6 CASE STUDY 2: Detection of ethyl acetate in wine

6.1 Introduction

Field asymmetric ion mobility spectrometry (FAIMS) can be used with other analytical techniques such as gas chromatography (GC) and mass spectrometry (MS) to enhance their performance. This improvement results from GC and MS separating compounds by different criteria to that of ion mobility, and so is complimentary through analysis.

The application of the Owlstone FAIMS sensor as a GC detector was investigated with regard to the detection of ethyl acetate within wine. This was to provide a focus and real world relevance to the study. Additionally, using lessons learnt from previous studies regarding the modification of the carrier flow, the ability to tailor an investigation to a single compound in a complex mixture was pursued.

6.2 The grading of wine

The global wine industry is worth billions of pounds [1-3], particularly the mass market to the interested public. Wine guides and reviews are often consulted by consumers to inform their selection of the wines for purchase. While there is broad agreement on the quality of a wine, in coarse terms (*e.g.* poor, good, very good or outstanding), between experts there is often disagreement of the particular ordering of wine within each subsection. This is not surprising considering the complexity of wines and the personal subjectivity that can accompany any grade given [4]. Robert Parker, the man responsible for first giving wine a mark on a hundred point scale, is quoted as saying [5],

"I really think probably the only difference between a 96-, 97-, 98-, 99-, and 100-point wine is really the emotion of the moment."

Further to this, consumers and experts alike can dramatically be swayed in their perception of a wine's smell and taste by visual cues. Studies have taken white wine and coloured them with an odourless dye, to disguise them as a rosé or red wine, and asked panels to report the sweetness of the wine. The researchers found that participants often discounted olfactory information in preference of visual information and drew further perceptions of varying sweetness despite all the wines presented to them being identical [6, 7]. Conversely, the identification of white and red wines was successfully accomplished when the wines were presented in dark glasses, where there was no additional visual clue. Rosé wine was still unsuccessfully identified [8].

The ability of skilled, and non-skilled, wine evaluation has been further tested with regards to their detection of particular scents and discrimination between sample wines. It was found that experts only surpassed novices in a small number of samples and even then they only correctly identified 76% of the test scents (perceived as strong, not threshold quantities) [9, 10]. Additionally, when presented with three test wines, two of which were identical and the third similar to the other two, experts correctly identified the unique wine approximately 70% of the time. Non-experts only identified the correct wine the same number of times as expected by chance alone.

It has also been shown that people can struggle to recognise even a small number of compounds within a solution. One such investigation, assessing the capacity of humans to identify odours in a mixture, presented one to five common but non-similar compounds to over a hundred and twenty test subjects [11]. It was found that correctly identifying even

two compounds within a mixture was extremely difficult for the participants. A plot from that study is presented in Figure 6.1.

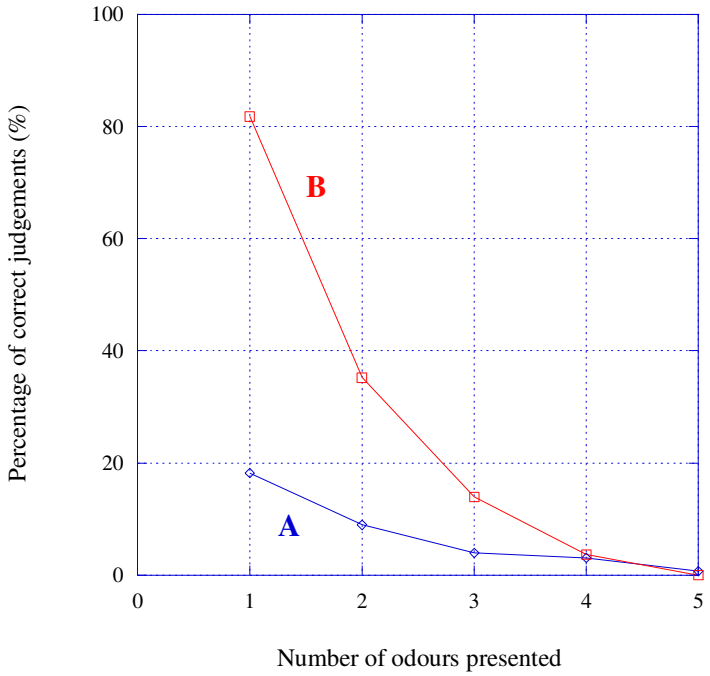


Figure 6.1 Percentage of judgements correctly identifying the components of stimuli consisting of 1-5 odourants. Function A indicates the percentage of times that the correct odour(s) were entirely unsuccessfully selected. Function B shows the percentage of times that the correct odour(s) were entirely selected successively. Originally from Laing and Francis [12].

The ambiguity and variation in the ability of the scent evaluation within these studies suggest that there is scope for a more standardised and analytical approach to inform the grading of wine, one which is removed from personal perceptions. While a complete methodology to implement such an enterprise is beyond the limits of this investigation, the aim was to demonstrate whether a technