98	9	107	26	5	31	1.57 NS

However, when comparing the ratios of the two populations in Figure 4.13, the magnitude of difference is perhaps not as large as would be expected. Assessors choosing A2 to be stronger had on average $C_{0.6}$ last peak heights, which were ~40% smaller than those from A2 (i.e. $60C_{0.6}/100A2 \times 100\%$), whereas assessors choosing $C_{0.6}$ had last peak heights from both streams that were very similar (i.e. near 100%). The expected percentage for the $C_{0.6}$ assessors might have been around 166% (i.e. $100C_{0.6}/60A2 \times 100\%$). This might have been due to the fact that stream $C_{0.6}$ was perceived in a different way to stream A2, or could be simply due to the differing numbers of data points in each population. Naturally, there were significantly more assessors who chose A2 as the stronger stream, and thus a larger population of instrumental results. This problem highlights one of many of the limitations in the data set that must be acknowledged before accepting any conclusions based on the tested hypotheses.

4.3 DISCUSSION

Discrimination testing has been used to test a relatively complicated hypothesis, where it was not the samples being tested, but the assessors themselves. This is in contrast to the original idea of this experiment, where it was assumed that a pulsed and constant stream of aroma could be tested as a simulation of retronasal pulsing. The change in test direction highlights problems that other researchers may have unknowingly experienced, where the stimulus tested becomes easily disrupted and in turn becomes the main source of variation. A solution to the problem was found to be the use of simultaneous instrumental analysis, however the difficulties in the data treatment meant that conclusions were far from unequivocal. An example of this difficulty was the method of data extraction, where only certain instrumental parameters were used. A sensory difference between two aroma streams may be decided by a number of comparisons between many parameters, and not necessarily just the five measurements used in this experiment alone.

This experiment has shown that breath by breath measurement of orthonasal delivery can be monitored in two ways, one that uses exhalation after the supposed point of

perception and one that uses inhalation before the perception. In particular, inhalation measurement highlighted the effect of assessor disruption of the stimulus, where the pulsed aroma stream in Figure 4.7 sometimes became a series of small peaks per sniff, as in Figure 4.9, or sometimes became tall single peaks where the pulsing was no longer apparent, as in Figure 4.10. Other differences exist between exhalation and inhalation measurements in the type of flow they simulate. Retronasal air flow has previously been shown to be laminar, i.e. odorants are released into air streams as plugs within the flow. This is in contrast to turbulent flow, in which odorant molecules would mix evenly into the stream (Keyhani, *et al.*, 1995; Keyhani, *et al.*, 1997). This is clearly shown in breath by breath exhalations, where the plug is monitored as a single peak within a breath on an APCI-MS trace. Orthonasal flow is also laminar, although in the inhalation breath by breath configuration it gave the impression of being more turbulent in the chromatograms because the molecules become more dispersed on their passage past the MS-interface. Nevertheless, the inhalation configuration is a more effective indication of pattern disruption by an individual than the exhalation configuration.

The simultaneous breath by breath paired comparisons showed that two people may differ in their sensory choice due to a difference in the last breath peak of each stream. This may be linked to aspects concerned with the method a person uses in making a comparison. They would store the impression of the first stream in the short term memory, and then the second stream impressions are compared against the first. Considering only 7 chunks of information can be stored in the short term memory, perhaps the brain limits the most important impressions of a perception to the final exposures i.e. the last breath peaks. (*Higbee, 2002*).

The findings in this study are related to other fields of sensory and psychophysical research. One related area is from a purely sensory perspective: the science behind the discrimination test. O'Mahony and colleagues have pioneered Thurstonian methods in modelling and understanding the mechanism and the power behind tests such as multiple paired comparisons (Cliff, *et al.*, 2000; O'Mahony, 1986; O'Mahony

and Rousseau, 2003; O'Mahony, 1992). These methods account for the factors that cause the effect of perceptual variance and response bias such as sequence presentation, adaptation and memory. Overall it calculates a normal distribution of responses in the context of a subject's criterion shift when discrimination tests are repeated. In this study, if the Thurstonian approach had been used on the sensory data only, it might have given a misleading picture of the degree of difference between a pulsed and constant aroma stream. It would have probably shown that the perceptual variation was quite large, thus yielding a lower d' value. A d' value is measured in terms of the standard deviations of the two distributions. However, this would not account for the drift in stimulus over repetitions observed in the results. As an extreme view, could it be possible that the person's criterion changed only slightly, whilst the stimuli were instead the main source of variation? There are other methods that account for variation in discrimination test data such as maximum likelihood estimation (Tsai and Bockenholt, 2001) and repetition dependency modelling (Dittrich, *et al.*, 2002). These two methods would also be misleading if it were the stimuli that differed between people and not the sensory response. This theory could also be applied to a retronasal discrimination test, as it is frequently observed that individuals produce significantly different in-nose profiles on the APCI-MS despite consuming the same samples (Hollowood, *et al.*, 2004). In this data set there was a link between stronger sample and an APCI-MS parameter. This meant that even though there was a significant sensory difference between constant and pulsed aroma streams, the assessors who chose the opposite to the majority, did in fact have a stimuli difference that supported their decision.

A limitation in the overall data set was that each instrumental parameter was treated as being independent of other measurements and also of previous events. Therefore, an experiment was set up to compare variation between people using time intensity experiments of constant versus pulsed aroma stimuli, which account for the changes over time (see chapter 5).

5 PERCEPTION OF PULSED AROMA - TIME INTENSITY

In chapter 4 it was observed that a repeatable aroma stimulus was unpredictably disrupted in many ways by different assessors and repetitions. One suggested explanation is that the differences may be one of the factors involved with the formation of background sensory noise. In other words, the assessors who contributed to the background noise of the discrimination tests, experienced aroma stimuli that were significantly different in the relative proportions of the two stimuli, that they responded in the opposite manner to the overall majority. This chapter takes this idea further, by using similar pulsing programmes to analyse sensory and instrumental differences between assessors and their corresponding repetitions. Whereas chapter 4 focussed on static judgements based on comparisons, this chapter focuses on the dynamic changes in perception over time and how these are related to the instrumental profiles.

In recent years, time intensity (TI) methods have become regarded as a powerful sensory tool, especially in conjunction with *in vivo* analysis. The difficulties begin with the analysis of the results, where the method can be quite different depending on whether products are being tested, whether the assessors are being tested or simply a correlation between the two (Piggott, 2000). For product testing, such as boiled sweets or flavoured gelatine, the result analysis is generally a TI curve parameter extraction method. Typical parameters include; time to reach maximum intensity, the maximum intensity, plateau measurements, decay constants and curve areas. These can be correlated to simultaneous instrumental measurements to varying degrees of success (Baek, *et al.*, 1999; Linforth, *et al.*, 1999). For a review on TI methods see section 1.2.3.

The aim of this experiment was to test the differences between individuals and not between products, therefore principal component analysis was used to assess the variation in the data in respect to time. Grouping of individuals and their repetitions was compared to the corresponding PCAs of the instrumental data to test whether the

variation in breath by breath could explain the sensory response. Two types of breath by breath measurements were used and compared; exhalation and inhalation configuration, as developed in chapter 4. Results have shown that the main source of variation in the data could not relate the sensory to the instrumental measurements. However, a minor component of variation could relate certain instrumental parameters to the differences between two groups of individuals.

5.1 METHOD

5.1.1 Olfactometer output programme

The olfactometer output programme used in every test was the same and produced a 30 second period of constant concentration of isoamyl acetate and then a pulsed presentation with a frequency of 0.6 seconds for 30 seconds and finally 30 seconds of further constant concentration. Figure 5.1 shows this raw output as measured by the APCI-MS. The repeatability of the olfactometer was tested in chapter 2.

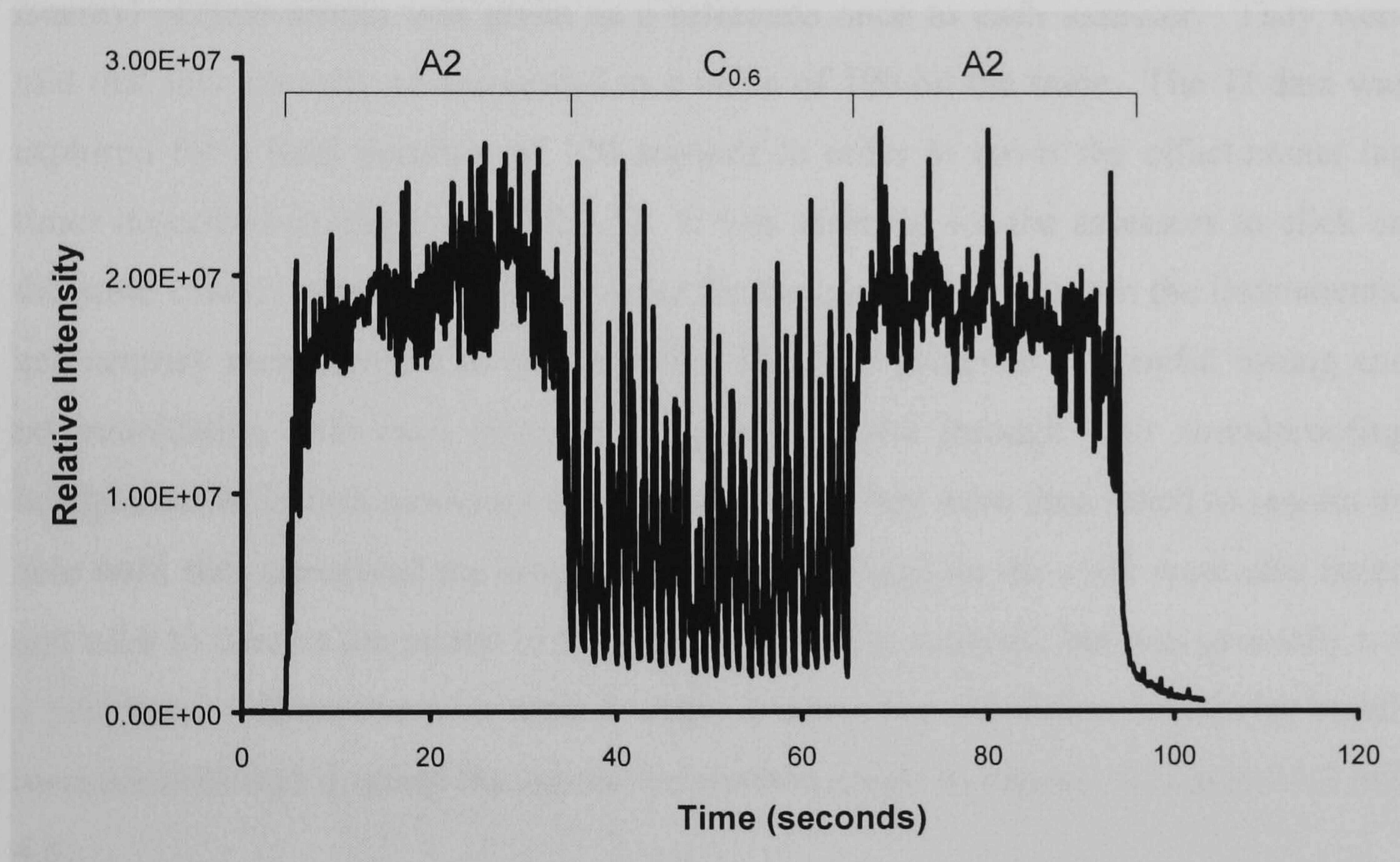


Figure 5.1 APCI-MS trace of raw olfactometer output programme for time intensity. 30 seconds of A2 (constant concentration), 30 seconds of $C_{0.6}$ (pulsed very 0.6 seconds), 30 seconds A2 (constant concentration).

Only one olfactometer output programme was used in this experiment because no two assessors or repetitions were assumed to be the same in their disruption of the stimulus. Therefore, the number of theoretical variations from one olfactometer programme alone was considered infinite.

5.1.2 Sensory procedure

A trained panel of 23 assessors was used for this experiment. This panel had been previously trained and used in many different time intensity experiments including: dynamic flavour-tastant interactions, flavoured gelatine, chewing gum and boiled sweets. Therefore, intensive training was not required for the experiment. Assessors were asked to monitor the intensity of banana/pear drop aroma using the mouse on a vertical line scale (FIZZ for Windows, Biosystemes, France). The scale began at zero and ended at 300, data points were taken every 0.12 seconds. A 5 second pulse of the isoamyl acetate aroma was given as a reference once to each assessor. They were told that this intensity corresponded to a value of 100 on the scale. The TI data was captured for a total duration of 120 seconds in order to cover the olfactometer lag times described in chapter 2 (2.2.3.5). It was essential for the assessors to click on the scale exactly when indicated in order for the correlation between the instrumental and sensory measurement to be accurate. This was achieved by careful timing and communication with each assessor using white noise through their soundproofing headphones to inform assessors to click on scale. They were then asked to remain on zero until they perceived the aroma. Delays in clicking on the scale were also timed and used to correct the points of the time intensity for analysis, but was generally not a problem. There were in total 6 tests: 3 using the exhalation breath by breath measurement and 3 using the inhalation method (refer to chapter 4, figures 4.2 and 4.8).

5.1.3 Instrumental analysis and data extraction

5.1.3.1 APCI-MS settings

The two methods of breath by breath measurement were the same as in chapter 4 (Figures 4.2 and 4.8). The APCI-MS settings in both configurations were as follows: source temperature, 75°C, corona pin voltage, 4 kV; sampling line flow, 15 mL min⁻¹; dwell time, 0.01 seconds. Monitored ions were isoamyl acetate, 131 [M+H]⁺, and acetone, 59.

5.1.3.2 APCI-MS trace interpretation

Data points were used to form smooth curves from the APCI-MS traces as peak heights and areas for the exhalation measurement (one peak per acetone peak). For the inhalation measurement there was often more than one isoamyl acetate peak per inhalation (refer to Figure 4.9). Therefore, average height was also calculated in addition to the maximum height per inhalation. Furthermore, the area per inhalation was calculated as the corresponding sum. The sensory data points occurred every 0.12 seconds, whereas instrumental data extractions occurred on average every 4 seconds and thus were far fewer. Therefore, the other time intensity data points that did not occur with an exhalation/inhalation event were not used in the analysis. If a trace was deemed problematic (unstable signal, coughing or overtly changeable breathing rates), it was neither repeated nor included in the analysis.

5.1.3.3 Data point time standardisation

Naturally no two assessors produced breath peaks at the same times or frequencies. Therefore, a method was used to standardise the data sets, in order that events all occurred on a comparable time scale using the following procedure. The average time for one breath cycle (exhalation + inhalation) was ~4 seconds (range = 2.35 to 7.26 s). Five second time brackets were used as a compromise because even the slowest breathers produced peaks that would fall into the majority of the brackets, i.e. 0-5 seconds, 5-10 seconds, 15-20 seconds etc. If four second brackets had been used, there would have been far more missing values from the slower breathers. The mid-value of these brackets was used as the X time point, i.e. 2.5, 7.5, 12.5 up to 17.5. Fast rate breathers produced more instrumental data points per 5 second bracket, which meant that the mean of the instrumental and the sensory results were taken to gain single Y points per time point. Slower breathers sometimes had peaks absent from some of the 5 second time brackets. These were not estimated and remained as missing data points.

5.1.4 Statistical analysis

The matrix contained the standardised time points (24 in total) as the variables and the assessors and repetitions as the samples. Principal component analysis was used to assess the sources of variation at each time point by correlation and was centred (The Unscrambler ® v8.0, CAMO Process AS, Oslo, Norway).

5.2 RESULTS

5.2.1 Raw results

5.2.1.1 Instrumental

Figure 5.2 demonstrates the types of stimulus disruption of the raw olfactometer output (from Figure 5.1) from the inhalation measurement. Four extreme examples of breath by breath APCI-MS traces are shown, where different assessors produced isoamyl acetate peaks that were not only very different in height, but also in general shape and profile. Plots (i) and (ii) contain inhalation peaks where the raw 0.6 second pulses could be observed during individual inhalations, whereas in (iii) and (iv) these raw output pulses are no longer visible, but have become single peaks during a single sniff.

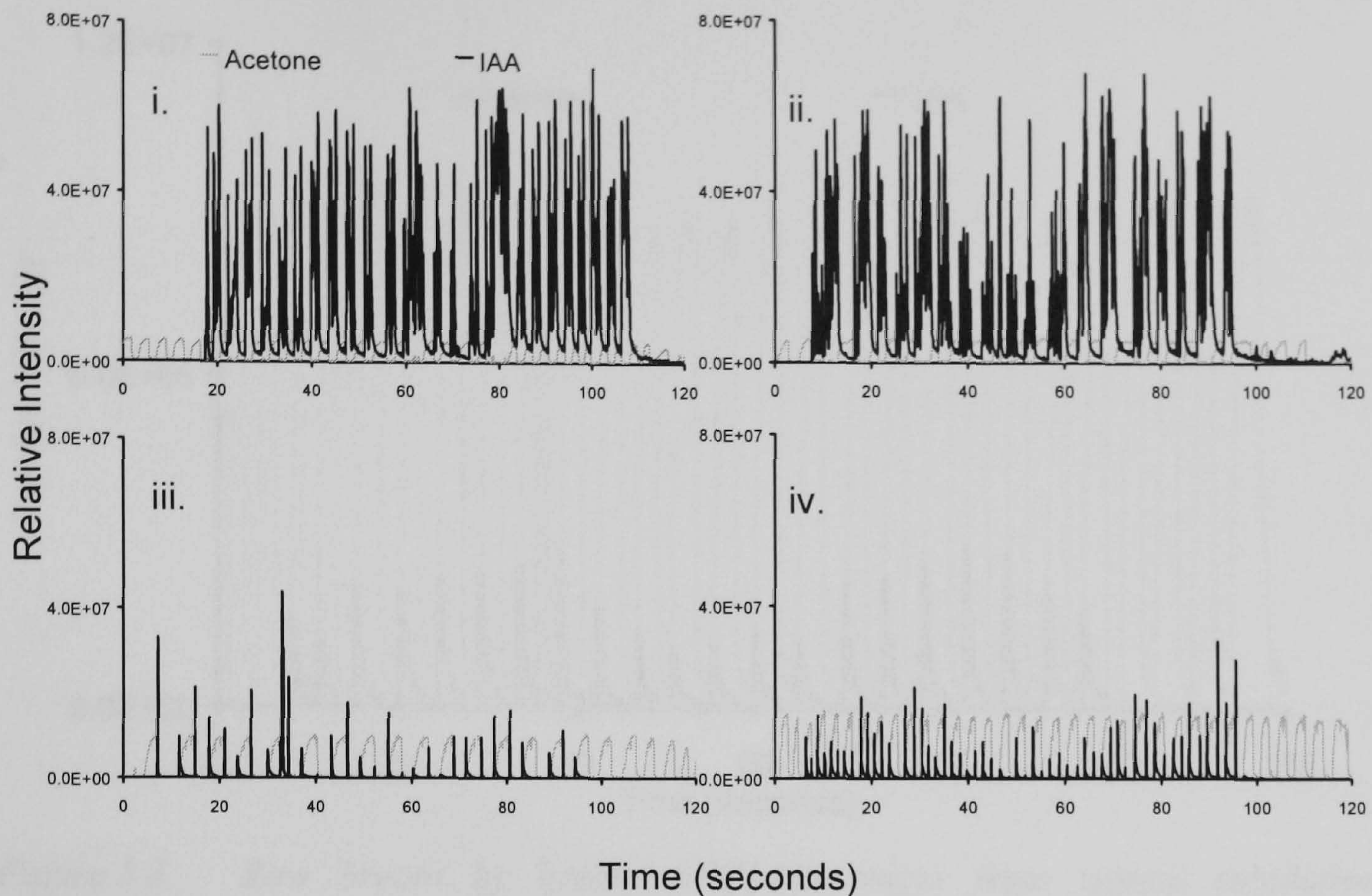


Figure 5.2 Raw breath by breath APCI-MS traces from inhalation measurement of A2-C_{0.6}-A2; i. peaks remain at constant height throughout constant and pulsed sections. ii. peak heights are relatively similar in height but change in area, iii. sharp narrow peaks and slow breathing, iv. Sharp narrow peaks and fast breathing.

Figure 5.3 shows a typical breath by breath trace of the olfactometer programme with APCI-MS in the exhalation configuration. With only a few exceptions, most assessors produced an exhalation profile that followed an m-shape where a volatile decrease occurred at times of pulsing.

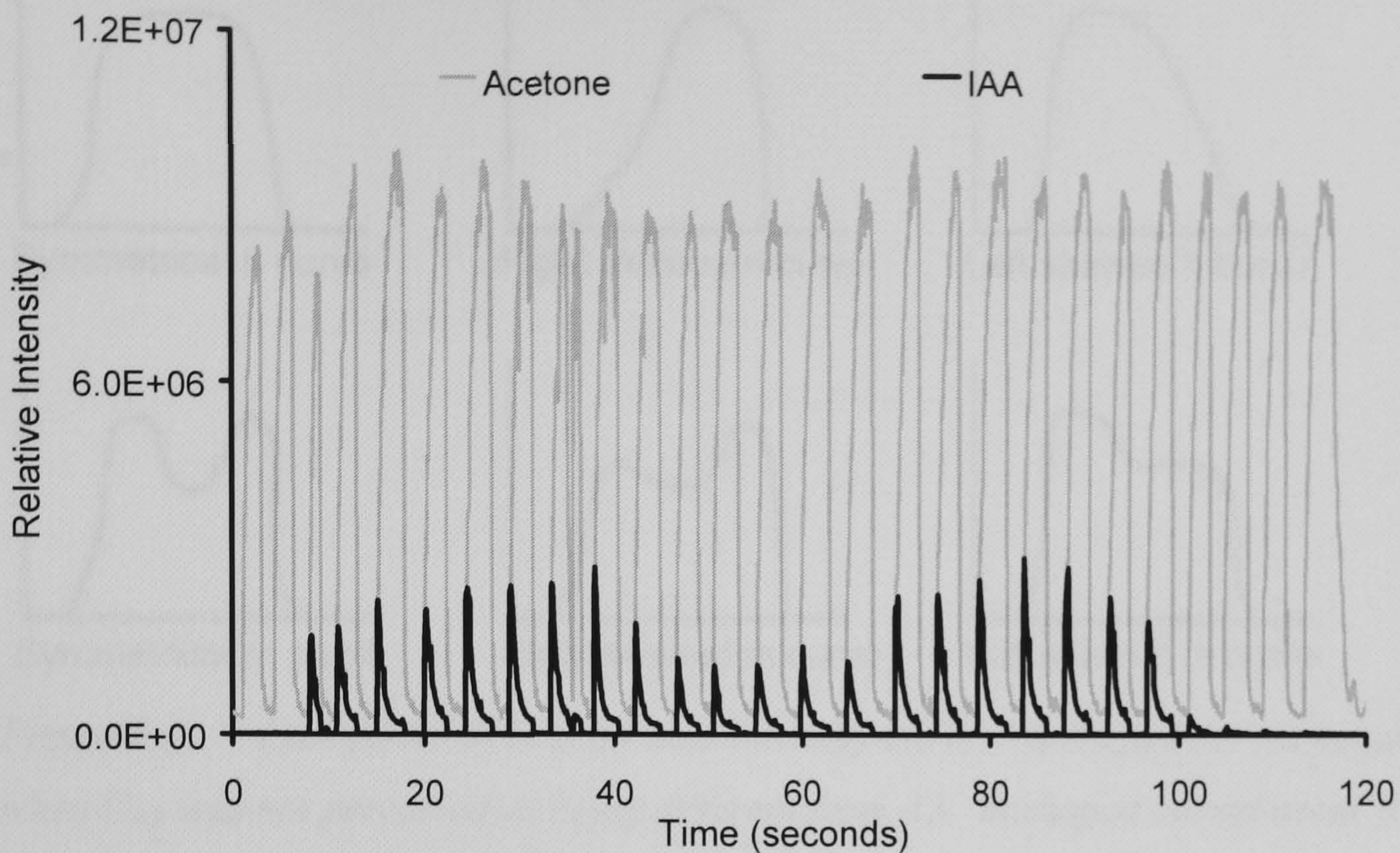


Figure 5.3 Raw breath by breath APCI-MS traces from typical exhalation measurement of A2-C_{0.6}-A2.

5.2.1.2 Sensory analysis

The typical time intensity curves observed in this experiment followed two patterns: curves where the assessors perceived the pulsed section to be weaker than the constant (m-shaped), and curves where the pulsed section was not perceived as different from the constant (n-shaped). Both curve shapes were then further categorised depending on whether the assessor produced a symmetrical curve or not. Figure 5.4 shows the 6 hypothetical categories of TI curves observed in this experiment.

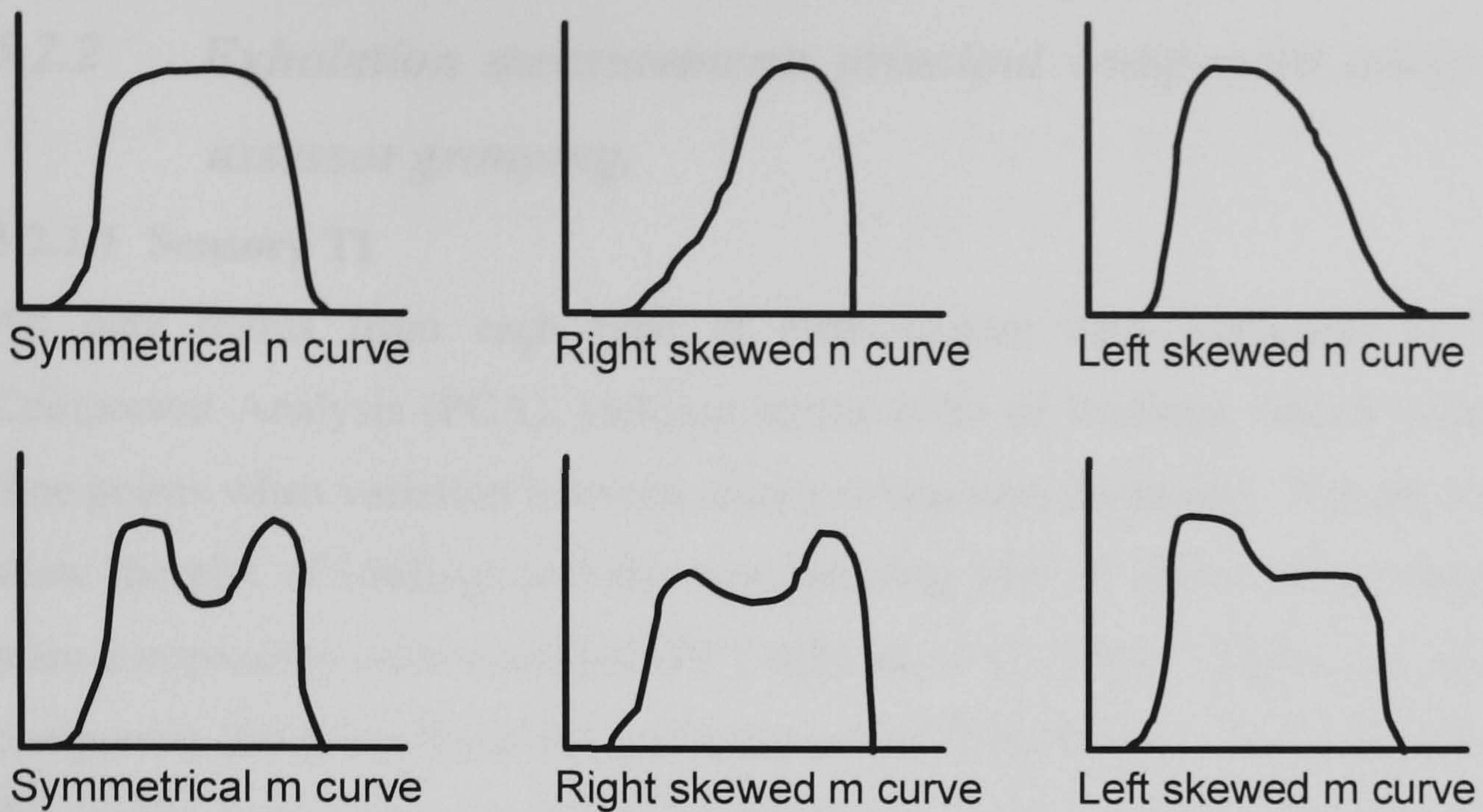


Figure 5.4 Categories of sensory time intensity curves. *n* shaped curves occurred when $C_{0.6}$ was not perceived as being different from A_2 . *m* shaped curves occur when $C_{0.6}$ perceived as being lower in intensity than A_2 .

Figure 5.5 shows 4 real examples of the TI together with the corresponding instrumental curves. In particular, in plot (i). an 'n' shaped TI curve has been produced, but the instrumental height curve is 'm' shaped. In contrast, plot (iv) shows that both sensory and TI curves were 'm' shaped.

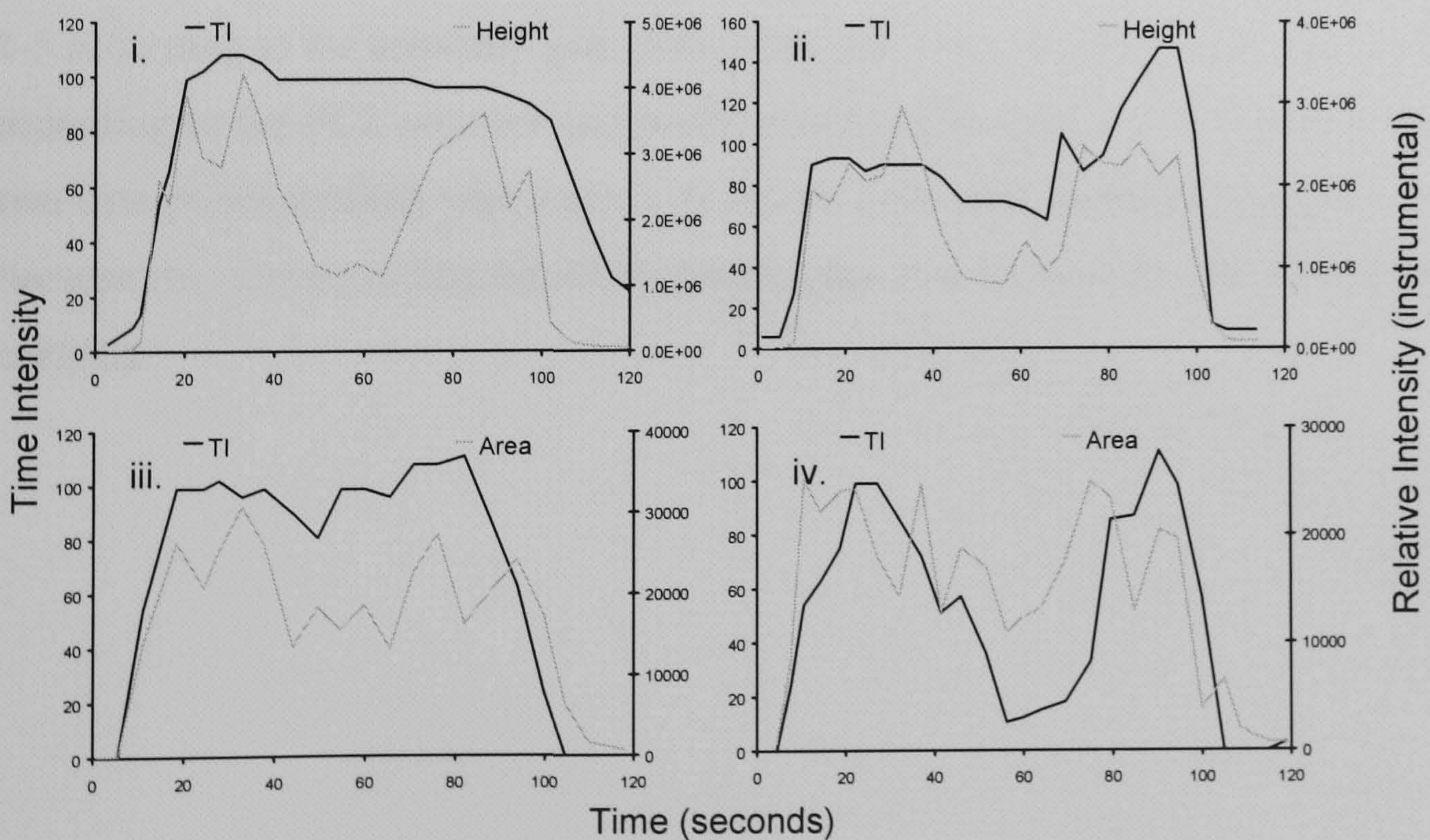


Figure 5.5 Examples of time intensity curves and exhalation instrumental heights (plots i and ii), and inhalation areas (plots iii and iv).

5.2.2 *Exhalation measurement: principal component analysis and assessor grouping.*

5.2.2.1 Sensory TI

All data points from each type of measurement were subjected to Principal Component Analysis (PCA), yielding scatter plots of loadings, which indicated the time points when variation between assessors was best explained. Figures 5.6a and b show the plot of loadings and the corresponding plot of scores, respectively. Two main components were extracted (PC1 46% and PC2 16%). There was also a third component (6%), but it yielded no further useful information. The time points where the results from assessors differed the most in respect to PC1 was during the A2 plateau sections, around 37.5 and 97.5 seconds respectively. Time points where the pulsed section or trough occurred (around 62.5 seconds) explained less of the variation between people than the A2 peaks. PC2 was best explained by variation between assessors at time points where the first A2 section was at its decay (27.5-32.5), and also the onset of the second A2 section (72.5-77.5). Figure 5.6b shows that the assessors were well distributed along these two components and could be arbitrarily divided into groups. Groups differing along PC1 were assigned a number 1-5 according to the arbitrary grid lines in the plot, then subdivided into either A or B according to the PC2 negative and positive sections as indicated in Figure 5.6b. The two data points marked with a circle in Figure 5.6b were removed from this analysis because the corresponding breath by breath data was difficult to interpret for further analysis.

A note on interpreting the scatter plots in this chapter.

The main rule with these plots is that as data moves further away from the origin (0,0) more variation between assessors is explained at those time points. Begin by reading along the X-axis. In the loadings plots, only positive PC1 values were obtained, thus only half the full plot is given to show more detail. At the time points to the far right, there is a lot of variation between the assessors in the perceived intensity of the signal or the instrumental measurements. Points on the left explain very little variation between individuals, i.e. assessors were using the scale in similar ways at these time points. When looking at the Y axis, the same rules apply, except that in all of the plots both negative and positive values are observed. Therefore, time points that explain maximum variation are at the positive and negative extremes. The time points at the extremes are then matched to their corresponding event, i.e. peak onset, second peak decay etc.

With regards to the plots of scores, both negative and positive X and Y values were obtained. Once again, where the assessors and their repetitions resolve in close proximity, it indicates that they produced similar results. Contrastingly, along PC1, if two assessors are at polar ends it would indicate that during the time points that explain the variation of PC1, they behaved very differently.

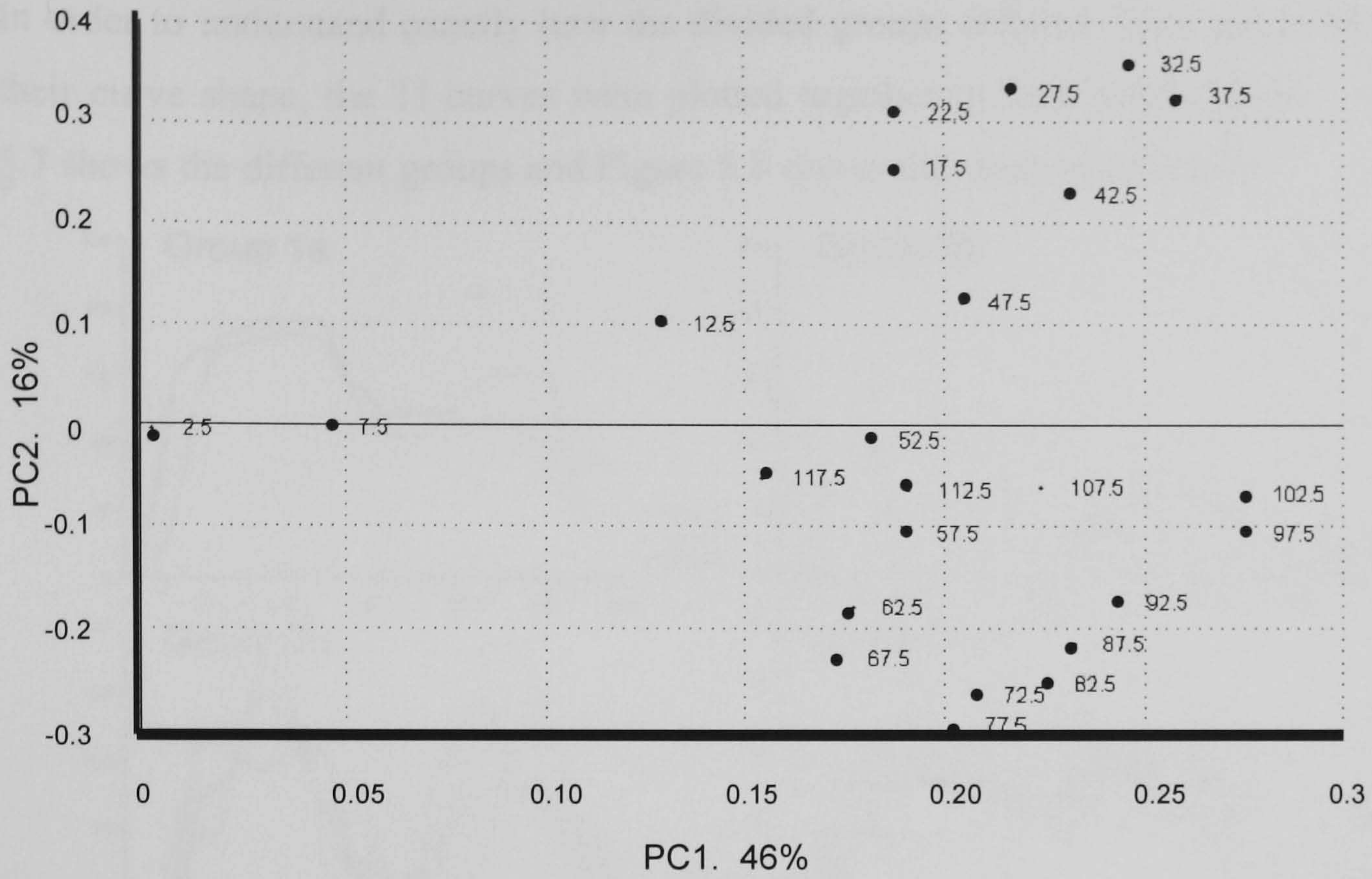


Figure 5.6a 2D scatter plot of loadings. X-Time points (seconds). Exhalation time intensity.

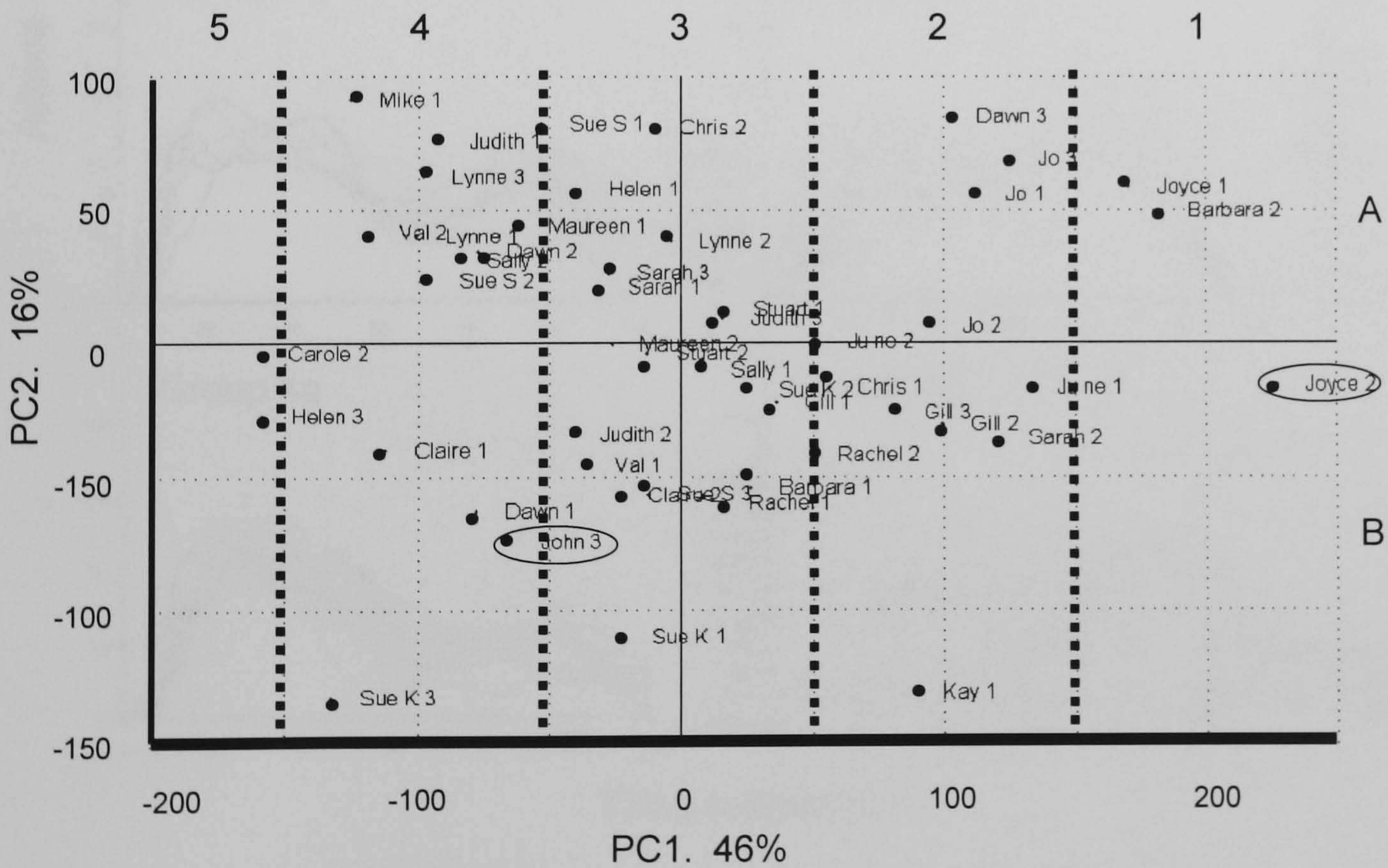


Figure 5.6b 2D scatter plot of scores. Assessors and repetitions. Exhalation Time Intensity data. Circled points excluded.

In order to understand exactly how the divided groups differed from one another in their curve shape, the TI curves were plotted together in their subdivisions. Figure 5.7 shows the different groups and Figure 5.8 shows the mean of the curves.

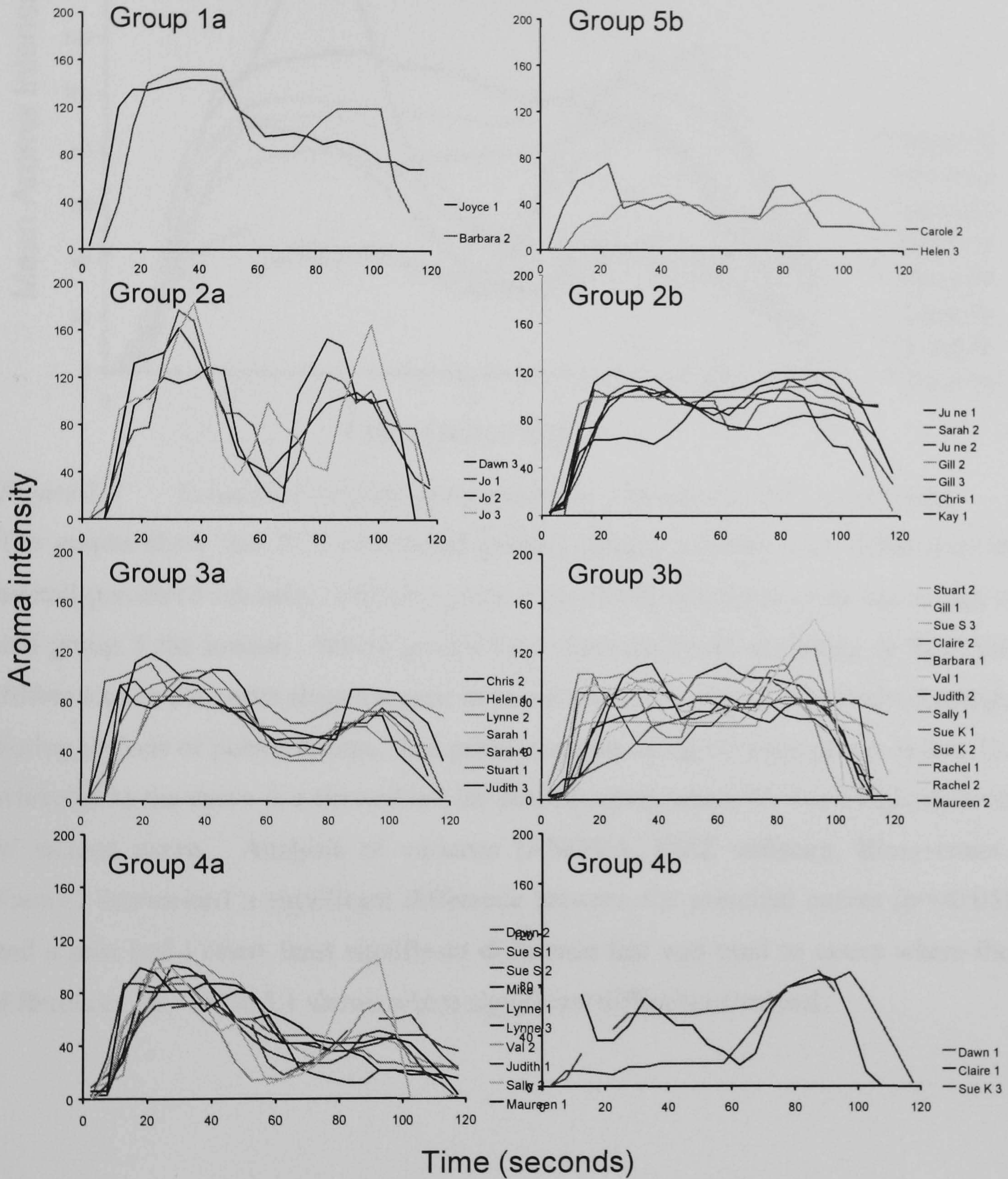


Figure 5.7 Exhalation sensory groups as formed from PCA scatter plot.

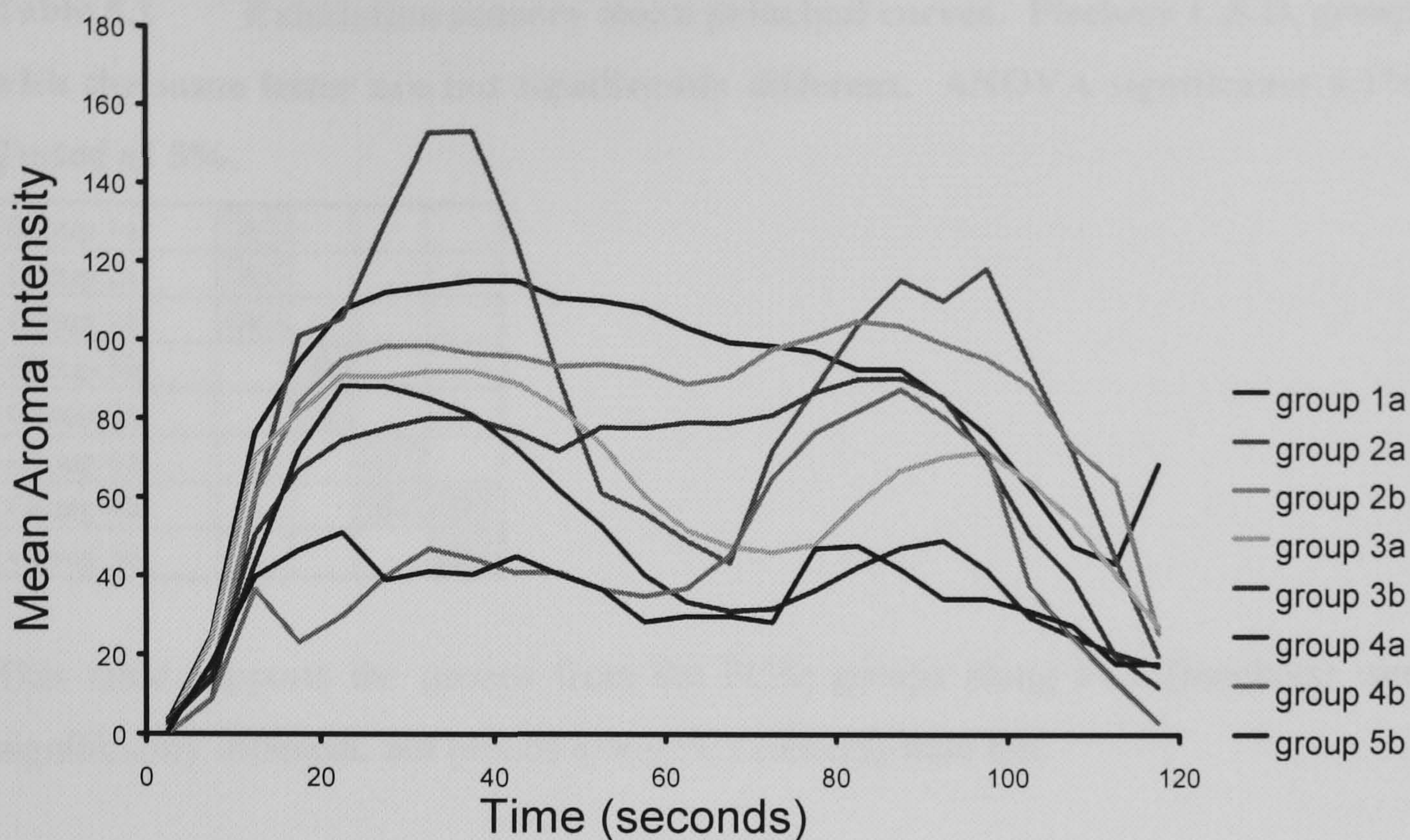


Figure 5.8 Exhalation sensory group means as formed from PCA scatter plot.

The graphs show that PC1 (numbered groups) mostly accounted for differences in overall perceived intensity, whereby group 1 perceived the aroma to be the strongest and group 5 the lowest. Where groups have been separated according to PC2, the differences in the curve shapes appear to be related to the degree of perceived trough during periods of pulsed aroma. The prime example being between group 3a and 3b, where in 3a the curve is a skewed left 'm' shaped curve, where 3b was a right skewed 'n' shaped curve. Analysis of variance (ANOVA, FIZZ software, Biosystemes, France) determined a significant difference between the principal curves ($p < 0.05$) and a post hoc Fishers least significant difference test was used to assess where the differences lie. Table 5.1 shows where significant differences existed.

Table 5.1 Exhalation sensory mean principal curves. Fischers L.S.D. groups with the same letter are not significantly different. ANOVA significance 0.1%. Tested at 5%.

Group 1a	A			
Group 2a	A			
Group 2b	A			
Group 3b		B		
Group 3a		B		
Group 4a			C	
Group 4b			C	D
Group 5b				D

This table supports the groups from the PCA; groups along PC1 (numbers) were significantly different, but groups along PC2 (letters) were not.

5.2.2.2 Comparing sensory principal curves to instrumental data

Using the same groups formed from the sensory principal component analysis, the mean instrumental (APCI-MS data) values for each group were calculated and then tested for significant differences. The mean curves were treated in the same way as the sensory and were subjected to ANOVA. Table 5.2 shows the result of this and where significant differences lay.

Table 5.2 Exhalation sensory groups as mean of instrumental heights. L.S.D. groups with the same letter are not significantly different. ANOVA significance 0.1%. Tested at 5%.

Group 5b	A					
Group 2a		B				
Group 3a			C			
Group 1a				D		
Group 4a				D	E	
Group 2b					E	
Group 3b					E	F
Group 4b						F

This result shows that even though the ANOVA model was highly significant, the actual differences between the group curves under their instrumental means were clearly different to the sensory. The model was significant because of differences in

the instrumental maximum levels as clearly seen in Figure 5.9, but the shapes were not obviously different. Figure 5.9 shows the sensory group curves according to the means of instrumental heights, and shows that in contrast to the sensory, the curves all had very similar shapes but differed mainly in overall magnitude.

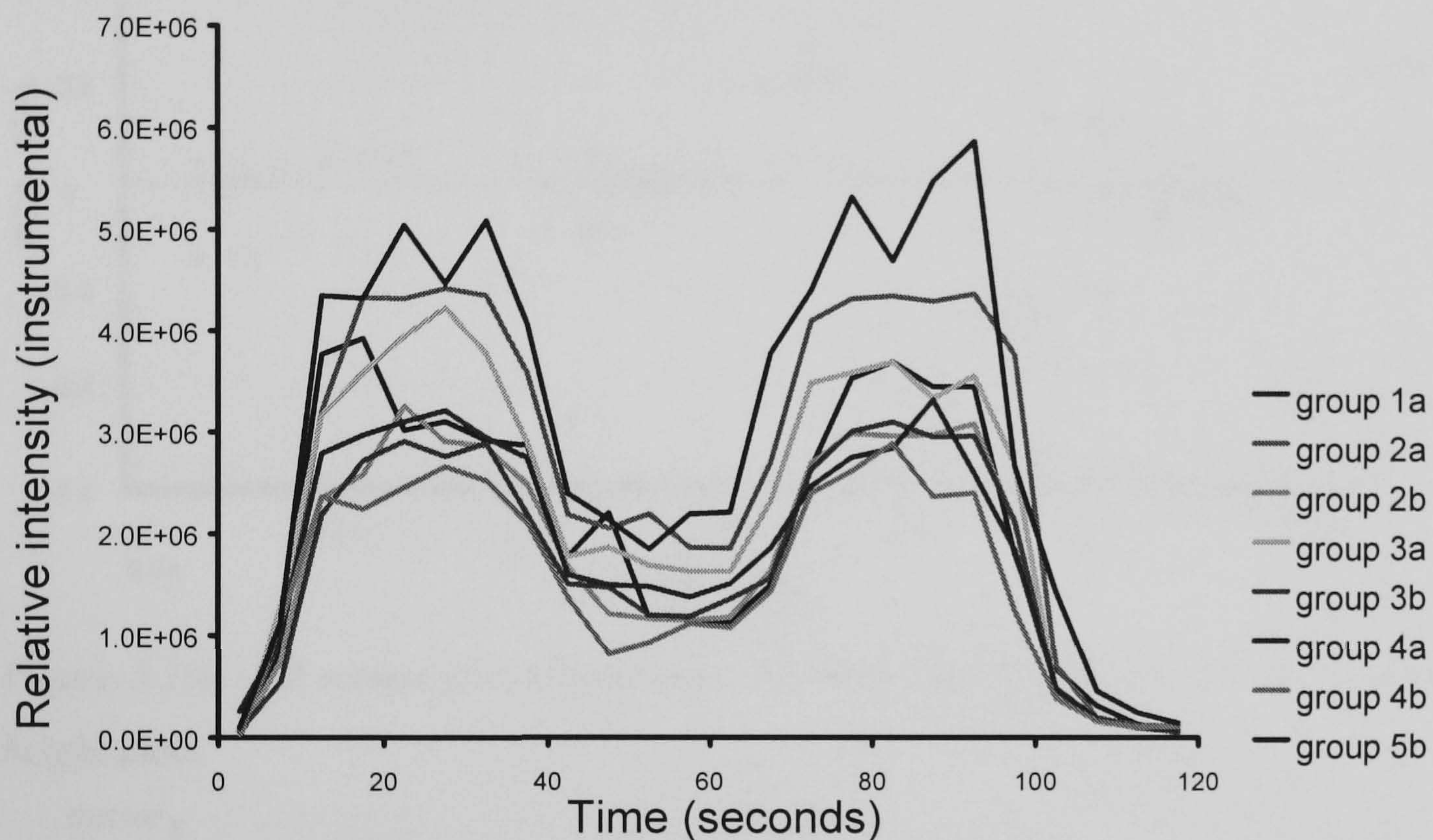


Figure 5.9 All exhalation sensory group mean instrumental height data.

The most striking difference between the sensory and the instrumental was that each pair of groups that differed by PC2 (Groups A and B) were shown to be significantly different, which is in contrast to Table 5.1. It is important to note that the same differences were found to exist when tested between groups using the instrumental areas instead of the heights. This is because the heights and the areas in exhalation measurement were highly correlated.

Figures 5.10a and b show the PCA of the instrumental heights and demonstrates time regions where the variation between assessors was differently explained to that in the TI. For example, instrumental PC1 (66%) and PC2 (10%) show more clustering of time points, although it is difficult to visually assess which curve events accounted for the majority of the variation between assessors. This is further shown in the plot of scores, where assessors are not evenly distributed, but clustered around the origin

with exception of a minority of assessors, who produced breath by breath data which explained most of the variation in the results.

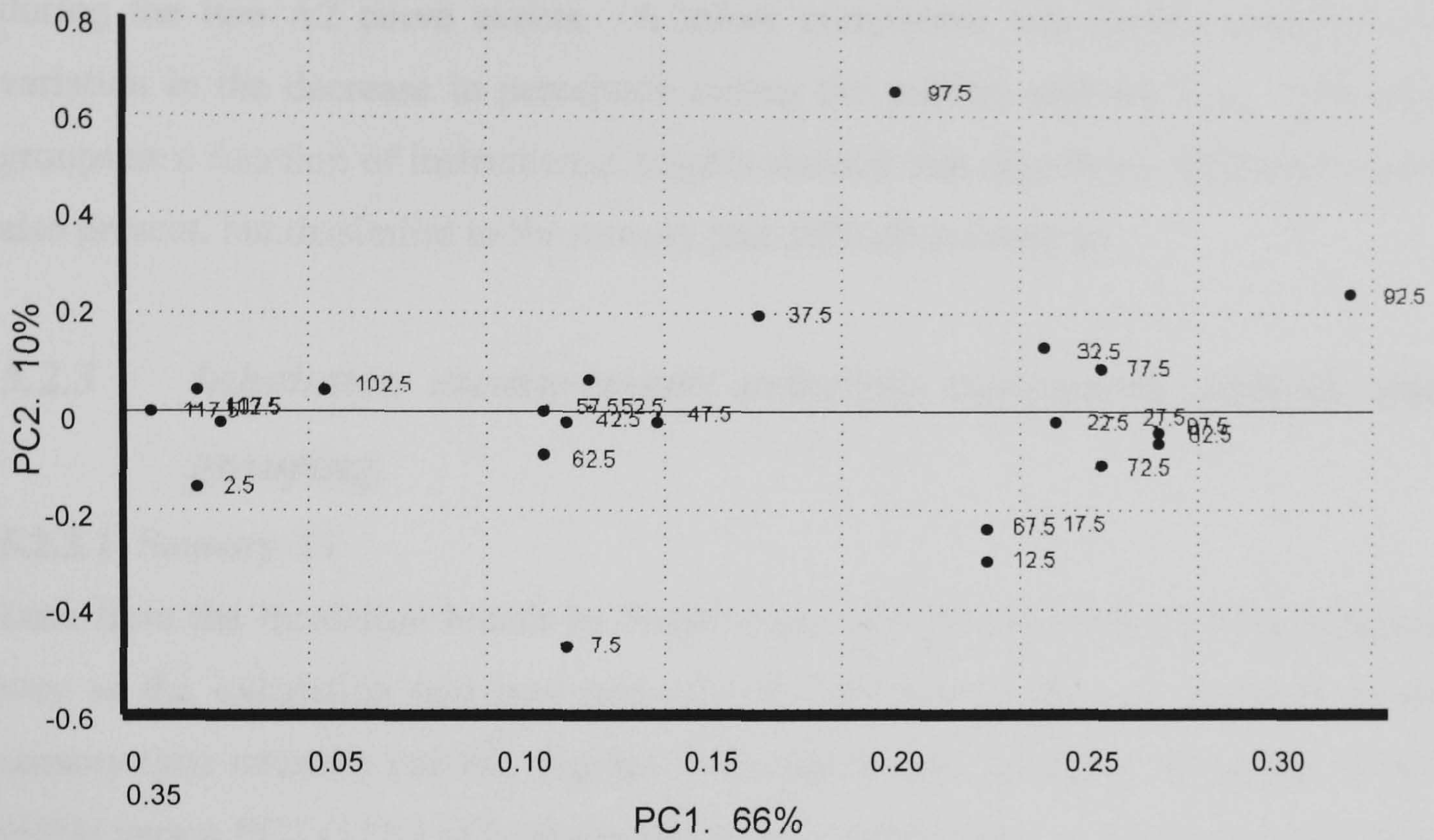


Figure 5.10a 2D scatter plot of loadings. X - Time points. Exhalation instrumental height data.

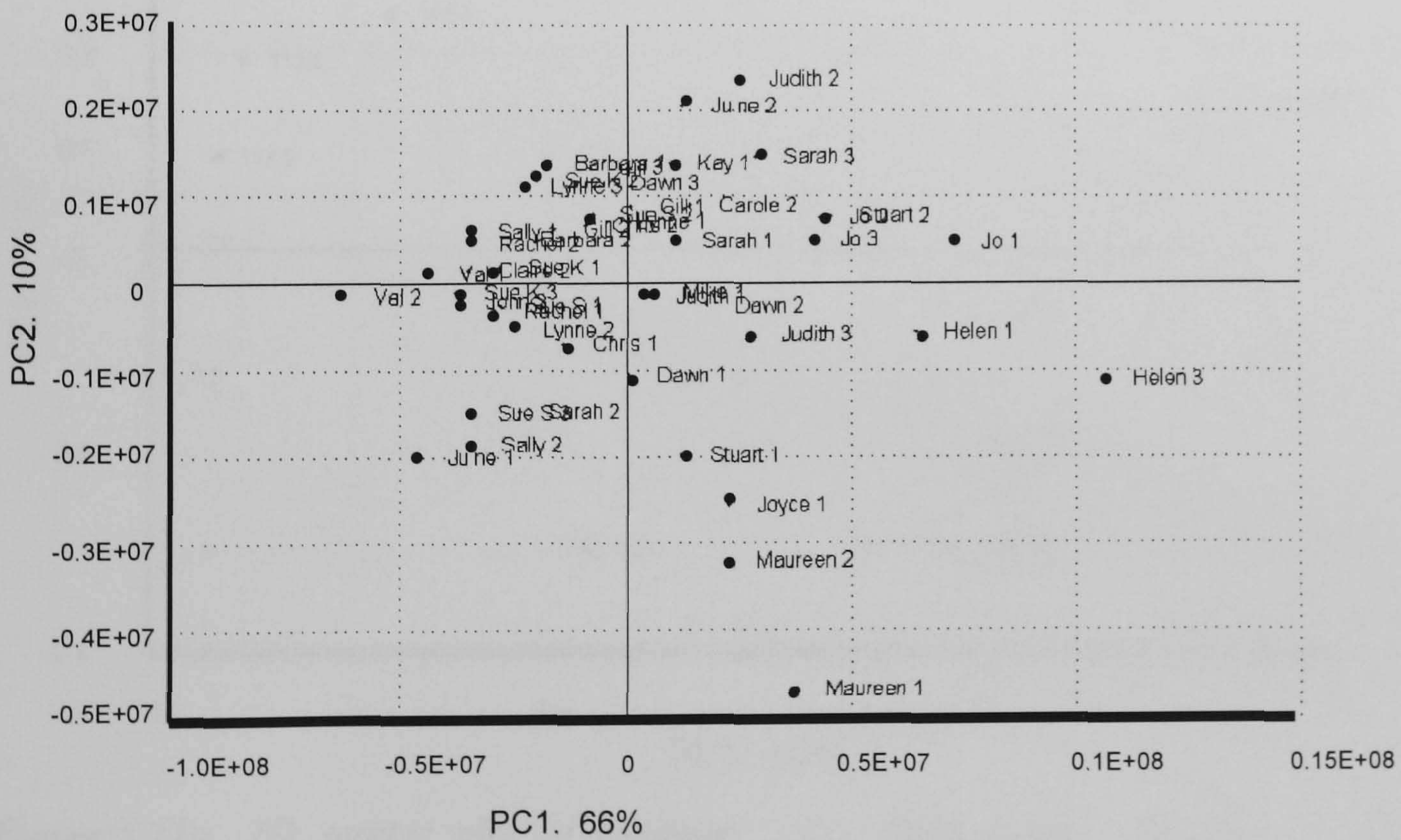


Figure 5.10b 2D scatter plot of scores. Assessors and reps. Exhalation instrumental height data.

In conclusion of the exhalation breath by breath analysis, the variation between assessors in their sensory perception was best explained by the maximum intensity during the two A2 curve events. A minor component was further explained by variation in the decrease in perception during the pulsing sections $C_{0.6}$. The same groups as a function of instrumental heights showed that significant differences were also present, but dissimilar to the sensory and difficult to interpret.

5.2.3 Inhalation measurement: principal component analysis and grouping.

5.2.3.1 Sensory TI

Data from the inhalation breath by breath measurement were treated in an identical way to the exhalation and was subjected to principal component analysis of the sensory time intensity curves. Figures 5.11a and b show the 2D scatter plots of PC1 (63%) versus PC2 (11%) of loadings and scores, respectively. Once again, PC3 (6%) yielded no further information about the data.

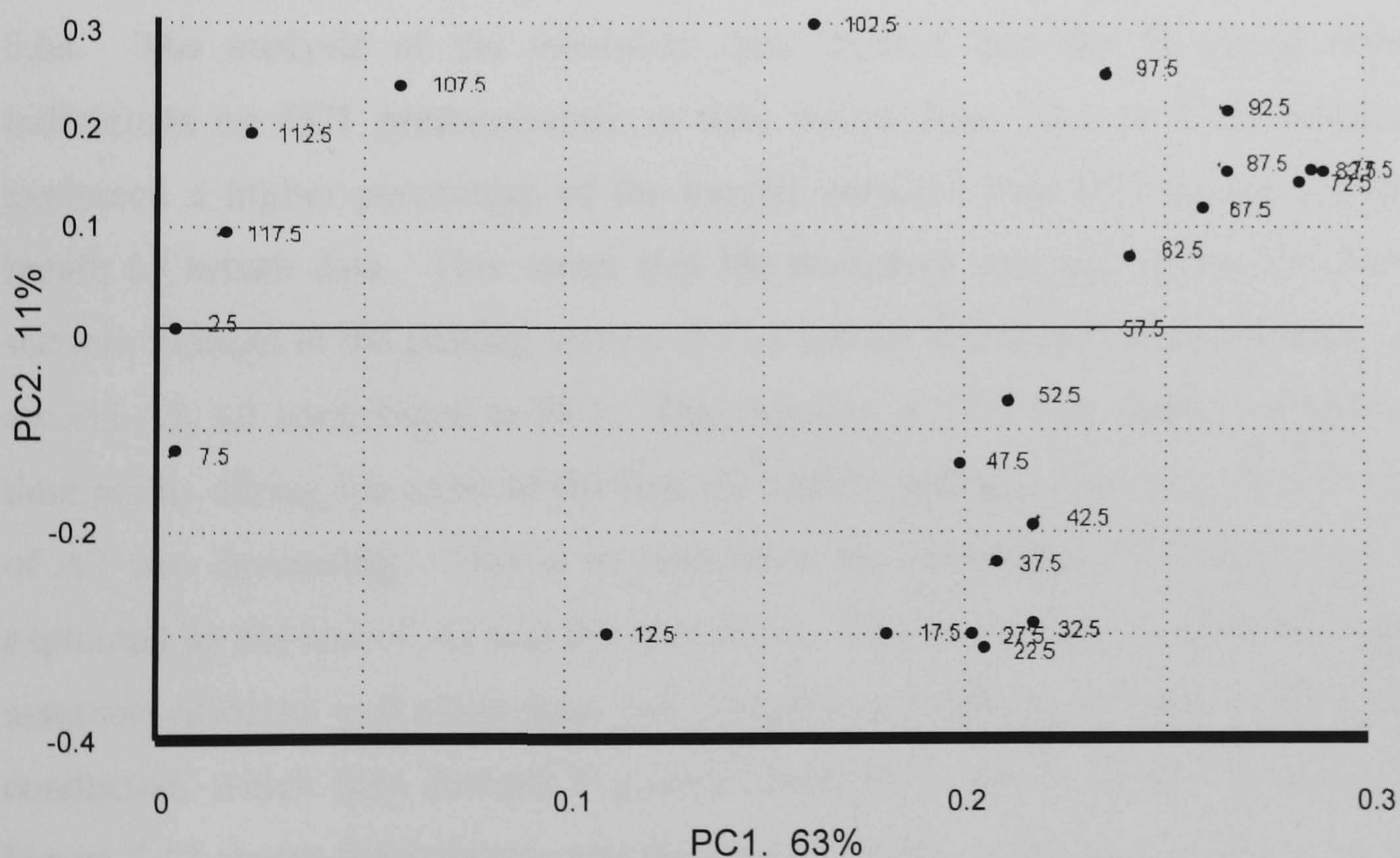


Figure 5.11a 2D scatter plot of loadings. X - Time points. Sensory TI data. Inhalation data.

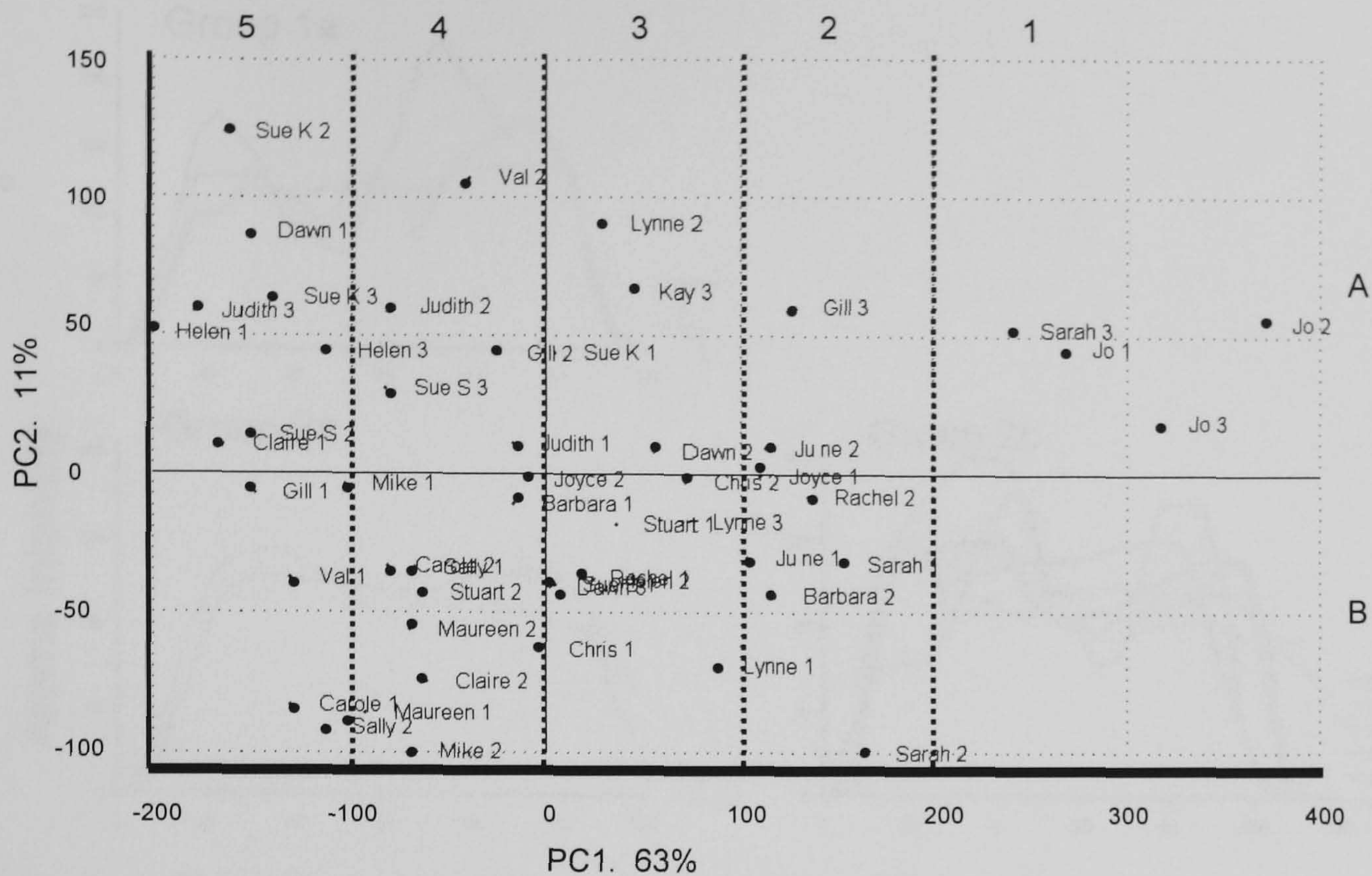


Figure 5.11b 2D scatter plot of scores. Assessors and reps. Sensory TI data. Inhalation.

It is interesting to note the differences between the plot in Figure 5.11a and that in 5.6a. The analysis of the inhalation data showed that the TI varied between individuals on PC1 predominantly at time points from 22.5 to 97.5, which also explained a higher percentage of the overall variation than PC1 of the exhalation breath by breath data. This meant that the maximum intensity during the first A2 section, through to the pulsing section of C_{0.6} and up to the maximum intensity of the second A2, all contributed to PC1. The variation in PC2 was mainly comprised of time points during the onset of the first A2 section and also when the second section of A2 was decreasing. This is in contrast to the exhalation data, which was best explained by the end of A1 and the start of A2. The plot of the scores shows that the assessors resolved well along these two components enabling a similar grouping to be conducted, which also formed 5 groups along PC1, and 2 (a and b) along PC2. Figure 5.12 shows these groups and their curve shapes, and Figure 5.13 shows group means plotted together.

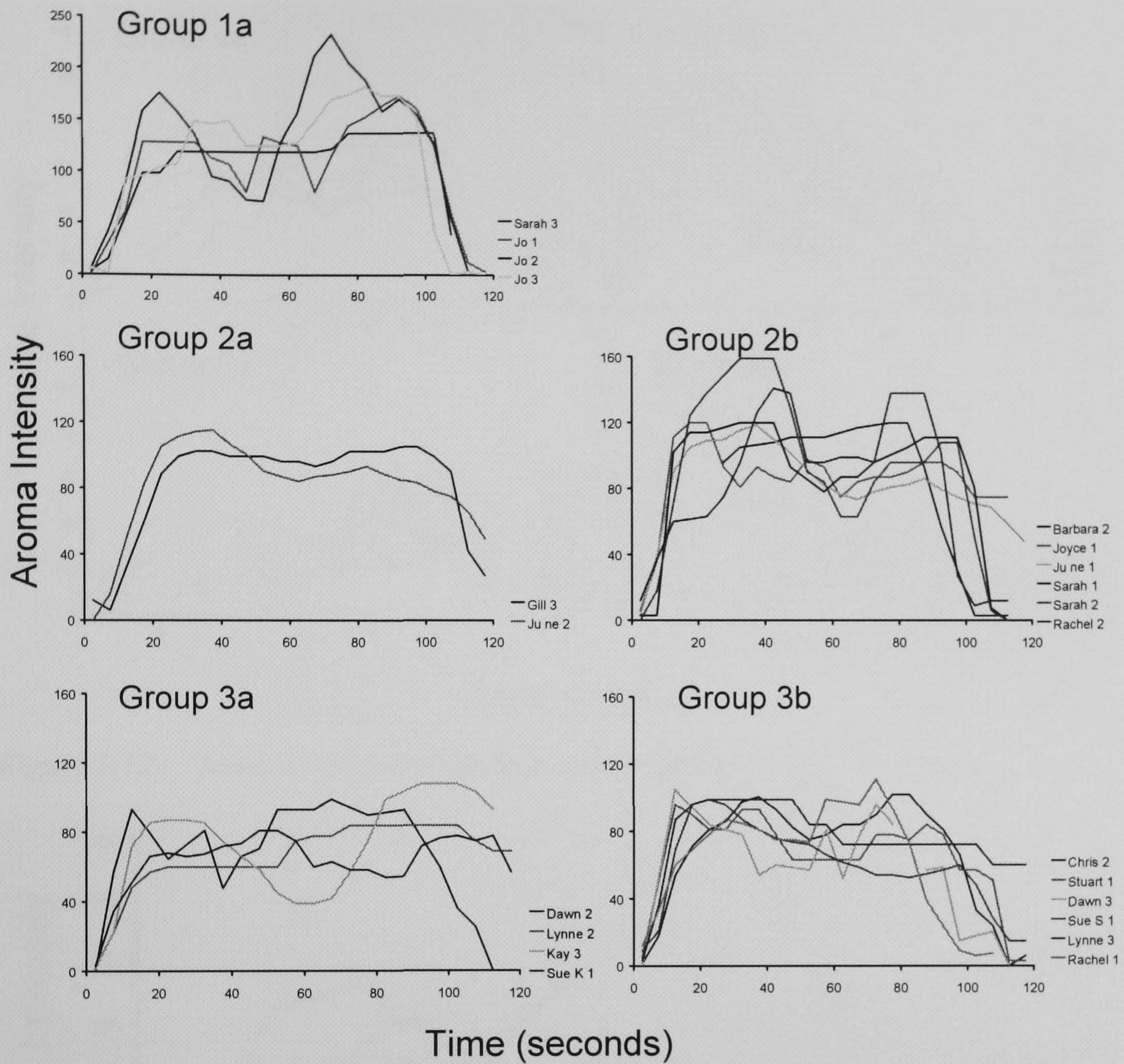


Figure 5.12 Inhalation sensory groups as formed from PCA scatter plot.

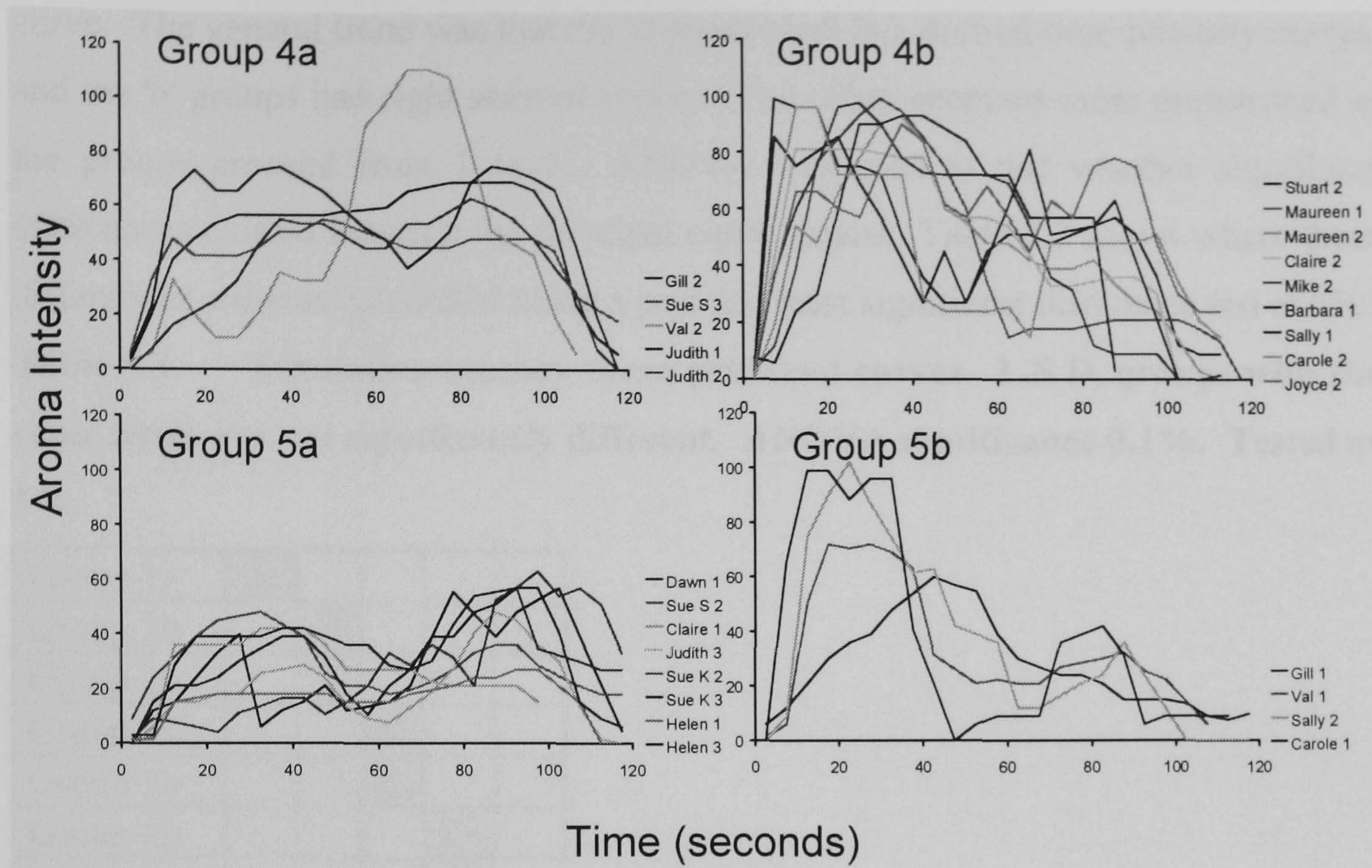


Figure 5.12 Inhalation sensory groups as formed from PCA scatter plot.

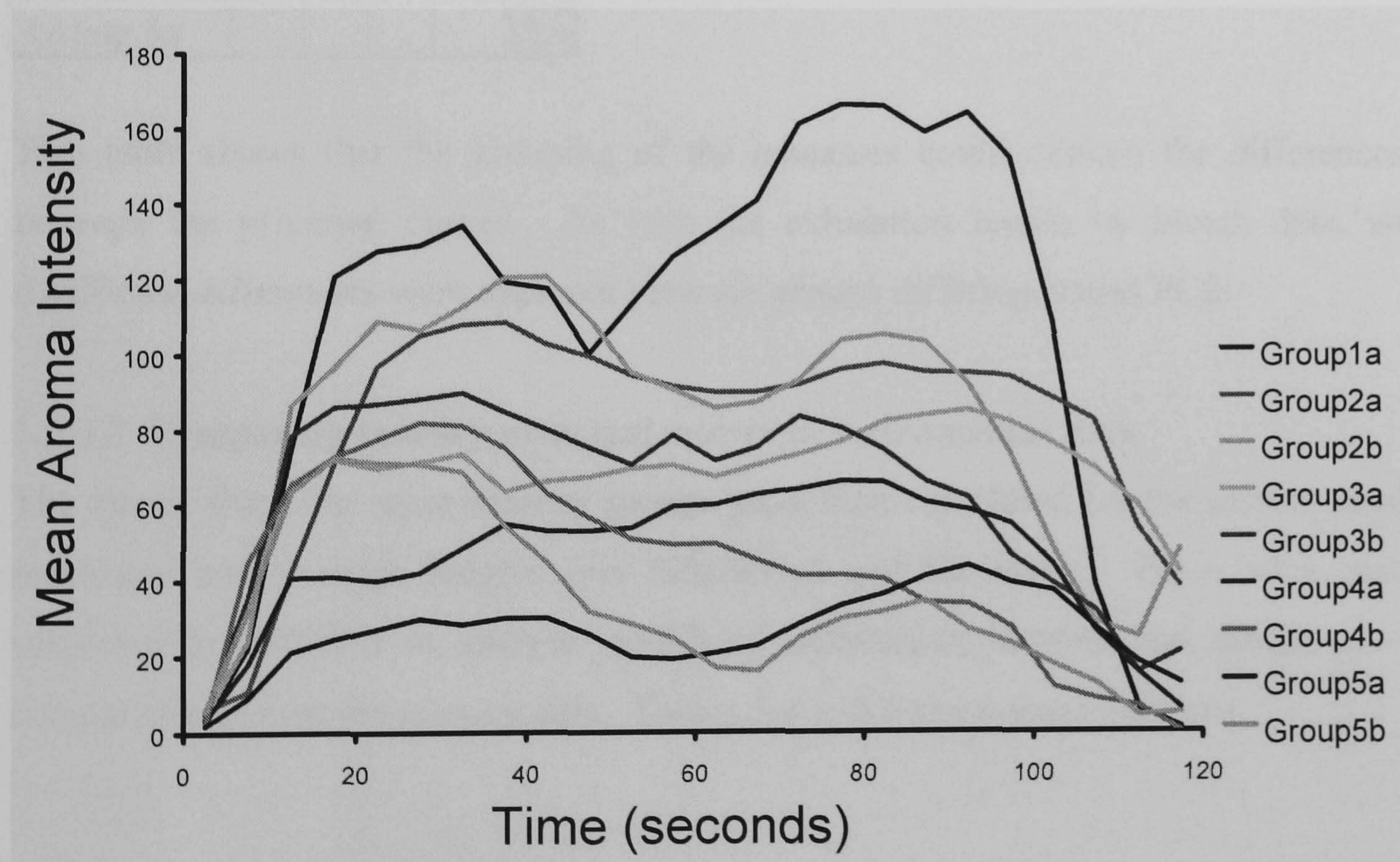


Figure 5.13 All inhalation sensory group means as formed from PCA scatter plot.

In a similar way to the exhalation TI group curves, the inhalation TI groups showed a decrease in the maximum intensities, where group 1 perceived the strongest intensities and group 5 the lowest. However, the main difference between the 'a' groups and 'b' groups appeared to be related to the direction of skew in the principal

curve. The general trend was that the 'a' groups had left skewed time intensity curves, and the 'b' groups had right skewed curves. The effect becomes more pronounced as the groups proceed from 1 to 5. ANOVA was used to test whether significant differences existed between the principal curve means. Table 5.3 shows where these differences arose as calculated using a post hoc least significant difference test at 5%.

Table 5.3 Inhalation sensory mean principal curves. L.S.D. groups with the same letter are not significantly different. ANOVA significance 0.1%. Tested at 5%.

Group 1a	A				
Group 2b		B			
Group 2a		B			
Group 3a			C		
Group 3b			C		
Group 4b				D	
Group 4a				D	
Group 5b					E
Group 5a					E

This table shows that the grouping of the assessors could explain the differences between the principal curves. As with the exhalation breath by breath data, no significant differences were apparent between groups differing across PC2.

5.2.3.2 Comparing sensory principal curves to instrumental data

The means from the same sensory groups were then calculated for the instrumental maximum and average heights (per inhalation) and the areas. These were also subjected to ANOVA to analyse significant differences between the groups as a comparison against the sensory data. Tables 5.4 to 5.6 show these analyses.

Table 5.4 Inhalation sensory groups as mean of instrumental maximum heights. L.S.D. groups with the same letter are not significantly different. ANOVA significance 0.1%. Tested at 5%.

Group 1a	A					
Group 4a		B				
Group 2b		B	C			
Group 5b		B	C			
Group 4b		B	C			
Group 2a			C	D		
Group 5a				D	E	
Group 3b					E	
Group 3a					E	

Table 5.5 Inhalation sensory groups as mean of instrumental heights per inhale. L.S.D. groups with the same letter are not significantly different. ANOVA significance 0.1%. Tested at 5%.

Group 1a	A					
Group 2b		B				
Group 4b		B	C			
Group 5b		B	C			
Group 4a			C			
Group 2a				D		
Group 3a				D		
Group 5a				D		
Group 3b				D		

Table 5.6 Inhalation sensory groups as mean of instrumental areas. L.S.D. groups with the same letter are not significantly different. ANOVA significance 0.1%. Tested at 5%.

Group 1a	A					
Group 2b	A					
Group 4b		B				
Group 5b		B				
Group 4a		B	C			
Group 3b			C			
Group 5a			C			
Group 3a			C			
Group 2a			C			

All three models were significant at the 0.1% level, but the actual differences between the groups according to the different instrumental parameters were again dissimilar to those between the sensory principal curves. Therefore, the PCA of the instrumental areas was calculated to observe how the data points were categorised, and whether any patterns could be seen. Figures 5.14a and b show the respective loadings and scores plots.

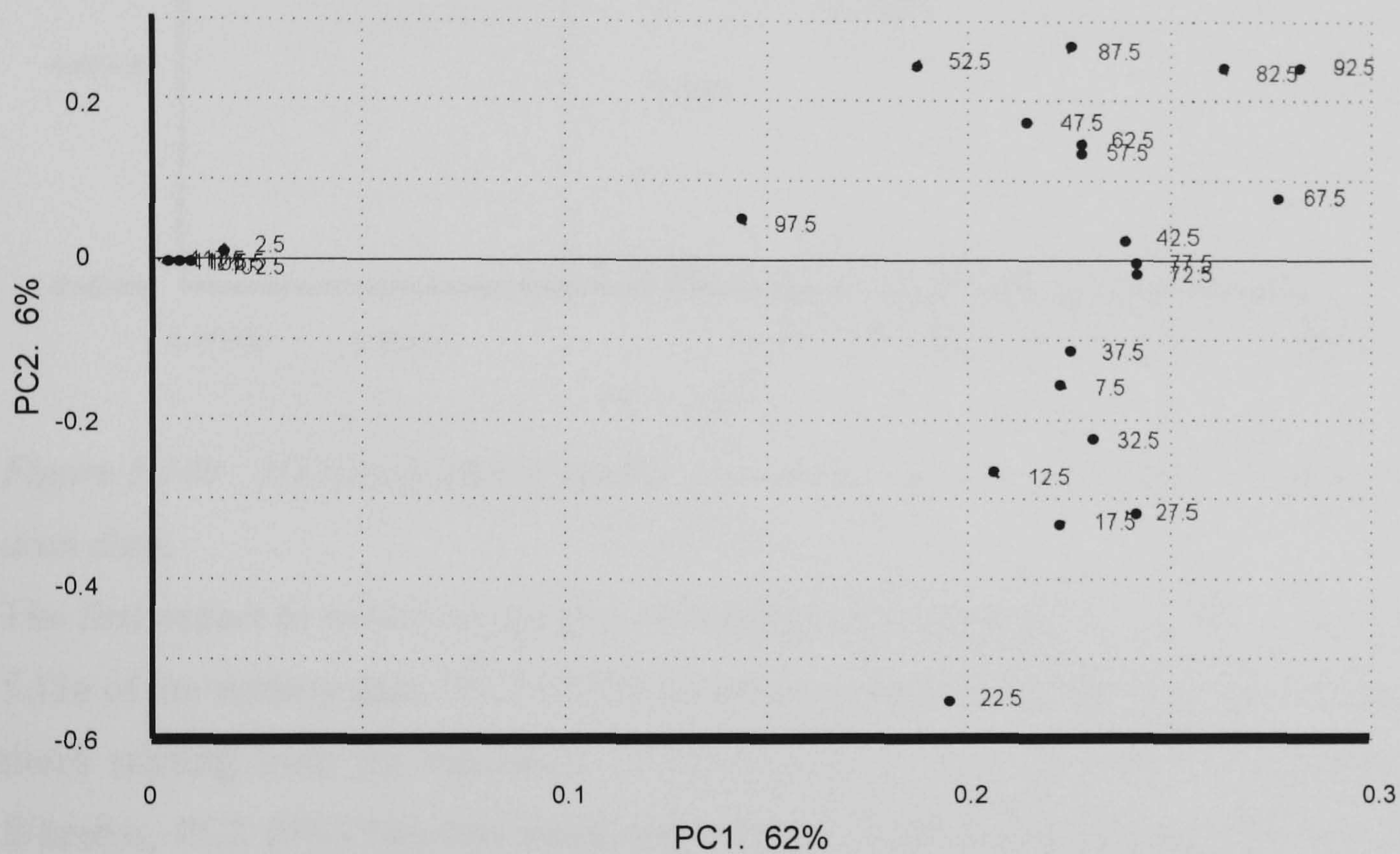


Figure 5.14a 2D scatter plot of loadings. X - time points. Inhalation instrumental area data.

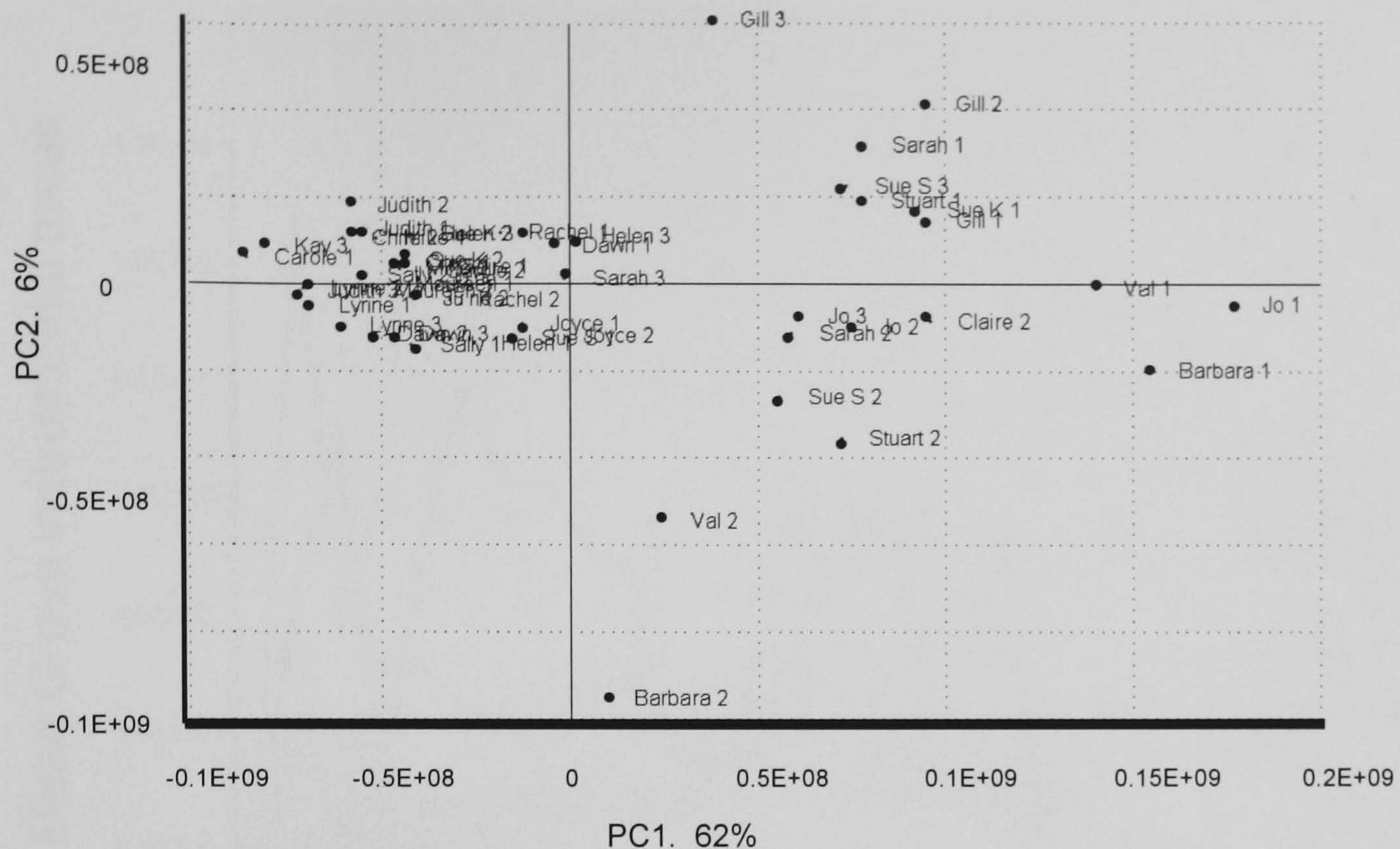


Figure 5.14b 2D scatter plot of scores. Assessors and reps. Inhalation instrumental area data.

The first aspect to notice on the plot of loadings is its similarity to the plot in Figure 5.11a of the sensory data. PC1 (62%) is mainly comprised of data points occurring at times starting from the maximum of the first A2 to the maximum of the second. Whereas, PC2 (6%) has two main contributions from points arising from first A2 onset and second A2 decay. The plot of scores is more difficult to visually assess as it does not discriminate between the assessors and their repetitions sufficiently, but there is a central cluster of individuals who do not significantly differ from one another. The remaining points show that the majority of the variation explained by the components was mainly attributed to a minority of assessors/reps. Assessors were grouped in a similar way to the exhalation data by setting arbitrary boundaries from the grid values as indicated on 5.11b. Figure 5.15 shows the means of the categorised groups in respect to the instrumental areas, and demonstrates further the poor resolution of the assessors along this measurement.

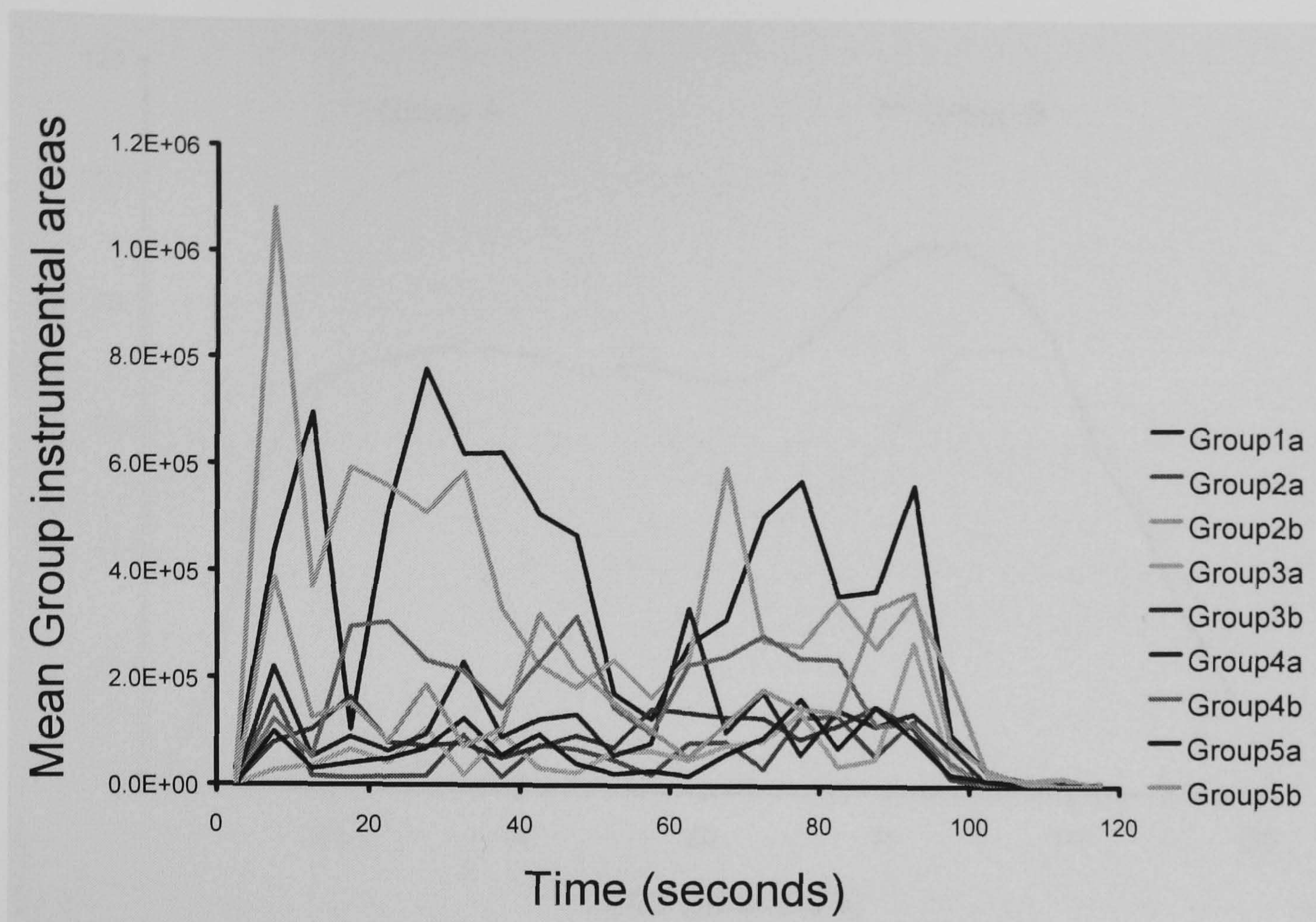


Figure 5.15 All inhalation sensory group mean instrumental area data.

This result showed that there was no apparent relationship between the sensory and instrumental variation in respect to the first principal component. However, across PC2 (minor source of variation) the assessors were well distributed and thus it was appropriate to subject the groups from this component in the same way as PC1. This would indicate whether the sensory and instrumental were related by a smaller source of variation.

5.2.4 Analysis of data sets according to the minor component (PC2)

In this analysis, both the exhalation and inhalation breath by breath data sets were once again categorised into groups using artificial boundaries (value zero of PC2 or X-axis) on the original scatter plots. However, this time the divide across PC2 was used to create just two sensory groupings: either A or B. It is first important to note that in both inhalation and exhalation sensory data sets, there were no significant differences between A and B groups. Nevertheless, the plots in Figures 5.16 and 5.17 show that in fact, two distinctly different curves were produced by groups A and B determined from each breath by breath measurement.

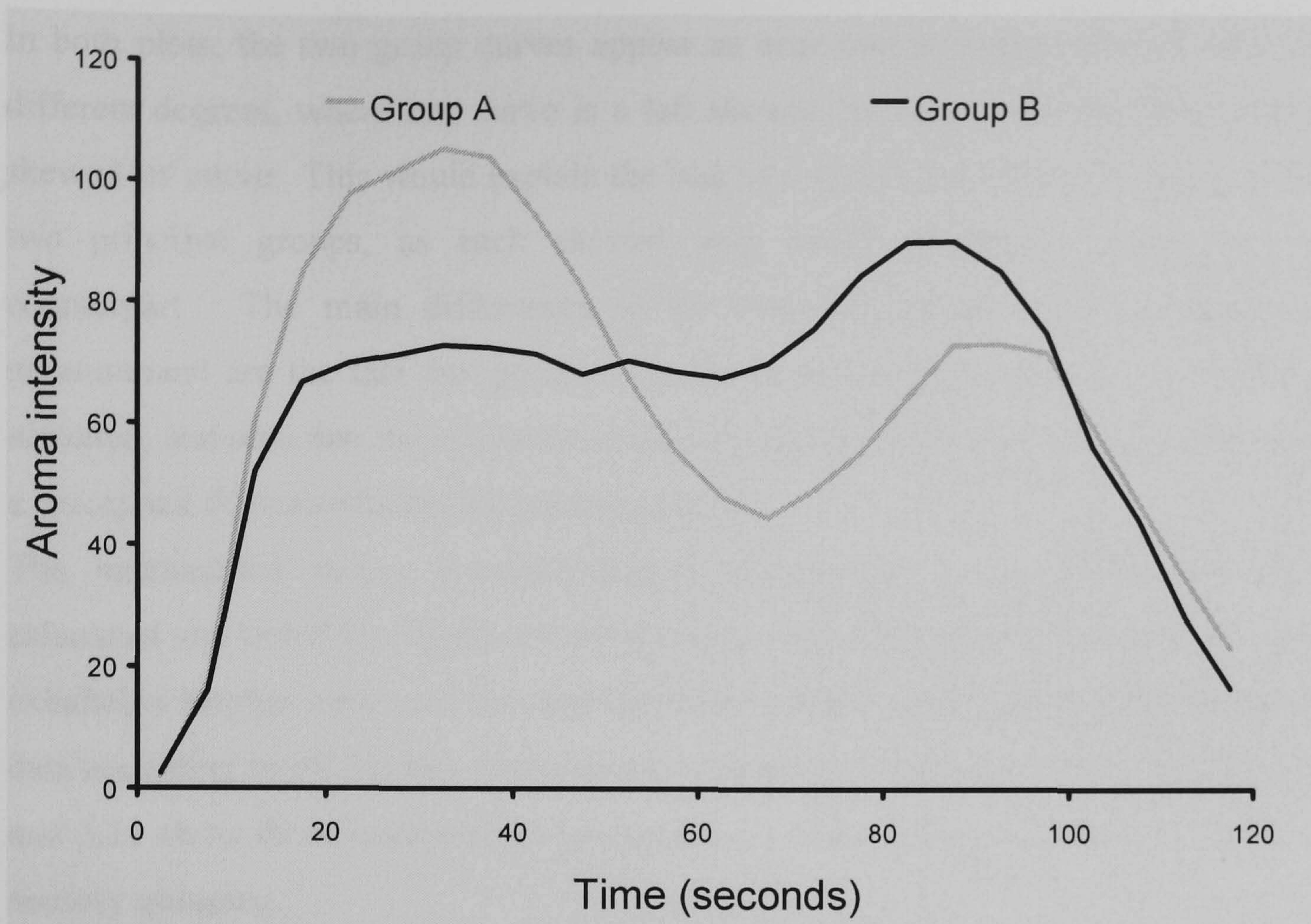


Figure 5.16 Sensory exhalation data. PC2 group curves of time intensity.

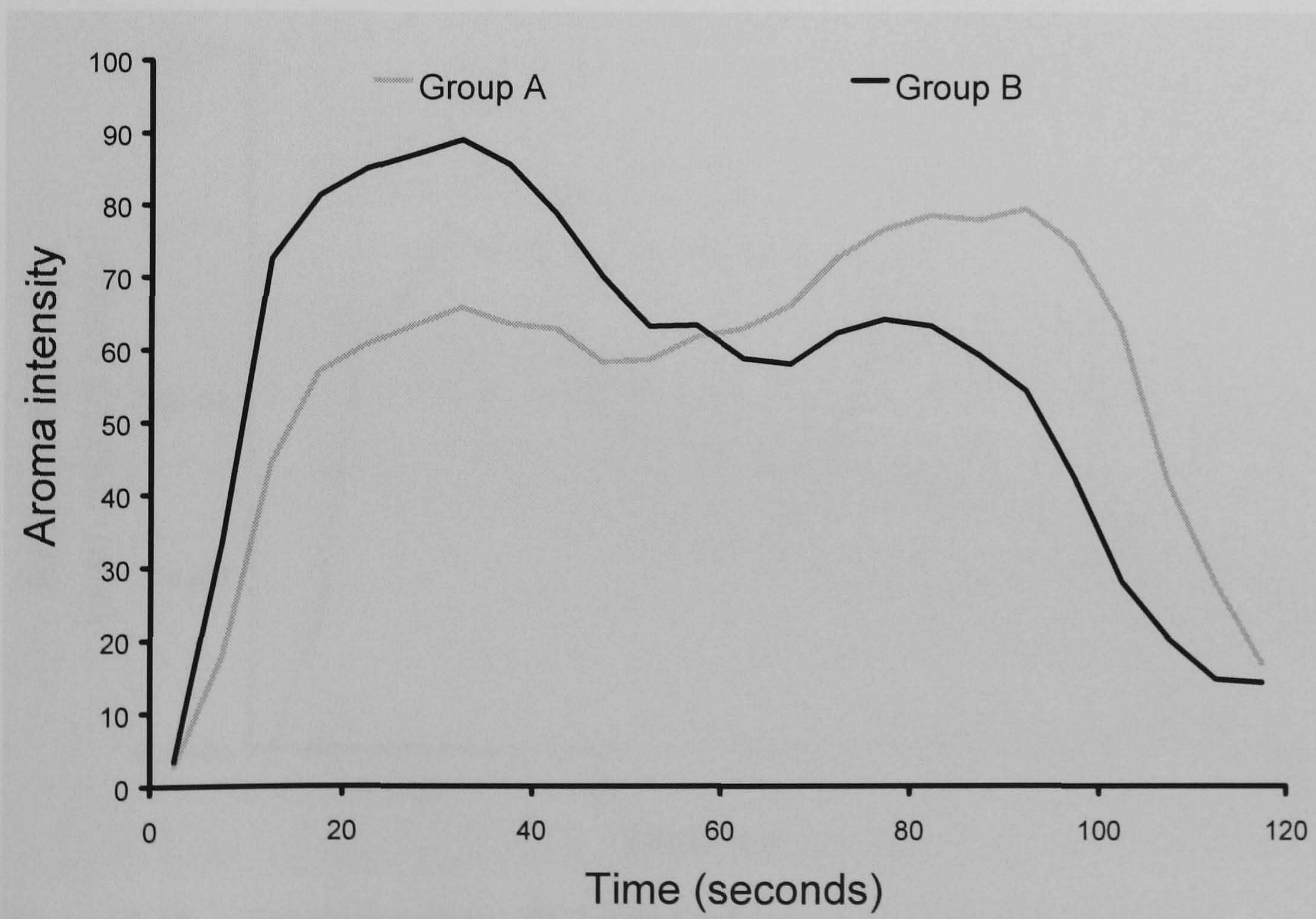


Figure 5.17 Sensory Inhalation data. PC2 curves of time intensity.

In both plots, the two group curves appear as near mirror images of each other to different degrees, where one curve is a left skewed 'm' curve, and the other a right skewed 'm' curve. This would explain the lack of a significant difference between the two principal groups, as each skewed side would essentially cancel out its counterpart. The main differences to be observed between the two types of measurement are the fact that groups with the same letter are skewed in a different direction, and also that the inhalation groups curves are both almost totally lacking in a perceptual decrease during the pulsing sections.

The instrumental means corresponding to these minor components were then calculated and tested for significant differences. In this instance, inhalation areas and exhalation heights were used because they both explained the highest variation in the data according to PC2 (other instrumental parameters plots not shown). Figure 5.18 and 5.19 show these instrumental parameters as mean curves according to the PC2 sensory grouping.

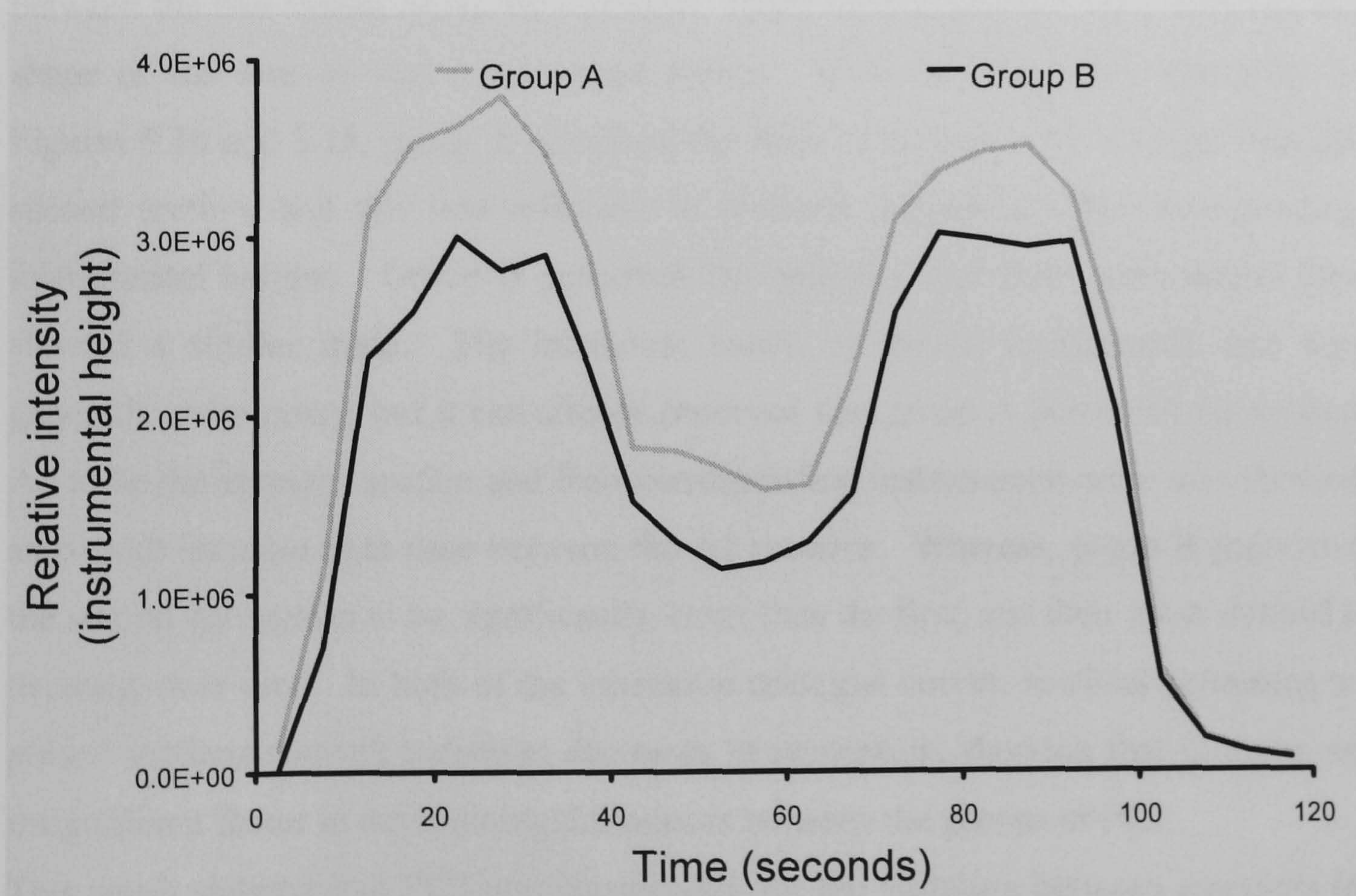


Figure 5.18 Exhalation data. PC2 principal curves of instrumental heights.

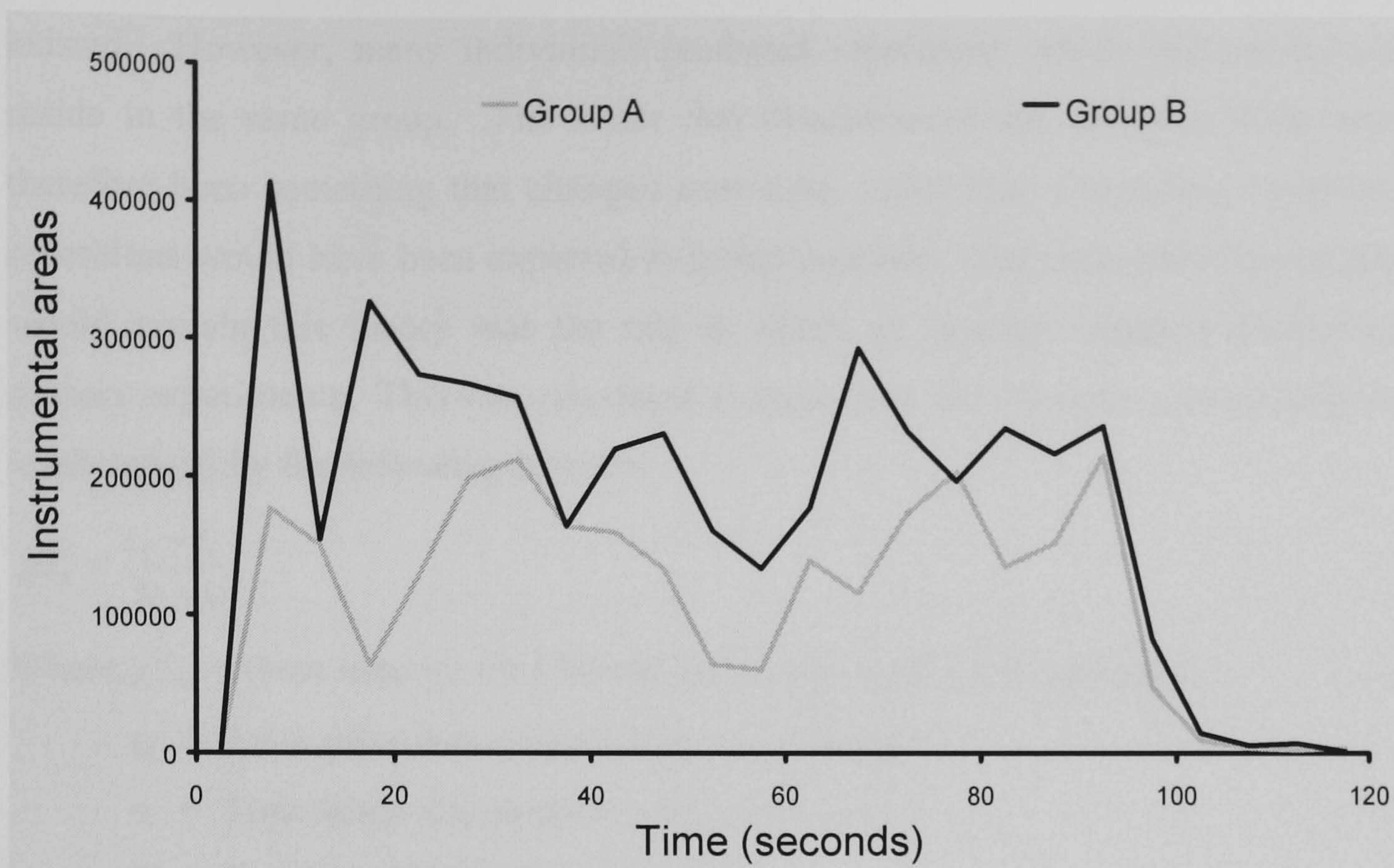


Figure 5.19 Inhalation data. PC2 principal curves of instrumental areas.

In both cases, a significant difference (5%, single factor ANOVA) was calculated between the two means of the PC2 groups. There were also similarities between the shape of the sensory and instrumental curves. With the exhalation data plots in Figures 5.16 and 5.18, group A perceived the first A2 section to be stronger than the second section, and this was reflected, to different degrees, by the corresponding instrumental heights. Group B perceived the opposite, and their instrumental data showed a similar trend. The inhalation breath by breath instrumental data was generally more noisy, but it can also be observed that group A perceived the second A2 to be the stronger section and their corresponding instrumental areas also showed an overall increase with time between the A2 sections. Whereas, group B perceived the second A2 section to be significantly lower than the first, and their areas showed a decrease over time. In both of the inhalation principal curves, sections pertaining to pulsed sections showed indistinct decreases in perception, showing that $C_{0.6}$ was an insignificant factor in determining differences between the groups of PC2.

This result showed that PC2 was accountable for the variation between assessors in their sensory response and the corresponding instrumental data. It could therefore be hypothesised that between these groups of individuals a physiological difference

existed. However, many individuals produced repetitions, which did not always reside in the same group. The factor that discriminates the assessors must have therefore been something that changed over time, rather than a constant, otherwise repetitions would have been expected to group together. One measurable factor that would explain this theory was the rate at which an assessor breathed during the sensory experiments. This was calculated as mean time for 1 breath cycle (exhalation + inhalation) by the following formula:

$$\chi T_b = \frac{t_L - t_1}{N - 1}$$

Where; χT_b = Mean time (s) for 1 breath cycle (exhalation plus inhalation)

t_L = Time when last acetone peak was recorded

t_1 = Time when first acetone peak was recorded

N = Number of acetone peaks

The mean breath cycle time was then calculated for each PC2 sensory group and then tested for significant differences. The bar chart in Figure 5.20 shows these means for the two types of breath by breath measurement.

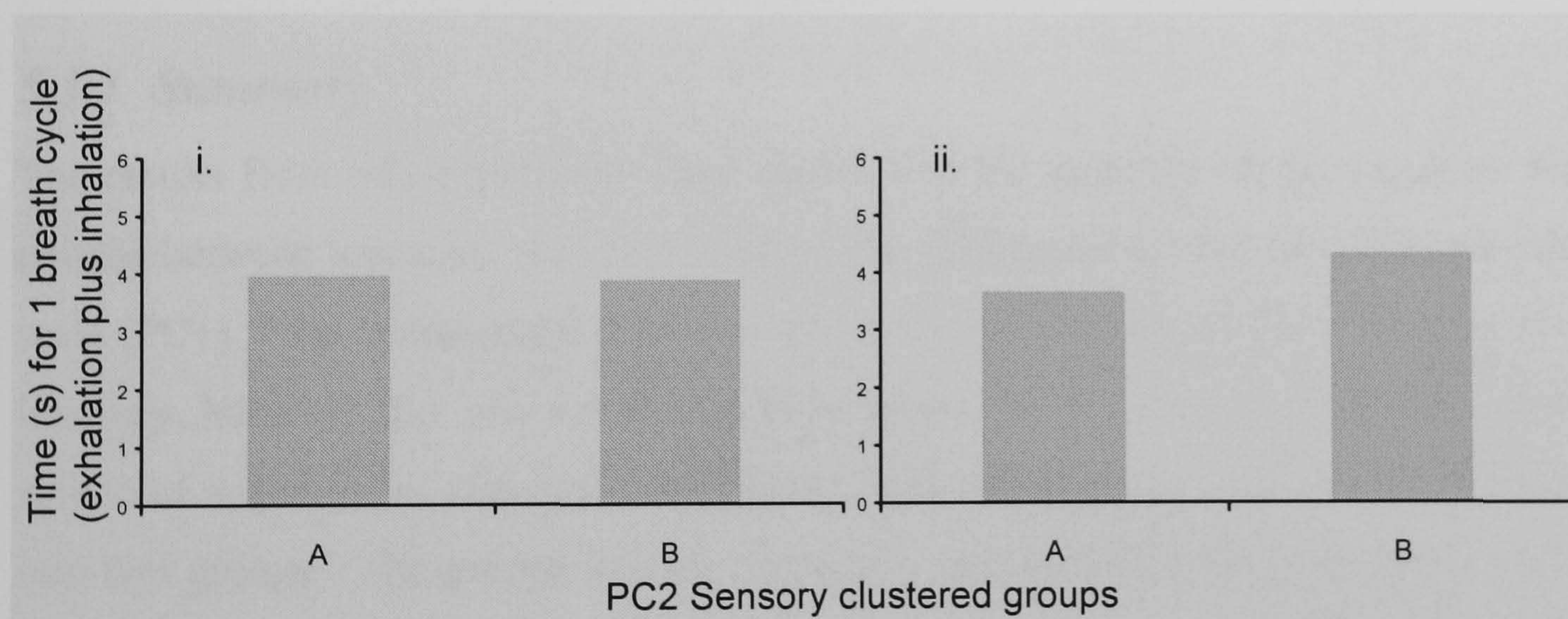


Figure 5.20 Mean breath cycle times for PC2 sensory groups. i. Exhalation data - not significantly different. ii. Inhalation data - significantly different (ANOVA 5%).

There was no significant difference in the breath cycle time between the groups in the exhalation data, but in the inhalation data it was shown that the assessors in group A breathed significantly faster than those in group B. The logical explanation for this connection between breathing rate, inhalation instrumental area and sensory response may be related to a person's subconscious behaviour when breathing on the APCI-

MS. When a person is asked to breathe on the APCI-MS, they have a tube inside their nostril, which might lead them to become more aware of their breathing than normal and consequently change their resting rate. However, over the course of a time intensity test, their breathing might drift back to the normal tidal resting rate as they become used to breathing on the instrument. As a hypothetical scenario the assessors in group A breathed significantly faster than those in group B. However, the values were based on the mean rates and gave no regards to changes in frequency over time. Therefore, they may have begun to breathe quickly at first and then slowed down to their respective tidal rates. Inhalation measurement has been shown to be affected by breathing rates and velocities (see chapter 6), where the height and area decrease with increasing breath rate and velocity. This theory would mean that over time the instrumental measurements from group A would show an increase in response to this respiration decrease.

5.3 DISCUSSION

5.3.1 Summary

The results from this experiment have shown that the majority of the variation that existed between assessors was explained by the differences in the way they used the scale (PC1). The instrumental data also showed large variation in the overall relative intensity, however this was not shown to be related to the sensory data. The minor source of variation in principal component 2 divided assessors and their repetitions into two groups. The groups in both exhalation and inhalation measurement mainly differed in the direction of the skew on their time intensity curves. Where one group had perceived the first A2 maximum to be stronger than the second, the other group had perceived the opposite. The instrumental data supported these TI curve differences, but due to the low amount of explained variation, the result cannot be considered conclusive.

In comparison of the two different breath by breath methods, exhalation measurement was more stable than the inhalation and far easier to extract the appropriate

parameters. However, the exhalation method produced very similar shaped instrumental curves between people, whereas the inhalation results were much more varied. In particular, inhalation was more affected by the rate at which an assessor breathed during an experiment. To the extent, that a minor link was shown between breathing rate, instrumental area and sensory response. Inhalation measurement was perhaps more suitable than exhalation measurement when attempting to simulate the process of retronasal olfaction because it captured the dynamic profiles of aroma compounds before they reached the nasal epithelium. Therefore, with respect to real time events, inhalation breath by breath approximation of olfaction would probably be closer to the actual event of perception than exhalation. This would be theoretically difficult to test, and the process speed of the mass spectrometer would become a limiting factor.

Overall, when a single distal stimulus was presented to assessors it becomes disrupted in infinite ways. This experiment has shown that a small amount of variation in breath by breath measurement between people explained why they might differ in their sensory response. This idea can be applied to descriptive panels who have consensus problems, where the issues may not necessarily be solely due to psychological error or training issues, but simply that the single product stimulus is prone to a high degree of variation between assessors once consumed.

5.3.2 Comparison of time intensity and paired comparison experiment

Chapter 4 showed that when assessors were given identical paired comparison tests, the individual ways in which they disrupted the stimuli by inhalation breath by breath measurement had an effect on their choice of perceived stronger aroma stream. This was only observed when a constant concentration stimulus was presented against a pulsed (A2 versus C_{0.6}), and was not significant when the same aroma stream was presented in a test (A2 versus A2, C_{0.6} versus C_{0.6}). Time intensity allowed these comparisons between constant and pulsed to be quantitatively and dynamically made, but the most important correlation between the sensory and instrumental data was the perceived difference between the two periods of constant concentration (A2 versus A2). In contrast, the perceived difference between the pulsed section and the constant

concentration was not significantly to the instrumental data (A2 versus C_{0.6}). The two types of experiment would therefore not appear to compliment each other in their respective conclusions. However, a discrimination test requires the subject to compare one set of information against another, whereas time intensity requires the subject to permanently compare a random points in time to previous ones (Overbosch, 1986; Hollowood, *et al.*, 2004). Therefore, a comparison between the two experimental methods may be unproductive.

5.3.3 Relevance to previous and current work

The original idea behind this work was to test whether pulsed stimuli are perceived as different or the same by assessors. Part of this work related to previous findings by authors investigating the application of electroencephalographic techniques in odour research (for a review see section 1.2.4). The research into chemosensory event-related potentials (CSERP) has frequently used olfactometer pulsing technology to relate stimulus strength and frequency to the actual electrophysiological response of the brain. The majority of the research is based on method development, in particular the breathing technique used. Kobal and co-workers pioneered the velopharyngeal closure breathing method, which uses an oral breathing method technique that minimises the disruption of an orthonasally delivered aroma (Kobal and Kettenmann, 2000). However, further testing has not absolutely confirmed whether oral breathing was more effective in such experiments compared to nasal breathing. The main example being that oral breathing gave higher CSERP amplitudes, but that could have been due to the unexpected and abnormal nature of the odour perception in this technique (Pause, *et al.*, 1999; Thesen and Murphy, 2001). The most relevant work also looked at the relationship between pulsed stimuli and continuous stimuli by presenting pulses in increasing frequency in order to calculate the time constant for adaptation when there was no perceived gap between the pulses. This time constant was shown to be primarily related to the onset of cognitive adaptation rather than the central process of habituation, which continues to relay information to the brain long after the former has ceased (Tateyama, *et al.*, 1998; Wang, *et al.*, 2002). The two experiments I conducted into the perception of pulsed odourants have shown the

importance of establishing an effective method to measure the actual concentration after its disruption. This relates to the current CSERP research by asking: how can the authors know what concentration was actually delivered to the assessor by their olfactometer? Other researchers also measured the latency of the signal and correlated it to nasal sensitivity, but again that would have required an estimation of when the stimulus reached the assessor rather than an actual measurement. Even with the breathing constraint techniques used in such studies, they would not be able to predict how their stimuli altered on their path from olfactometer outlet source to nasal epithelium. Perhaps the CSERP researchers would benefit from the employment of an APCI-MS to compliment their results. My research could not test whether pulsed stimuli near the flicker fusion frequency were perceived as the same or different to constant stimuli, but it could explain why this was difficult to test.

The other relevant areas of research to mention are the studies of instrumental and sensory correlation. Time intensity has been used with simultaneous volatile in nose release studies to study; how different fat contents affect flavour release and subsequent perception (Brauss, *et al.*, 1999; Roberts, *et al.*, 2003; Charles, *et al.*, 2000), rate of flavour release from models gels and perception (Baek, *et al.*, 1999; Linforth, *et al.*, 1999) and modelling of adaptation functions (Overbosch and Dejong, 1989; Overbosch, 1986; Overbosch, *et al.*, 1986; Hollowood, *et al.*, 2004). The majority of the work uses a parameter extraction method on the TI curves, yielding measurements such as Time to reach maximum intensity T_{max} etc. These can then be correlated to the corresponding measurement as extracted from the instrumental data. This method does not attempt to explain the variation between people, but generally compensates for their differences instead. Conversely, the TI research pertaining to understanding fundamental psychophysical functions does account for the variation between people and also correlates the response in respect to time. The technique was highly successful in relating an individual's response to the stimulus as their sensitivity changed in accordance with their adaptation, but showed unexplainable issues concerning time lag between the aroma release and the TI data. This type of modelling with respect to time was not attempted in my study mainly due to the

difficulties in deciphering curves with such irregular shapes (i.e. predominantly 'm' shaped), which would have required the employment of highly advanced mathematical techniques that were beyond my capabilities. It might be worth considering a collaborated project with mathematicians in future work of this type.

6 PHYSICAL DISRUPTION OF THE AROMA STIMULUS

It was shown in chapters 4 and 5 that the physical disruption of an aroma stimulus can impact on the subsequent perception. However, these chapters have mainly focussed on the variation between people as a function of their breathing for orthonasally delivered stimuli, whereas there are also other physiological aspects in retronasal delivery that lead to individual differences. Two of the main mechanical aspects include the act of chewing and swallowing, both of which have been shown to actively affect the aroma concentration in the nose. ElectroMyoGraphical (EMG) analysis of subjects gave information on chewing frequency which correlated well with the nasal air fluctuations of the volatile laden air, thereby indicating that the air was pumped from the mouth into the nasal pharynx as the subject exhaled (Hodgson, *et al.*, 2003). This transport could be dramatically increased during swallowing events, where the nasal airflow temporarily ceased during a swallow and was then followed by a sharp pulse of aroma in the breath (Hodgson, *et al.*, 2004). Other researchers have suggested that swallowing events alone are important for retronasal perception, because the mouth is essentially a ‘closed’ system at any other time points (Buettner and Schieberle, 2000b; Buettner and Schieberle, 2000a). They suggest that the reason behind the prolonged retronasal perception, despite the apparent intermittency of aroma delivery, is due to the adsorption of the odorant to the food matrix and the oral mucosa (Buettner, *et al.*, 2002). However as discussed previously, the state of the velum depends on the nature of the food and the individual and it is therefore difficult to generalise. The effect of modelled air flow on olfactory ability has been described by many researchers and is discussed in chapter 1.

The aim of this chapter was not to prove or disprove the aforementioned hypotheses, but rather to use some of the techniques to demonstrate the types of variation associated with measurements of chewing and swallowing. It also investigated the theory behind the conclusion in chapter 5, where it was indicated that the perception

of inhaled aroma was related to the instrumental data, which in turn was partly a consequence of the breathing rate. This was investigated using measurements of breath and nitrogen gas flow with simultaneous online monitoring of volatiles in exhaled breath.

The theoretical disruption of an aroma stimulus when presented retronasally was also simulated. The pulsing olfactometer emission tube was presented to the mouth. Individuals were asked to either chew non-flavoured gum to establish a regular chewing pattern or instigate swallowing actions according to a set protocol. The subsequent exhalations of the disrupted aroma were measured using APCI-MS. Chewing and swallowing were monitored by EMG and laryngography, respectively (Brown, 1994; Firmin, *et al.*, 1999).

6.1 METHODS AND MATERIALS

Breath flow, mastication and swallowing were measured according to methods developed by Hodgson *et al.* (2003)

6.1.1 Breath volatile measurement

See section 4.1.2.1 of chapter 4.

6.1.2 Breath flow measurement

The rate and volume of the nasal airflow was monitored using a digital volume transducer (World Precision Instruments, Stevenage, UK), suitable for flow rates up to 3 L.sec⁻¹. It consists of an opto-electronic pick up assembly contained within a small cartridge. The cartridge was placed in-line with the nitrogen flow or the breath flow as depicted in Figure 6.1. The air movement associated with inhalations and exhalations caused an ultra light weight impeller blade to spin, in one direction for exhalations and the opposite for inhalations. As the impeller rotated, it sequentially interrupted light beams, generated by light emitting diodes and these interruptions were detected by photo transistors. These signals were input into a 1401 Mk II (Cambridge Electronic Design (CED), Cambridge, UK) Analogue to Digital

Converter (ADC), and then processed using computer software package (Spike 2 from CED), specifically designed to handle physiological data.

The olfactometer output programme employed was A2 versus $C_{0.6}$, which is described in chapter 4; Figure 4.7.

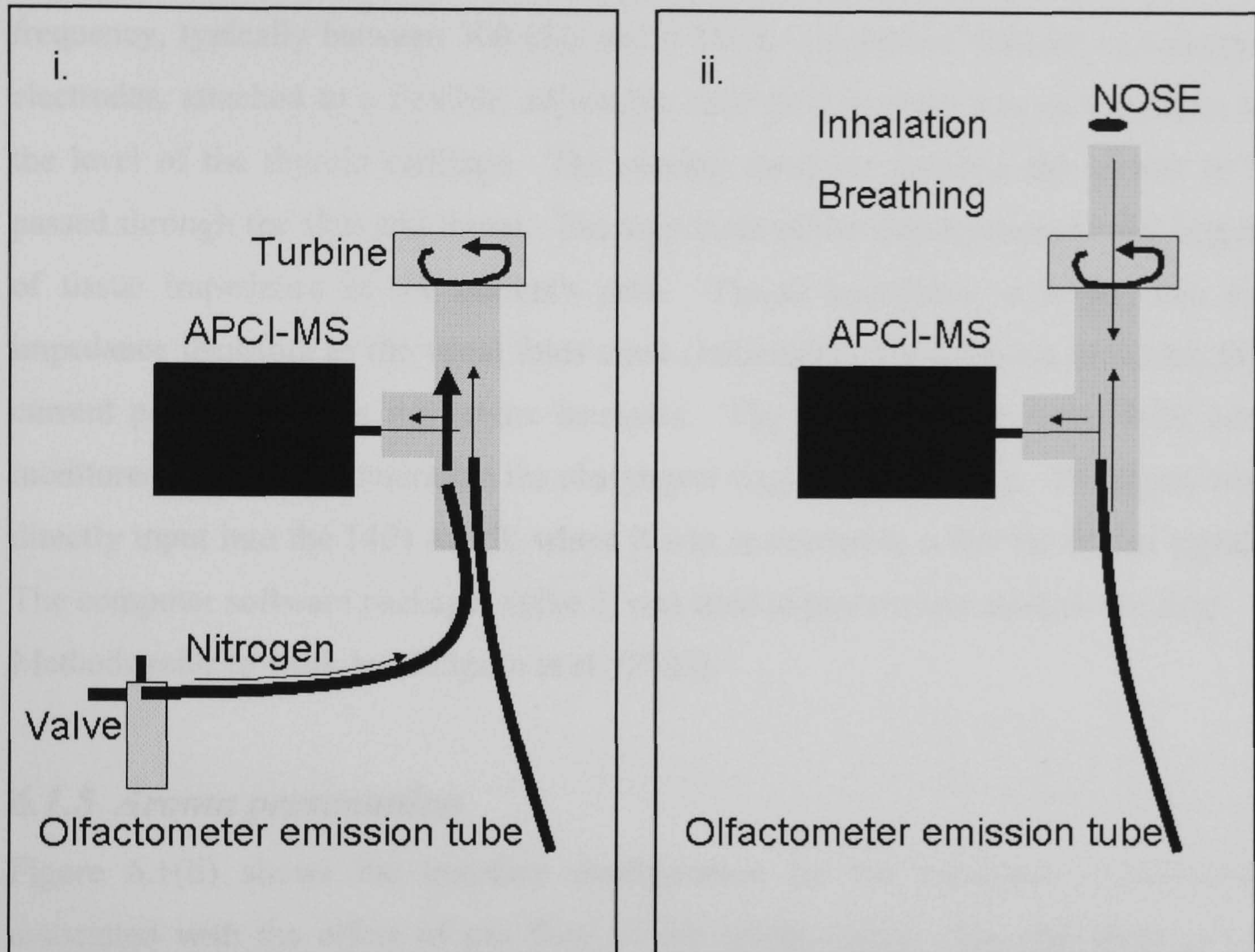


Figure 6.1 Configurations of turbine flow measurement with APCI-MS interface to calibrate inhalation breath by breath measurements. i. nitrogen gas flow. ii. using an individual.

6.1.3 Mastication measurements (Electromyography; EMG)

Mastication was measured using electromyography (EMG) by detecting the electrical activity associated with the contractions of the masseter muscle. This was achieved by placing bipolar surface electrodes (Medicotest UK Ltd) at either end of the muscle. The raw signal was fed into a 1902 signal conditioner (CED), which filtered out low frequency noise, cut off threshold 0.05 KHz. The filtered signal was then input into the 1401 Mk II and converted to a 5 KHz digital signal. Finally, the data was collected and analysed using the computer software package, Spike 2 (CED).

6.1.4 Swallowing measurements

Electroglottography (ECG) was used to monitor the pharyngeal stage of swallowing. A laryngograph (Laryngograph Ltd, London, UK) measured the electrical impedance across the throat during the eating process. An AC sinusoidal current of alternating frequency, typically between 300 kHz and 5 MHz, was passed between two copper electrodes, attached to a flexible, adjustable band held in place around the throat at the level of the thyroid cartilage. The sensing electrode detected the current as it passed through the skin and throat. The amplitude of the signal relates to the degree of tissue impedance in the current's path. Tissue impedance is lower than air impedance therefore as the vocal folds close (initiated by the reflexive swallow), the current passing through the larynx increases. The activity of the vocal folds was monitored and used to determine the pharyngeal stage of swallowing. The signal was directly input into the 1401 Mk II, where it was converted to a 800 Hz digital signal. The computer software package, Spike 2, was used to process and analyse the data. Methods refer to work by Hodgson et al. (2003).

6.1.5 Aroma presentation

Figure 6.1(ii) shows the interface configuration for the inhalation experiments associated with the effect of gas flow on the aroma signal. Six individuals were asked to perform to repetitions of the same programme but with breathing at different rates and velocities. Figure 6.2 shows the interface configuration for the remaining experiments on mouth presented aromas and APCI-MS measured exhalations. The same individuals performed both experiments. Isoamyl acetate was the volatile used in the experiment and was produced by the pulsing olfactometer described in chapter 2. Apart from the chewing experiment, the raw olfactometer output for all the tests was a constant concentration 270 mg.m^{-3} and not pulsed. For the chewing experiment, the same olfactometer programme measured in Figure 4.7.

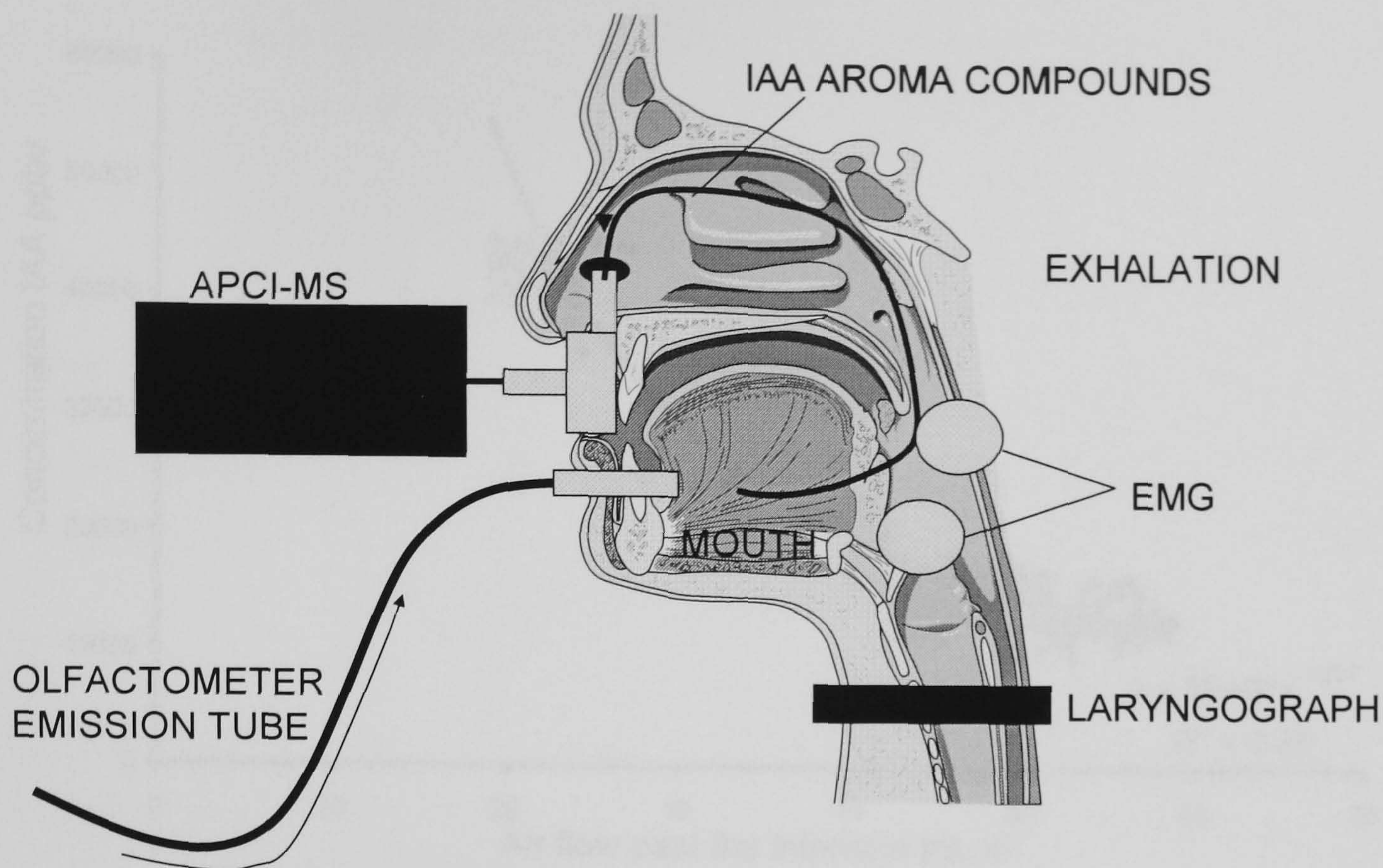


Figure 6.2 Configuration of chewing and swallowing experiments.

6.2 RESULTS

6.2.1 *The effect of air flow on the volatile concentration measured by the APCI-MS in the inhalation configuration*

6.2.1.1 **Controlled flow of nitrogen past the interface**

The aim of this experiment was essentially to calibrate the effect of air flow on the concentration of volatiles reaching the mass spectrometer through the Venturi. Nitrogen was used prior to using actual subjects as a comparison because the air flow could be controlled and unlike a person, did not fluctuate.

The range of flow rates of the nitrogen was 20-60 mL.s⁻¹. Even with this relatively small range compared to the range of flow rates observed from the breathing measurement, there was an effect of reducing the aroma signal as the flow increased. Figure 6.3 shows that a negative power function best explained the relationship between nitrogen flow and aroma signal with an R² value of 0.86. The explanation for this was that as the orthogonal gas flow increased past the interface silica, the low venturi flow into the mass spectrometer sampled a decreasing fraction of the aroma molecules.

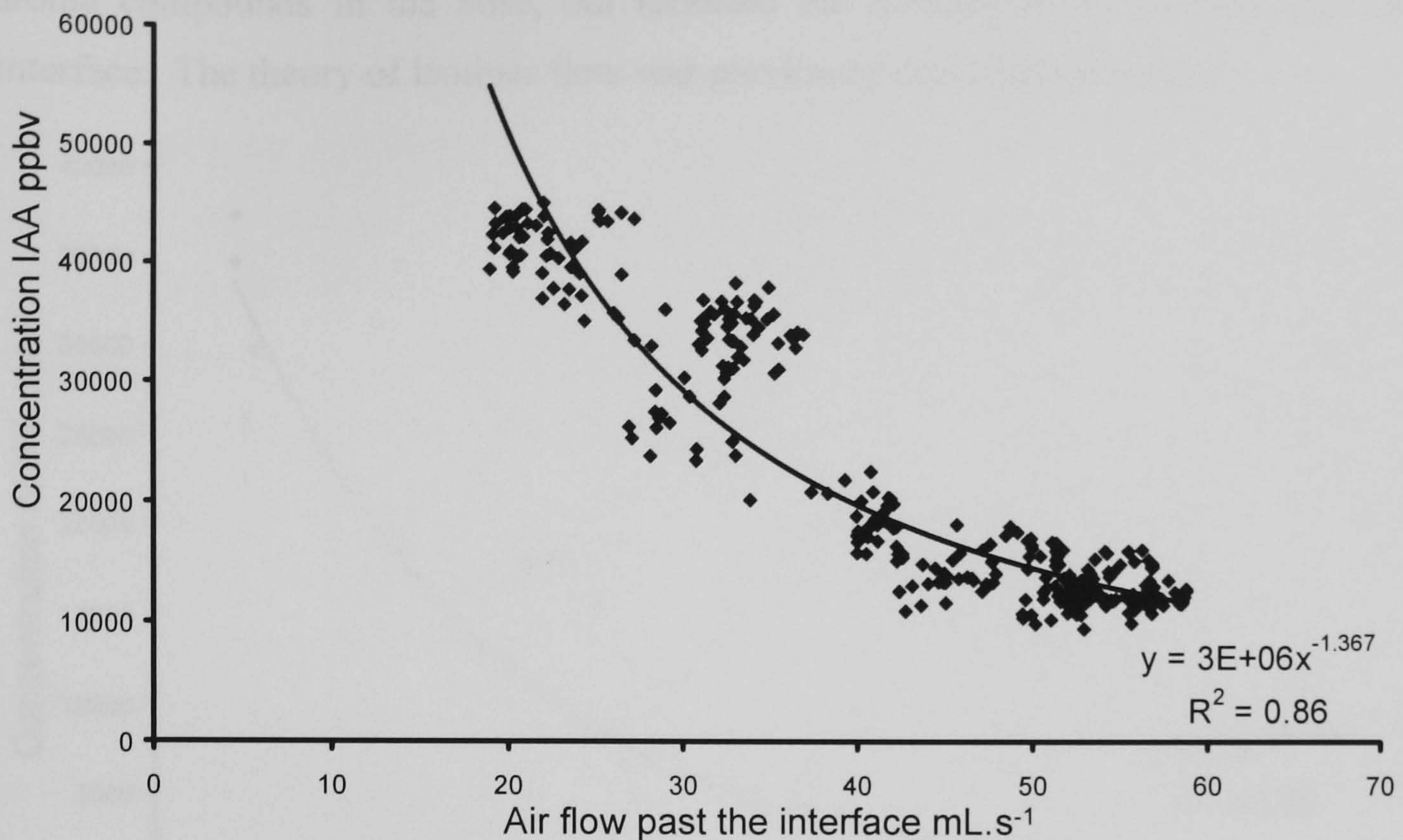


Figure 6.3 The effect of air flow on the volatile concentration measured by the APCI-MS

6.2.1.2 Different breathing rates and velocities

The effect of controlled gas flow on the measurement of volatiles by the APCI-MS was compared to real situations where individuals naturally breathed with different rates and velocities. It was expected that a similar inverse relationship would be seen between the two measurements, which would confirm certain theories in chapter 5 that were used to describe the potential relationship between the olfactometer stimulus and the response over time.

The range of gas flow rates taken at the maximum point in a breath was ~ 50 - 350 mL.s⁻¹. In support of the relationship observed with the nitrogen flow, Figure 6.4 also demonstrated a negative power function in respect to the aroma signal (R^2 value = 0.89). However, the power function of the curve was significantly smaller than the nitrogen flow function. This was likely to be due to the differences in the gas flow ranges, which meant that the nitrogen flow values corresponded to the steeper gradient regions of the breath flow curve. The two plots can therefore be used to demonstrate an effect, but cannot be used for calibration of flow versus relative intensity. It is also pertinent to reiterate that the inhalation breath by breath measurement of gas phase volatiles did not simulate the true laminar flow nature of

aroma compounds in the nose, but reflected the turbulence on the path past the interface. The theory of laminar flow was previously described in chapter 4.

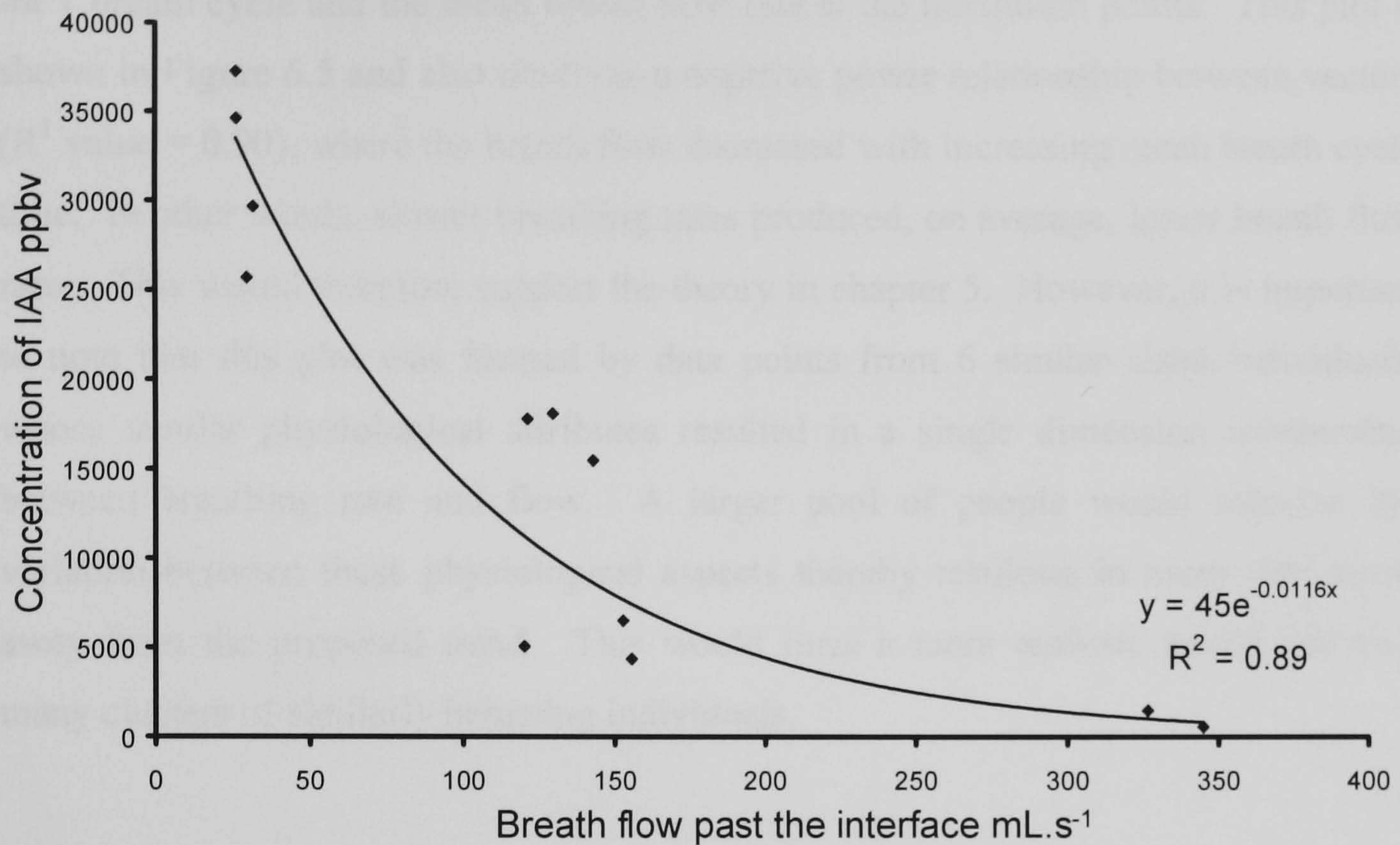


Figure 6.4 The effect of breath flow on the volatile concentration measured by the APCI-MS in the inhalation configuration.

6.2.1.3 The relationship between breathing rate and breath flow rate

In chapter 5, a theory was postulated to understand the reason behind the relationship between breathing rate and instrumental analyses. It was assumed that the presence of the nose tube and mass spectrometer interface might have resulted in assessor breathing rates that were slightly different to the normal resting rates. As a consequence, individual breathing rates may have changed over the course of a TI test. Moreover, this change in flow resulted in a change in the aroma disruption pattern and finally made an impact on perception. A hypothetical example of this would be an assessor who initially breathed on the APCI-MS at a faster rate than their normal resting rate. Over the course of the sensory test, their breathing rate resumed back to its resting rate as their attention focussed on the test and they became accustomed. The opposite effect occurred if an assessor initially breathed slower than the resting rate. However, only breathing rate (i.e. Time (secs) for 1 breath cycle) was measured in this experiment, which only assumed a relationship between

breathing rate and actual flow rate/velocity, but showed no evidence to support this. Therefore it was necessary to test whether there was in fact a trend between the time for 1 breath cycle and the mean breath flow rate at the maximum points. This plot is shown in Figure 6.5 and also observes a negative power relationship between vectors (R^2 value = 0.90), where the breath flow decreased with increasing mean breath cycle time. In other words, slower breathing rates produced, on average, lower breath flow rates. This would therefore support the theory in chapter 5. However, it is important to note that this plot was formed by data points from 6 similar sized individuals, whose similar physiological attributes resulted in a single dimension relationship between breathing rate and flow. A larger pool of people would increase the variation between these physiological aspects thereby resulting in many deviations away from the proposed trend. This would form a more realistic model showing many clusters of similarly behaving individuals.

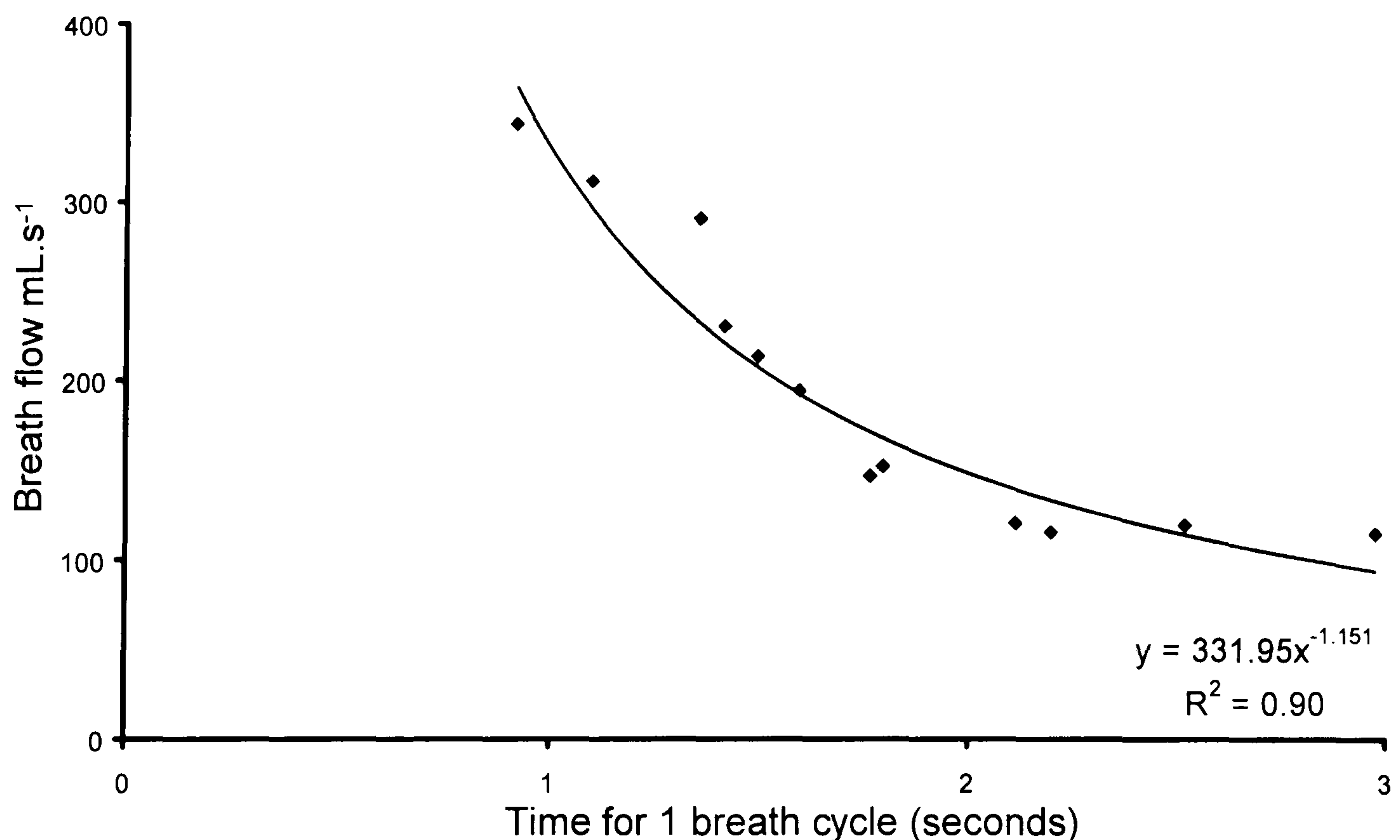


Figure 6.5 Relationship between breathing rate and breath flow velocity. Six assessors and 2 repetitions.

6.2.2 *The disruption of the stimulus from retronasally presented aromas by mastication*

This experiment and the following section describe the effect of disrupting a controlled aroma pattern by physiological measurements normally associated with mastication.

Aroma streams of gas phase isoamyl acetate (constant concentration: 270 mg.m^{-3} , flow 5 mL.min^{-1}) were presented to the mouth of individuals and the volatiles measured by APCI-MS; without chewing (Figure 6.6a) and then with chewing (Figure 6.6b).

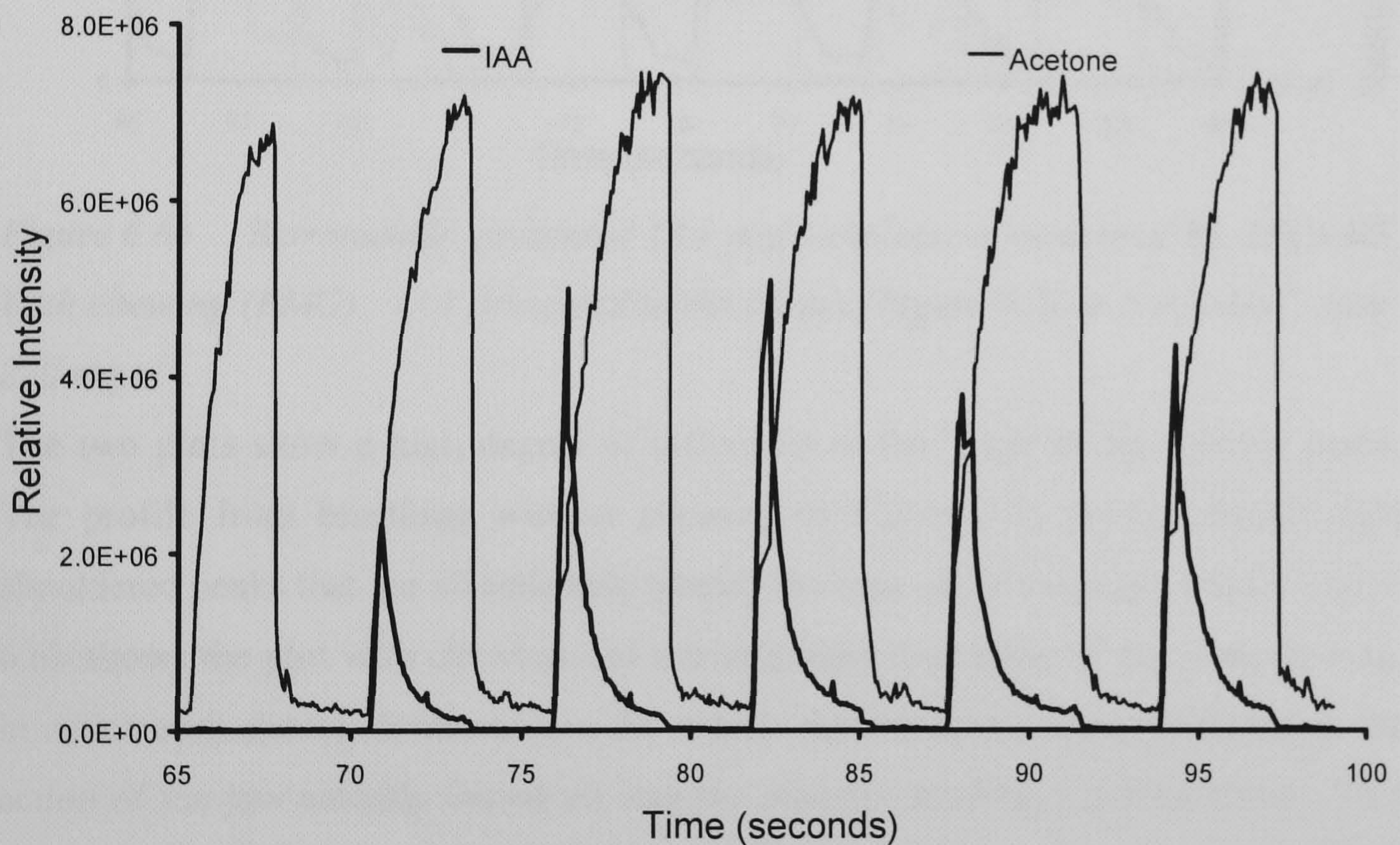


Figure 6.6a *Retronasally presented IAA and exhalations measured by APCI-MS: without chewing. IAA presented to the mouth (Figure 6.2) at 5 mL.min^{-1} , conc. 270 mg.m^{-3} .*

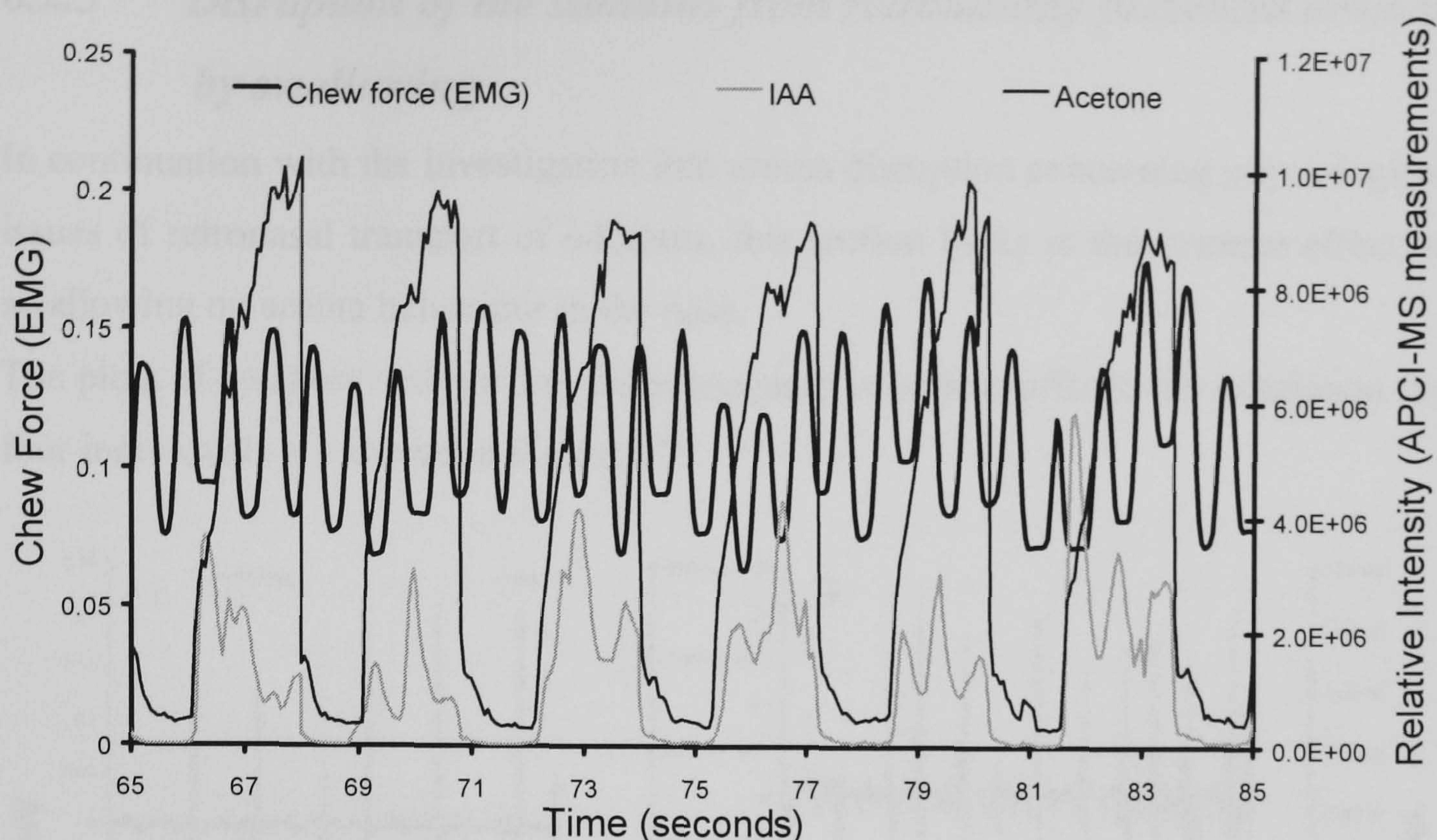


Figure 6.6b Retronasally presented IAA and exhalations measured by APCI-MS. With chewing (EMG). IAA presented to the mouth (Figure 6.2) at $5 \text{ mL} \cdot \text{min}^{-1}$, conc. $270 \text{ mg} \cdot \text{m}^{-3}$.

The two plots show a high degree of difference in the shape of the volatile peaks. The profile from breathing without chewing in Figure 6.6a shows smooth right shouldered peaks that are all relatively similar in shape and form single peaks. Figure 6.6b shows the plot with chewing and shows a large disruption of the peaks in 6.6a, in which each chew is followed by a tall narrow peak of aroma in an exhalation as the action of the jaw actually forced air into the pharynx creating a pulsed effect. This supports previous research, which showed the same effect with real food products (Hodgson, *et al.*, 2003), and demonstrates how chewing can increase the person to person variability in breath by breath studies, thereby affecting potential studies with correlated sensory analyses. Nevertheless, there is currently little evidence to suggest that the disruption caused by mastication has a significant impact on flavour perception.

6.2.3 *Disruption of the stimulus from retronasally presented aromas by swallowing.*

In continuation with the investigation into aroma disruption concerning physiological issues of retronasal transport of odorants, this section looks at the extreme effect of swallowing on aroma behaviour in the nose.

The plots of swallow activity and the subsequent volatile profile in the exhalation for four individuals are shown in Figure 6.7.

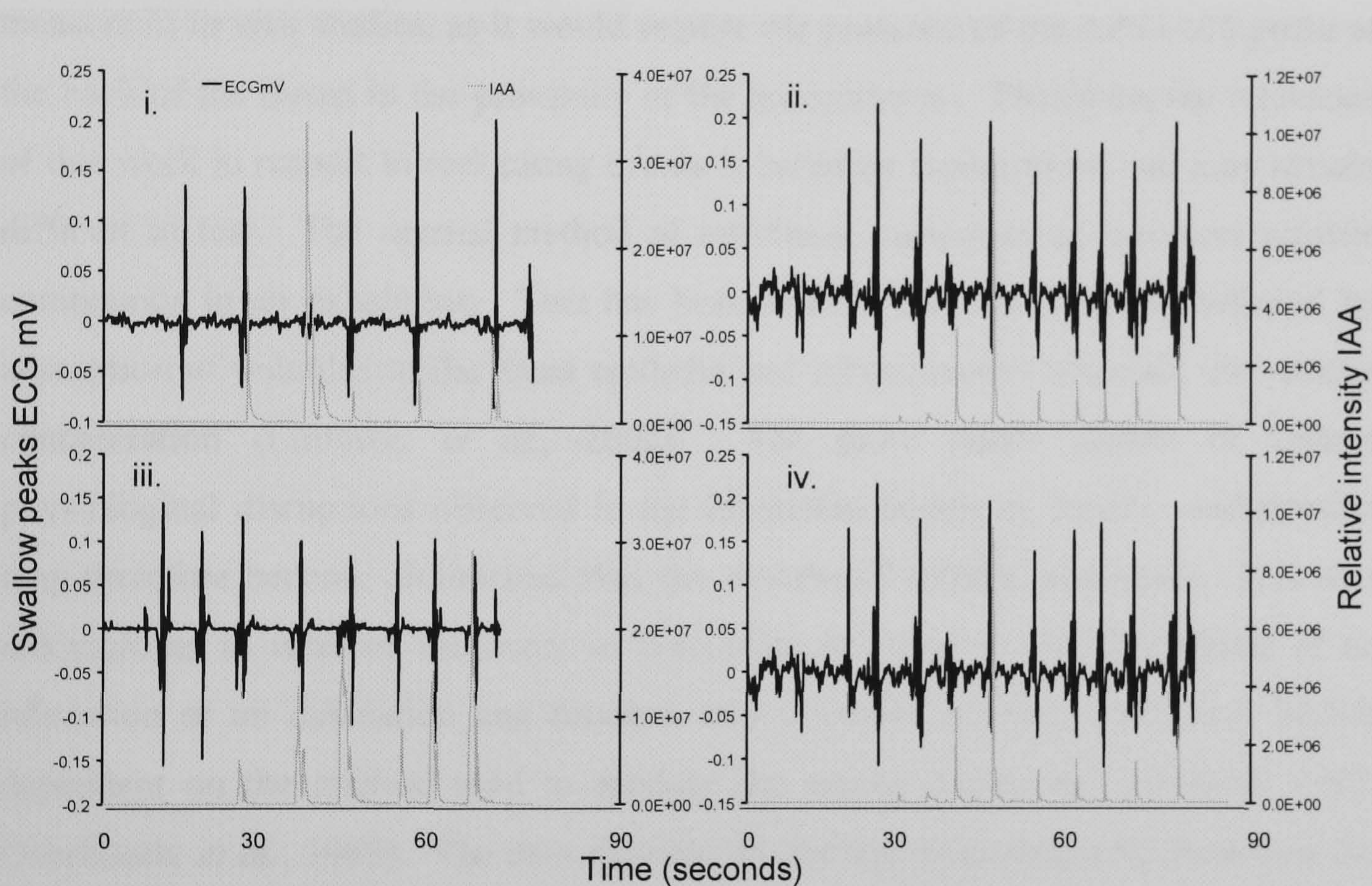


Figure 6.7 The effect of swallowing on the exhaled IAA stimulus after retronasal presentation of IAA from 4 different individuals. Individuals (i) and (iii) show less frequent swallowing than (ii) and (iv).

This demonstrates how the identical olfactometer output (see Figure 4.7) was totally disrupted from its original profile due to the act of swallowing and this differed greatly between people. In this data set, the individual plots of (i) and (iii) show less frequent swallowing and the volatile concentration in the breath was much higher than the concentration in the breath from individuals who swallowed more frequently (plots (ii) and (iv)). This effect is not observed when actual samples are consumed, such as flavoured aliquots of solution. In these instances, frequency of swallowing

has not been shown to effect the concentration of the volatile in the nose (Private communication with Michael Hodgson).

6.3 DISCUSSION

This chapter showed that breathing rate and flow disrupted aroma stimuli when measured in the inhalation configuration. This effect may exist retronasally as well (i.e. before the entrance into the anterior nares), however, it would be very difficult to measure in *in vivo* studies, as it would require the presence of the APCI-MS probe at the back of the throat in the proximity of the nasopharynx. Therefore, the relevance of this work in respect to real eating events is based on assumptions that may remain difficult to test. The normal method of retronasal measurement monitors volatile compounds in an exhalation. This has been shown to be dramatically affected by adsorption of volatiles to the nasal epithelia and subsequently decreases the volatile concentration (Linforth, *et al.*, 2002). The more subtle effects of certain physiological disruptions observed in the inhalation breath by breath configuration may therefore become diminished after the process of volatile adsorption. It is also unknown as to whether the event of perception is closer to the time points of an inhalation or an exhalation and research into olfaction reaction times may be too dependent on the method used to produce the aroma (Laing and Macleod, 1992; Overbosch, *et al.*, 1989). The data obtained by the measurement of air flow past the mass spectrometer interface may be discredited by papers that have modelled air flow through the upper respiratory airways. Firstly, one finding suggests that regardless of exhalation or inhalation, flow through the nasal cavity is essentially laminar and thus odorants occur as diffusible plugs rather than constant concentrations created by mixing in turbulent flow (Keyhani, *et al.*, 1995; Keyhani, *et al.*, 1997). In this study, the effect of an inhalation on the stimulus could only indicate the effect of a turbulent mixing flow. Despite this deviation from recent postulations, the inhalation patterns of the volatiles were shown to explain the sensory variation between individuals (chapter 5). A related point to note comes from another much more recent study where it was shown by human nasal cavity modelling that two individuals could inhale identical amounts of air at the same rate yet one could have up to 50 times

greater local air flow thereby increasing the supply of odorant (Zhao, *et al.*, 2004). Perhaps this explains why the sensory line scale usage in chapter 5 did not correlate to the instrumental measurements of volatile inhalation. In other words, the way in which the volatiles were inhaled was irrelevant to perception because the events inside the nose were far more important.

In this study, chewing and swallowing were shown to significantly disrupt a retronasally delivered aroma stimulus in a subject dependent manner. The effect of this disruption on perception could be tested using a similar method of data analysis to chapter 5. This could be performed by analysing the instrumental data of the different sensory principal groups and mapping this onto the variation observed between their mastication and swallowing patterns.

In conclusions, we can measure with relative accuracy the disruptions of an aroma stimulus associated with breathing swallowing and chewing once they have been exposed to the individual. Physiological consequences of breathing were shown to affect the measurements on the APCI-MS, and some of these disruptions lead to a stimulus being perceived in different ways. The impact and relevancy of the other physiological measurements on flavour perception remain as yet unclear.

7 GENERAL DISCUSSION

My principal aim was to investigate the APCI-MS in order to model the sensory responses to food/aroma stimuli.

The broad title of the thesis meant that the project could have taken many different directions and initially, it was quite difficult to establish the area of a very large surface that I ought to be scratching. Many of my decisions stemmed from the experiment on retronasal and orthonasal thresholds, which meant that I produced a thesis that was predominantly psychophysical rather than conventionally sensory.

The initial aim of the thresholds chapter was to generate data for use in a link project that was modelling time intensity responses by using the Overbosch equation and its derivatives (*see section 1.3.3.3*). In order to do this, the majority of my first year was spent developing appropriate techniques, which is why I also wanted to use the data to test a different, albeit related, set of theories to those proposed by the link project. It was at this point that I began to focus on the APCI-MS as a tool to measure the variation between people rather than volatiles or products. It was already widely acknowledged that two individuals would differ in their breath by breath profiles despite consuming the same solution, but the consequence on perception had not been extensively studied. By obtaining threshold data I hypothesised that if two individuals had very different thresholds, their breath by breath profiles would be significantly different. This conclusion was not shown by my threshold results probably because the hypothesis was simply not true. However, certain unavoidable compromises in the methodology meant that I could never be totally confident in the data. Firstly, the orthonasal gas phase thresholds were calculated according to the air-water partition coefficient and actual in nose volatile concentration differences between individuals were not measured. This would have been impossible at the concentration range used, thus a compromise was made. Secondly, retronasal thresholds were based on breath by breath measurements, but only as a mean value from consumptions of higher concentration aqueous samples. As exposed in chapters 4, 5 and 6, individual breath by breath repetitions can also differ dramatically and

therefore using a mean value to form a partition coefficient would not reflect these changes over time. Once again, this would have been simply impossible to measure for two reasons: the volatile concentration ranges were too low for the sensitivities of the mass spectrometer and it would have also been uncomfortable for a panellist to conduct all threshold tests on the breath by breath interface. A future experiment of this kind could be improved if dose response curves were employed to extrapolate towards the threshold rather than to measure it directly. A similar method was used in an experiment by Vuilleumier and colleagues (2000) to much success. In this way, volatile concentrations could be high enough for online measurement of retronasal consumption and more importantly to monitor how an individual inhales the headspace for orthonasal determination. Despite the obvious problems encountered in measuring the variation between individuals, overall the thresholds were shown to be highly repeatable measurements even over an 8 month period. More importantly, the experiment demonstrated that individuals could be clustered according to their psychophysical measurements when a larger group of individuals was analysed. This result was supported by the experiments in the subsequent chapters.

The experiments conducted on perception of pulsed odorants had two origins; from work on other modalities and the observed discrepancy between breath by breath data and time intensity. The other modalities to be investigated for their responses to pulsed stimuli were mainly vision and hearing under the heading of 'flicker fusion theory'. This psychophysical theory suggests that the perceptual systems have a minimum number of time frames upon which a piece of information is imprinted. If this rate is exceeded then those perceptual frames will coalesce into one dynamic experience, the prime example being cinema. Similarly, breath by breath data produces volatile peaks with the tidal respiration, yet the perceptual response is smoothed as if the captured information imprints itself long enough to allow for the subsequent wave of aroma to arrive without a decrease being perceived. It was these ideas that produced the question; are pulsed odorants perceived as being the same or different to constant odorants?

The problems involved in answering this question were perhaps even more extreme than those in the thresholds investigation and were obvious from the start.

-The olfactometer needed to pulse at a rate quick enough to produce a sufficient amount of pulses within the time for an average inhalation.

-Even when this rate was set at 0.6 seconds, it was shown that each person received different versions of this aroma output due to their individual breathing patterns.

-Analysing the aromas in the nasal exhalations reflected conventional retronasal *in vivo* measurement but could not monitor the pattern of pulses on their path to the nose.

Measuring the pattern of aroma inhalation by an individual solved part of these problems was also invaluable in comparisons against exhalation measurements. Furthermore, conducting these experiments simultaneously with instrumental measurement lead full circle back to the threshold related hypotheses that an individual's perception would differ depending on how they received the inhaled aroma patterns. This could also take into account the variability between repetitions from the point of view that a single olfactometer emission pattern was disrupted in a infinite number of ways by individuals at different points in time. Therefore, the procedure could not be used to model the perceptual response, but instead was highly effective in understanding why two people may differ in their sensory responses.

The results of these pulsing experiments showed that in a discrimination test with a significant outcome, there were individual repetitions that went against the overall consensus. However, the instrumental analysis revealed that these statistical deviants had in fact inhaled significantly different patterns of aroma to the majority. Therefore, it was not their cognition that went against the consensus, it was in fact the consequence of their physiology. In other words, when two people breathe in an aroma stimulus, they disrupt it in different ways. This disruption directly affects their perception, and causes their sensory responses to be different.

This result was further supported by looking at the perception of these aroma patterns over time. Unlike the two way decision of a discrimination test, time intensity revealed the infinite number of different perceptual profiles between people and how

this was partly a consequence of differences in their aroma inhalations. Overall differences between people and their repetitions in respect to maximum aroma intensity and average scale usage could not be explained by the instrumental data. However, the overall shape and skew of the TI curve was shown to be indicative of the pattern of aroma they received in their inhalations.

These theories can be extended to normal eating events; two people may differ in their perceptual experiences due to the dissimilarity of the aroma patterns on their path to the nasal receptors. In addition to breathing, other factors such as swallowing and chewing may enhance this pattern disruption, as shown in chapter 6, but their true effects on perception remain to be seen.

The drawback in conducting these pulsing experiments was the laborious analysis of the instrumental data. To put this into perspective, the time intensity experiment alone took three days to capture all the data but took five months to analyse, extract and format the results. I would have ideally wanted to conduct further experiments using other volatiles or on a consumer scale, but the time for analysis prevented me from doing so.

For this reason, I believe a major advance in understanding flavour release and behaviour in the mouth and nasal cavity would be in the development of analytical software that could extract relevant points in real time. In this way, far greater data sets could be handled and would certainly increase the likelihood of revealing further links between the stimulus and the response.

In retrospect, I am satisfied with the path my research has taken and the reasons behind my experimental decisions. It also seemed highly appropriate to be able to return to original hypotheses regarding the reasons for person to person sensory variation.

Through these relatively complicated experiments an interesting conclusion about olfaction has been revealed, which sets this modality apart from the other senses: the

disruption of the aroma patterns from source to receptor are so powerful that they result in sensory differences between people.

BIBLIOGRAPHY

Abraham M.H., Gola J.M.R., Cometto-Muniz J.E. and Cain W.S. (2002) *A model for odour thresholds*. *Chemical Senses*, 27, 95-104

Aubry V., Etievant P., Sauvageot F. and Issanchou S. (1999) *Sensory analysis of burgundy pinot noir wines: A comparison of orthonasal and retronasal profiling*. *Journal of Sensory Studies*, 14, 97-117

Bacon A.W., Bondi M.W., Salmon D.P. and Murphy C. (1998) *Olfaction and taste xii, Very early changes in olfactory functioning due to alzheimer's disease and the role of apolipoprotein e in olfaction*. 723-731

Baek I., Linforth R.S.T., Blake A. and Taylor A.J. (1999) *Sensory perception is related to the rate of change of volatile concentration in-nose during eating of model gels*. *Chemical Senses*, 24, 155-160

Bensafi M., Rouby C., Farget V., Bertrand B., Vigouroux M. and Holley A. (2002) *Autonomic nervous system responses to odours: The role of pleasantness and arousal*. *Chemical Senses*, 27, 703-709

Boccorh R.K. and Paterson A. (2002) *An artificial neural network model for predicting flavour intensity in blackcurrant concentrates*. *Food Quality and Preference*, 13, 117-128

Brauss M.S., Linforth R.S.T., Cayeux I., Harvey B. and Taylor A.J. (1999) *Altering the fat content affects flavor release in a model yogurt system*. Journal of Agricultural and Food Chemistry, 47, 2055-2059

Breer H. (2003) *Sense of smell: Recognition and transduction of olfactory signals*. Biochemical Society Transactions, 31, 113-116

Breslin P.A.S. (2001) *Human gustation and flavour*. Flavour and Fragrance Journal, 16, 439-456

Brockerhoff E.G. and Grant G.G. (1999) *Correction for differences in volatility among olfactory stimuli and effect on eeg responses of dioryctria abietivorella to plant volatiles*. Journal of Chemical Ecology, 25, 1353-1367

Brown W.E. (1994) *Method to investigate differences in chewing behaviour in humans. 1. Use of electromyography in measuring chewing*. Journal of Texture Studies, 25, 1-16

Buettner A., Beer A., Hannig C., Settles M. and Schieberle P. (2002) *Physiological and analytical studies on flavour perception dynamics as induced by the eating and swallowing process*. Food Quality and Preference, 13, 497-504

Buettner A. and Schieberle P. (2000a) *Exhaled odorant measurement (exom) - a new approach to quantify the degree of in-mouth release of food aroma compounds*. Lebensmittel Wissenschaft und Technologie, 33, 553-559

Buettner A. and Schieberle P. (2000b) *Influence of mastication on the concentrations of aroma volatiles - some aspects of flavour release and flavour perception*. Food Chemistry, 71, 347-354

Buck L. and Axel R. (1991) *Odorant receptors and the organisation of the olfactory system*. Cell, 65, 175-187.

Caporale G., Policastro S. and Monteleone E. (2004) *Bitterness enhancement induced by cut grass odorant (cis-3-hexen-1-ol) in a model olive oil*. Food Quality and Preference, 15, 219-227

Carrie S., Scannell J.W. and Dawes P.J.D. (1999) *The smell map: Is there a commonality of odour perception?* Clinical Otolaryngology, 24, 184-189

Carstens E., Iodi Carstens M., Dessirier J.-M., O'Mahony M., Simons C.T., Sudo M. and Sudo S. (2002) *It hurts so good: Oral irritation by spices and carbonated drinks and the underlying neural mechanisms*. Food Quality and Preference, 13, 431-443

Castle P.C., Van Toller S. and Milligan G.J. (2000) *The effect of odour priming on cortical eeg and visual erp responses*. International Journal of Psychophysiology, 36, 123-131

Cerf-Ducastel B. and Murphy C. (2001) *Fmri activation in response to odorants orally delivered in aqueous solutions*. Chemical Senses, 26, 625-637

Charles M., Rosselin V., Beck L., Sauvageot F. and Guichard E. (2000) *Flavor release from salad dressings: Sensory and physicochemical approaches in relation with the structure*. Journal of Agricultural and Food Chemistry, 48, 1810-1816

Chaya C., Perez-Hugalde C., Judez L., Wee C.S. and Guinard J.X. (2004) *Use of the statis method to analyze time-intensity profiling data*. Food Quality and Preference, 15, 3-12

Chen Y.C., Bundy D.S. and Hoff S.J. (1999) *Using olfactometry to measure intensity and threshold dilution ratio for evaluating swine odor*. Journal of the Air & Waste Management Association, 49, 847-853

Cliff M.A. and Heymann H. (1993) *Development and use of time-intensity methodology for sensory evaluation - a review*. Food Research International, 26, 375-385

Cliff M.A., O'Mahony M., Fukumoto L. and King M.C. (2000) *Development of a 'bipolar' r-index*. Journal of Sensory Studies, 15, 219-229

Coile D.C., Pollitz C.H. and Smith J.C. (1989) *Behavioural determination of critical flicker fusion frequency in dogs*. Physiology & Behavior, 45, 1087-1092

Colin C., Radeau M., Soquet A., Demolin D., Colin F. and Deltenre P. (2002) *Mismatch negativity evoked by the mcgurk-macdonald effect: A phonetic representation within short-term memory*. *Clinical Neurophysiology*, 113, 495-506

Cook D.J., Hollowood T.A., Linforth R.S.T. and Taylor A.J. (2002) *Perception of taste intensity in solutions of random-coil polysaccharides above and below c**. *Food Quality and Preference*, 13, 473-480

Cook D.J., Hollowood T.A., Linforth R.S.T. and Taylor A.J. (2003) *Oral shear stress predicts flavour perception in viscous solutions*. *Chem. Senses*, 28, 11-23

Coren S., Ward L.M. and Enns J.T. (1999) *Sensation and perception fifth edition*, Psychophysics. Harcourt College Publishers, Orlando. 15-49

Cornsweet T.N. (1965) *The staircase method in psychophysics*. *American Journal of Psychology*, 75, 485-491

Dade L.A., Zatorre R.J. and Jones-Gotman M. (2002) *Olfactory learning: Convergent findings from lesion and brain imaging studies in humans*. *Brain*, 125, 86-101

Dalton P., Doolittle N., Nagata H. and Breslin P.A.S. (2000) *The merging of the senses: Integration of subthreshold taste and smell*. *Nature Neuroscience*, 3, 431-432

Davidson J.M., Linforth R.S.T., Hollowood T.A. and Taylor A.J. (1999) *Effect of sucrose on the perceived flavor intensity of chewing gum*. Journal of Agricultural and Food Chemistry, 47, 4336-4340

Davy E. (1952) *The intensity-time relation for multiple flashes of light in the peripheral retina*. Journal of the Optical Society of America, 42, 937-941

de Araujo I.E.T., Rolls E.T., Kringelbach M.L., McGlone F. and Phillips N. (2003) *Taste-olfactory convergence, and the representation of the pleasantness of flavour, in the human brain*. European Journal of Neuroscience, 18, 2059-2068

de Kruijk J.R., Leffers P., Menheere P., Meerhoff S., Rutten J. and Twijnstra A. (2003) *Olfactory function after mild traumatic brain injury*. Brain Injury, 17, 73-78

Deisingh A.K., Stone D.C. and Thompson M. (2004) *Applications of electronic noses and tongues in food analysis*. International Journal of Food Science and Technology, 39, 587-604

Delwiche J. (2004) *The impact of perceptual interactions on perceived flavor*. Food Quality and Preference, 15, 137-146

Devos M., Patte F., Rouault J., Laffort P. and Van Gemert L.J. (1990) Standardized human olfactory thresholds. IRL Press at Oxford University Press, Oxford. 165

Dijksterhuis G.B. and Piggott J.R. (2000) *Dynamic methods of sensory analysis*. Trends in Food Science & Technology, 11, 284-290

Dittrich R., Hatzinger R. and Katzenbeisser W. (2002) *Modelling dependencies in paired comparison data - a log-linear approach*. Computational Statistics & Data Analysis, 40, 39-57

Doleman B.J., Severin E.J. and Lewis N.S. (1998) *Trends in odor intensity for human and electronic noses: Relative roles of odorant vapor pressure vs molecularly specific odorant binding*. Proceedings of the National Academy of Sciences of the United States of America, 95, 5442-5447

Doty R.L. (2001) *Olfaction*. Annual Review of Psychology, 52, 423-452

Doty R.L. (2002) *Chemistry of taste: Mechanisms, behaviours, and mimics, Olfactory psychophysics*. American Chemical Society, Washington D.C. 123-139

Doty R.L., McKeown D.A., Lee W.W. and Shaman P. (1995a) *A comparative study of the reliability of 10 olfactory tests and mathematical models of the relationship between reliability and test length*. Chemical Senses, 20, 67-67

Doty R.L., McKeown D.A., Lee W.W. and Shaman P. (1995b) *A study of the test-retest reliability of ten olfactory tests*. Chemical Senses, 20, 645-656

Doyen K., Carey M., Linforth R.S.T., Marin M. and Taylor A.J. (2001) *Volatile release from an emulsion: Headspace and in-mouth studies*. Journal of Agricultural and Food Chemistry, 49, 804-810

Dryer L. and Berghard A. (1999) *Odorant receptors: A plethora of g-protein-coupled receptors*. TiPS, 20, 413-418

Elsner R.J.F. (2001) *Odor threshold, recognition, discrimination and identification in centenarians*. Archives of Gerontology and Geriatrics, 33, 81-94

Evans W.J., Cui L. and Starr A. (1995) *Olfactory event-related potentials in normal human subjects: Effects of age and gender*. Electroencephalography and Clinical Neurophysiology, 95, 293-301

Ferrari J.R. and Dovidio J.F. (2000) *Examining behavioral processes in indecision: Decisional procrastination and decision-making style*. Journal of Research in Personality, 34, 127-137

Firestein S. (2002) *Chemistry of taste: Mechanisms, behaviours, and mimics, Olfactory receptor neuron signaling*. American Chemical Society, Washington D.C. 110-122

Firmin H., Reilly S. and Fourcin A. (1999) *Non-invasive monitoring of reflexive swallowing*. Speech hearing and language, 10, 171-184

Fortier I., Ferraris J. and Mergler D. (1991) *Measurement precision of an olfactory perception threshold test for use in field studies*. American Journal of Industrial Medicine, 20, 495-504

Garrido D., Calvino A. and Hough G. (2001) *A parametric model to average time-intensity taste data*. Food Quality and Preference, 12, 1-8

Gold G.H. (1999) *Controversial issues in vertebrate olfactory transduction*. Annual Review of Physiology, 61, 857-871

Goldstein E.B. (1999) *Sensation and perception*. Brooks/ Cole Publishing Company.

Goldstone R.L. (1998) *Perceptual learning*. Annual Review of Psychology, 49, 585-612

Gomez G., Voigt R. and Atema J. (1999) *Temporal resolution in olfaction iii: Flicker fusion and concentration-dependent synchronization with stimulus pulse trains of antennular chemoreceptor cells in the american lobster*. Journal of Comparative Physiology A-Sensory Neural and Behavioural Psychology, 185, 427-436

Gopel W. (2000) *From electronic to bioelectronic olfaction, or: From artificial "moses" to real noses*. Sensors and Actuators B-Chemical, 65, 70-72

-
- Gottfried J.A. and Dolan R.J. (2003) *The nose smells what the eye sees: Crossmodal visual facilitation of human olfactory perception*. *Neuron*, 39, 375-386
- Griep M.I., Mets T.F., Vercruyse A., Cromphout I., Ponjaert I., Toft J. and Massart D.L. (1995) *Food odor thresholds in relation to age, nutritional, and health-status*. *Journals of Gerontology Series a-Biological Sciences and Medical Sciences*, 50, B407-B414
- Grosch W. (2001) *Evaluation of the key odorants of foods by dilution experiments, aroma models and omission*. *Chemical Senses*, 26, 533-545
- Haffenden L.J.W., Yaylayan V.A. and Fortin J. (2001) *Investigation of vibrational theory of olfaction with variously labelled benzaldehydes*. *Food Chemistry*, 73, 67-72
- Hamada T. and Yamaguchi M. (2001) *Evoked and oscillatory neuromagnetic responses to sniffing odour in human subjects*. *Behavioural Brain Research*, 123, 219-223
- Hanaoka K., Vallet N., Giampaoli P., Heyd B. and MacLeod P. (2001) *Possible influence of breathing on detection frequency and intensity rating in gas chromatography-olfactometry*. *Food Chemistry*, 72, 97-103
- Harrison M., Hills B.P., Bakker J. and Clothier T. (1997) *Mathematical models of flavor release from liquid emulsions*. *Journal of Food Science*, 62, 653-&

Harvey B.A. and Barra J. (2003) *Real time breath and headspace analysis of flavour volatiles*. European Journal of Pharmaceutics and Biopharmaceutics, 55, 261-269

Heilmann S. and Hummel T. (2004) *A new method for comparing orthonasal and retronasal olfaction*. Behavioral Neuroscience, 118, 412-419

Heilmann S., Strehle G., Rosenheim K., Damm M. and Hummel T. (2002) *Clinical assessment of retronasal olfactory function*. Archives of Otolaryngology-Head & Neck Surgery, 128, 414-418

Herz R.S. and Cupchik G.C. (1992) *An experimental characterization of odor-evoked memories in humans*. Chemical Senses, 17, 519-528

Herz R.S. and Cupchik G.C. (1995) *The emotional distinctiveness of odor-evoked memories*. Chemical Senses, 20, 517-528

Herz R.S., McCall C. and Cahill L. (1999) *Hemispheric lateralization in the processing of odor pleasantness versus odor names*. Chemical Senses, 24, 691-695

Herz R.S. and Zeitlin S.B. (1995) *Emotion experienced during encoding enhances odor retrieval cue efficacy*. Chemical Senses, 20, 116-116

Higbee K.L. (2002) *Your memory: How it works and how to improve it*. Englewood Cliffs, N.J. Prentice Hall.

Hirsch A.R. and Tranel T.J. (1996) *Chemosensory disorders and psychiatric diagnoses*. *Journal of Neurological and Orthopaedic Medicine and Surgery*, 17, 25-30

Hodgson M., Langridge J.P., Linforth R.S.T. and Taylor A.J. (2004) *Aroma release and delivery following the consumption of beverages*.

Hodgson M., Linforth R.S.T. and Taylor A.J. (2003) *Simultaneous real time measurements of mastication, swallowing, nasal airflow and aroma release*. *Journal of Agricultural and Food Chemistry*, 51, 5052-5057

Hollowood T.A., Chang K.M., Hort J., Langridge J.P., Fearn T. and Taylor A.J. (2004) *Temporal flavour perception in vivo*. Wartberg conference, *in press*,

Hollowood T.A., Linforth R.S.T. and Taylor A.J. (2000) *Flavor release, The relationship between carvone release and the perception of mintyness in gelatine gels*. American Chemical Society, Washington.

Hollowood T.A., Linforth R.S.T. and Taylor A.J. (2002) *The effect of viscosity on the perception of flavour*. *Chemical Senses*, 27, 583-591

Hornung D. and Mozell M. (1977) *Factors influencing the differential sorption of odorant molecules across the olfactory mucosa*. Journal of General Physiology, 69, 343-361

Hornung D.E., Kurtz D.B., Bradshaw C.B., Seipel D.M., Kent P.F., Blair D.C. and Emko P. (1998) *The olfactory loss that accompanies an hiv infection*. Physiology & Behavior, 64, 549-556

Hornung D.E., Youngentob S.L. and Mozell M.M. (1984) *Odorant uptake by the olfactory mucosa*. Chemical Senses, 8, 251-252

Hornung D.E., Youngentob S.L. and Mozell M.M. (1987) *Olfactory mucosa-air partitioning of odorants*. Brain Research, 413, 147-154

Hort J. and Hollowood T.A. (2004) *Controlled continuous flow delivery system for investigating taste-aroma interactions*. Journal of Agricultural and Food Chemistry, 52, 4834-4843

Hummel T. (2000a) *Assessment of intranasal trigeminal function*. International Journal of Psychophysiology, 36, 147-155

Hummel T. (2000b) *Clinical aspects of olfactometry*. International Journal of Psychophysiology, 35, 29-30

Jacob T.J.C., Fraser C., Wang L.W., Walker V. and O'Connor S. (2003) *Psychophysical evaluation of responses to pleasant and mal- odour stimulation in human subjects; adaptation, dose response and gender differences*. International Journal of Psychophysiology, 48, 67-80

Jacquot L., Monnin J. and Brand G. (2004) *Influence of nasal trigeminal stimuli on olfactory sensitivity*. C. R. Biologies, 327, 305-311

Jaubert J.N., Tapiero C. and Dore J. (1995) *The field of odors: Toward a universal language for odor relationships*. Perfumer and Flavorist, 20, 1-16

Jehl C., Royet J.P. and Holley A. (1995) *Odor discrimination and recognition memory as a function of familiarization*. Perception & Psychophysics, 57, 1002-1011

Jinks A. and Laing D.G. (1999a) *A limit in the processing of components in odour mixtures*. Perception, 28, 395-404

Jinks A. and Laing D.G. (1999b) *Temporal processing reveals a mechanism for limiting the capacity of humans to analyze odor mixtures*. Cognitive Brain Research, 8, 311-325

Jinks A. and Laing D.G. (2001) *The analysis of odor mixtures by humans: Evidence for a configurational process*. Physiology & Behavior, 72, 51-63

Jinks A., Laing D.G., Hutchinson I. and Oram N. (1998) *Olfaction and taste xii, Temporal processing of odor mixtures reveals that identification of components takes precedence over temporal information in olfactory memory*. NEW YORK ACAD SCIENCES, New York. 834-836

Kaernbach C. (2001) *Adaptive threshold estimation with unforced-choice tasks*. Perception & Psychophysics, 63, 1377-1388

Karlsen A.M., Aaby K., Sivertsen H., Baardseth P. and Ellekjaer M.R. (1999) *Instrumental and sensory analysis of fresh norwegian and imported apples*. Food Quality and Preference, 10, 305-314

Keast R.S.J., Dalton P.H. and Breslin P.A.S. (2003) *Flavor interactions*. Abstracts of Papers of the American Chemical Society, 226, 163-AGFD

Kelly J.T., Prasad A.K. and Wexler A.S. (2000) *Detailed flow patterns in the nasal cavity*. Journal of Applied Physiology, 89, 323-337

Keyhani K., Scherer P.W. and Mozell M.M. (1995) *Numerical simulation of airflow in the human nasal cavity*. Journal of Biomechanical Engineering-Transactions of the Asme, 117, 429-441

Keyhani K., Scherer P.W. and Mozell M.M. (1997) *A numerical model of nasal odorant transport for the analysis of human olfaction*. Journal of Theoretical Biology, 186, 279-301

Kobal G. and Kettenmann B. (2000) *Olfactory functional imaging and physiology*. International Journal of Psychophysiology, 36, 157-163

Laing D.G. (1985a) *The characteristics of human sniffing behavior that provide optimum perception of odors*. Chemical Senses, 10, 412-412

Laing D.G. (1985b) *Optimum perception of odor intensity by humans*. Physiology & Behavior, 34, 569-574

Laing D.G. (1986) *Identification of single dissimilar odors is achieved by humans with a single sniff*. Physiology & Behavior, 37, 163-170

Laing D.G. (1988) *Relationship between the differential adsorption of odorants by the olfactory mucus and their perception in mixtures*. Chemical Senses, 13, 463-471

Laing D.G. and Francis G.W. (1989) *The capacity of humans to identify odors in mixtures*. Physiology & Behavior, 46, 809-814

Laing D.G., Legha P.K., Jinks A.L. and Hutchinson I. (2003) *Relationship between molecular structure, concentration and odor qualities of oxygenated aliphatic molecules*. Chem. Senses, 28, 57-69

Laing D.G., Link C., Jinks A.L. and Hutchinson I. (2002) *The limited capacity of humans to identify the components of taste mixtures and taste-odour mixtures.* Perception, 31, 617-635

Laing D.G. and Macleod P. (1992) *Reaction-time for the recognition of odor quality.* Chemical Senses, 17, 337-346

Laska M. (2002) *Olfactory discrimination ability for aromatic odorants as a function of oxygen moiety.* Chem. Senses, 27, 23-29

Laurent G., Stopfer M., Friedrich R.W., Rabinovich M.I., Volkovskii A. and Abarbanel H.D.I. (2001) *Odor encoding as an active, dynamical process: Experiments, computation, and theory.* Annual Review of Neuroscience, 24, 263-297

Lawless H.T., Thomas C.J.C. and Johnston M. (1995) *Variation in odor thresholds for l-carvone and cineole and correlations with suprathreshold intensity ratings.* Chemical Senses, 20, 9-17

Lehrner J.P., Brucke T., DalBianco P., Gatterer G. and KryspinExner I. (1997) *Olfactory functions in parkinson's disease and alzheimer's disease.* Chemical Senses, 22, 105-110

Lindemann B. (2001) *Receptors and transduction in taste.* Nature, 413, 219-225

Linforth R.S.T. (2000) *Developments in instrumental techniques for food flavour evaluation: Future prospects*. Journal of the Science of Food and Agriculture, 80, 2044-2048

Linforth R.S.T., Baek I. and Taylor A.J. (1999) *Simultaneous instrumental and sensory analysis of volatile release from gelatine and pectin/gelatine gels*. Food Chemistry, 65, 77-83

Linforth R.S.T., Martin F., Carey M., Davidson J. and Taylor A.J. (2002) *Retronasal transport of aroma compounds*. Journal of Agricultural and Food Chemistry, 50, 1111-1117

Linforth R.S.T. and Taylor A.J. (2000) *Persistence of volatile compounds in the breath after their consumption in aqueous solutions*. Journal of Agricultural and Food Chemistry, 48, 5419-5423

Linschoten M.R., Harvey L.O., Eller P.M. and Jafek B.W. (2001) *Fast and accurate measurement of taste and smell thresholds using a maximum-likelihood adaptive staircase procedure*. Perception & Psychophysics, 63, 1330-1347

Lobel D., Jacob M., Volkner M. and Breer H. (2002) *Odorants of different chemical classes interact with distinct odorant binding protein subtypes*. Chem. Senses, 27, 39-44

Lorig T.S. (2000) *The application of electroencephalographic techniques to the study of human olfaction: A review and tutorial*. International Journal of Psychophysiology, 36, 91-104

Lowe G. and Gold G.H. (1995) *Olfactory transduction is intrinsically noisy*. Chemical Senses, 20, 174-174

Lucchini R., Bergamaschi E., Smargiassi A., Festa D. and Apostoli P. (1997) *Motor function, olfactory threshold, and hematological indices in manganese-exposed ferroalloy workers*. Environmental Research, 73, 175-180

Macrae A.W. and Falahee M. (1995) *Theoretical note on a practical problem - effective screening of drinking-water for taints*. Food Quality and Preference, 6, 69-74

Maeztu L., Sanz C., Andueza S., Paz De Pena M., Bello J. and Cid C. (2001) *Characterization of espresso coffee aroma by static headspace gc-ms and sensory flavor profile*. Journal of Agricultural and Food Chemistry, 49, 5437-5444

McGurk H., and MacDonald J.W. (1976) *Hearing lips and seeing voices*. Nature, 264, 746-748

Martin N. (2002) *Sweet sour balance in champagne wine and dependence on taste/odour interactions*. Food Quality and Preference, 13, 295-305

Martonen T.B., Zhang Z.Q., Yu G.Q. and Musante C.J. (2001) *Three-dimensional computer modeling of the human upper respiratory tract*. Cell Biochemistry and Biophysics, 35, 255-261

Martonen T.B., Zhang Z.Q., Yue G. and Musante C.J. (2002) *3-d particle transport within the human upper respiratory tract*. Aerosol Science, 33, 1095-1110

Moskowitz H.R. (2003) *The intertwining of psychophysics and sensory analysis: Historical perspectives and future opportunities - a personal view*. Food Quality and Preference, 14, 87-98

Murphy C., Bacon A.W., Bondi M.W. and Salmon D.P. (1998) *Olfaction and taste xii, Apolipoprotein e status is associated with odor identification deficits in nondemented older persons*. Physiology, 26, 744-750

Murphy C., Morgan C.D., Geisler M.W., Wetter S., Covington J.W., Madowitz M.D., Nordin S. and Polich J.M. (2000) *Olfactory event-related potentials and aging: Normative data*. International Journal of Psychophysiology, 36, 133-145

Nahon D.F., Roozen J.P. and de Graaf C. (1998) *Sensory evaluation of mixtures of sodium cyclamate, sucrose, and an orange aroma*. Journal of Agricultural and Food Chemistry, 46, 3426-3430

Nahon D.F., Roozen J.P. and DeGraaf C. (1996) *Sweetness flavour interactions in soft drinks*. Food Chemistry, 56, 283-289

Normand V., Avison S. and Parker A. (2004) *Modeling the kinetics of flavour release during drinking*. Chemical Senses, 29, 235-245

O'Mahony M. (1986) Sensory evaluation of food. Statistical methods and procedures., Sensory multiple-difference testing, using the r-index to ascertain degrees of difference. Washington D.C. 389-397

O'Mahony M. (1992) *Understanding discrimination tests: A user friendly treatment of response bias, rating and ranking r-index tests and their relationship to signal detection*. Journal of Sensory Studies, 7, 1-47

O'Mahony M. and Rousseau B. (2003) *Discrimination testing: A few ideas, old and new*. Food Quality and Preference, 14, 157-164

O'Sullivan M.G., Byrne D.V., Jensen M.T., Andersen H.J. and Vestergaard J. (2003) *A comparison of warmed-over flavour in pork by sensory analysis, gc/ms and the electronic nose*. Meat Science, 65, 1125-1138

Overbosch P. (1986) *A theoretical-model for perceived intensity in human taste and smell as a function of time*. Chemical Senses, 11, 315-329

Overbosch P., Afterof W.G.M. and Haring P.G.M. (1991) *Flavor release in the mouth*. Food Reviews International, 7, 137-184

Overbosch P. and Dejong S. (1989) *A theoretical-model for perceived intensity in human taste and smell* .2. Temporal integration and reaction-times. Physiology & Behavior, 45, 607-613

Overbosch P., Dewijk R., Dejonge T.J.R. and Koster E.P. (1989) *Temporal integration and reaction-times in human smell*. Physiology & Behavior, 45, 615-626

Overbosch P., Vandenenden J.C. and Keur B.M. (1986) *An improved method for measuring perceived intensity time relationships in human taste and smell*. Chemical Senses, 11, 331-338

Owen C.M. and Patterson J. (2002) *Odour liking physiological indices: A correlation of sensory and electrophysiological responses to odour*. Food Quality and Preference, 13, 307-316

Owen C.M., Patterson J. and Simpson D.G. (2002) *Development of a continuous respiration olfactometer for odorant delivery synchronous with natural respiration during recordings of brain electrical activity*. Ieee Transactions on Biomedical Engineering, 49, 852-858

Pause B.M. and Krauel K. (2000) *Chemosensory event-related potentials (cserp) as a key to the psychology of odors*. International Journal of Psychophysiology, 36, 105-122

Pause B.M., Krauel K., Sojka B. and Ferstl R. (1999) *Is odor processing related to oral breathing?* International Journal of Psychophysiology, 32, 251-260

Philpott C., Goodenough P., Passant C., Robertson A. and Murty G. (2004) *The effect of temperature, humidity and peak inspiratory nasal flow on olfactory thresholds*. Clinical Otolaryngology, 29, 24-31

Pierce J. and Halpern B.P. (1996) *Orthonasal and retronasal odorant identification based upon vapor phase input from common substances*. Chemical Senses, 21, 529-543

Piggott J.R. (2000) *Dynamism in flavour science and sensory methodology*. Food Research International, 33, 191-197

Roberts D.D., Pollien P., Antille N., Lindinger C. and Yeretzian C. (2003) *Comparison of nosespace, headspace, and sensory intensity ratings for the evaluation of flavor absorption by fat*. Journal of Agricultural and Food Chemistry, 52, 3636-3642

Sakai N., Kobayakawa T., Gotow N., Saito S. and Imada S. (2001) *Enhancement of sweetness ratings of aspartame by a vanilla odor presented either by orthonasal or retronasal routes*. *Perceptual and Motor Skills*, 92, 1002-1008

Schieberle P. (1991) *Aroma compounds of popcorn*. *Journal of Agricultural and Food Chemistry*, 39, 1141-1144

Shimojo S. and Shams L. (2001) *Sensory modalities are not separate modalities: Plasticity and interactions*. *Current Opinion in Neurobiology*, 11, 505-509

Snow N.H. and Slack G.C. (2002) *Head-space analysis in modern gas chromatography*. *Trends in Analytical Chemistry*, 21, 608-617

Stevens D.A. and Lawless H.T. (1987) *Enhancement of responses to sequential presentation of oral chemical irritants*. *Physiology & Behavior*, 39, 63-65

Stevens J.C., Cain W.S. and Burke R.J. (1988) *Variability of olfactory thresholds*. *Chemical Senses*, 13, 643-653

Stevens J.C. and Cruz I.A. (1995) *Taste and touch in aging: Assessment by repeated threshold measures*. *Chemical Senses*, 20, 285-285

Stevens J.C., Cruz L.A., Marks L.E. and Lakatos S. (1998) *A multimodal assessment of sensory thresholds in aging*. Journals of Gerontology Series B-Psychological Sciences and Social Sciences, 53, P263-P272

Stevens J.C. and Dadarwala A.D. (1993) *Variability of olfactory threshold and its role in assessment of aging*. Perception & Psychophysics, 54, 296-302

Stevenson R.J. (2001) *Associative learning and odor quality perception: How sniffing an odor mixture can later the smell of its parts*. Learning and motivation, 32, 154-177

Stevenson R.J. and Boakes R.A. (2003) *A mnemonic theory of odor perception*. Psychological Review, 110, 340-364

Stevenson R.J., Prescott J. and Boakes R.A. (1999) *Confusing tastes and smells: How odours can influence the perception of sweet and sour tastes*. Chemical Senses, 24, 627-635

Stillman J.A. (2002) *Gustation: Intersensory experience par excellence*. Perception, Advanced online publication,

Sulmont C., Issanchou S. and Koster E.P. (2002) *Selection of odorants for memory tests on the basis of familiarity, perceived complexity, pleasantness, similarity and identification*. Chemical Senses, 27, 307-317

Takeoka G., Buttery R.G. and Ling L. (1996) *Odour thresholds of various branched and straight chain acetates*. Food Science and Technology-Lebensmittel-Wissenschaft & Technologie, 29, 677-680

Takeoka G.R., Buttery R.G., Ling L.C., Wong R.Y., Dao L.T., Edwards R.H. and Berrios J.D. (1998) *Odor thresholds of various unsaturated branched esters*. Food Science and Technology-Lebensmittel-Wissenschaft & Technologie, 31, 443-448

Tateyama T., Hummel T., Roscher S., Post H. and Kobal G. (1998) *Relation of olfactory event-related potentials to changes in stimulus concentration*. Evoked Potentials-Electroencephalography and Clinical Neurophysiology, 108, 449-455

Taylor A.J. (2002) *Release and transport of flavors in vivo: Physicochemical, psychological, and perceptual considerations*. Comprehensive reviews in food science and food safety, 1, 45-57

Taylor A.J. and Linforth R.S.T. (2001) *Modelling flavour release through quantitative structure property relationships (qspr)*. Chimia, 55, 448-452

Taylor A.J., Linforth R.S.T., Harvey B.A. and Blake B. (2000) *Atmospheric pressure chemical ionisation mass spectrometry for in vivo analysis of volatile flavour release*. Food Chemistry, 71, 327-338

Tegoni M., Pelosi P., Vincent F., Spinelli S., Campanacci V., Grolli S., Ramoni R. and Cambillau C. (2000) *Mammalian odorant binding proteins*. Biochimica et

Biophysica Acta (BBA) - Protein Structure and Molecular Enzymology, 1482, 229-240

Thesen T. and Murphy C. (2001) *Age-related changes in olfactory processing detected with olfactory event-related brain potentials using velopharyngeal closure and natural breathing*. International Journal of Psychophysiology, 40, 119-127

Tsai R.C. and Bockenholt U. (2001) *Maximum likelihood estimation of factor and ideal point models for paired comparison data*. Journal of Mathematical Psychology, 45, 795-811

Tsukatani T., Miwa T., Furukawa M. and Costanzo R.M. (2003) *Detection thresholds for phenyl ethyl alcohol using serial dilutions in different solvents*. Chemical Senses, 28, 25-32

Turin L. (2002) *A method for the calculation of odor character from molecular structure*. Journal of Theoretical Biology, 216, 367-385

Voirol E. and Daget N. (1986) *Comparative study of nasal and retronasal olfactory perception*. Lebensmittel Wissenschaft und Technologie, 19, 316-319

Vuilleumier C., Cayeux I. and Velazco M.I. (2000) *Dose-response curves of odor and taste stimuli: Influence of sweetening agents*. Abstracts of Papers of the American Chemical Society, 219, 145-AGFD

Walker J.C., Hall S.B., Walker D.B., Kendal-Reed M.S., Hood A.F. and Niu X. (2003) *Human odor detectability: New methodology used to determine threshold and variation*. *Chemical Senses*, 28, 817-826

Walker J.C., Kurtz D.B., Shore F.M., Ogden M.W. and Reynolds J.H. (1990) *Apparatus for the automated measurement of the responses of humans to odorants*. *Chemical Senses*, 15, 165-177

Wang L.W., Walker V.E., Sardi H., Fraser C. and Jacob T.J.C. (2002) *The correlation between physiological and psychological responses to odour stimulation in human subjects*. *Clinical Neurophysiology*, 113, 542-551

Welge-Lussen A., Wolfensberger M., Kobal G. and Hummel T. (2002) *Basics, methods and indications for objective olfactometry*. *Laryngo-Rhino-Otologie*, 81, 661-667

Wilkinson G. and Yuksel D. (1997) *Using artificial neural networks to develop prediction models for sensory-instrumental relationships; an overview*. *Food Quality and Preference*, 8, 439-445

Wilson D.A. and Stevenson R.J. (2003) *The fundamental role of memory in olfactory perception*. *Trends in Neurosciences*, 26, 243-247

Wolfensberger M., Schnieper I. and Welge-Lussen A. (2000) *Sniffin' sticks: A new olfactory test battery*. *Acta Otolaryngology*, 120, 303-306

Zhao K., Scherer P.W., Hajiloo S.A. and Dalton P. (2004) *Effect of anatomy on human nasal air flow and odorant transport patterns: Implications for olfaction*. *Chemical Senses*, 29, 365-379

Zhou M., Robards K., Glennie-Holmes M. and Helliwell S. (2000) *Contribution of volatiles to the flavour of oatmeal*. *Journal of the Science of Food and Agriculture*, 80, 247-254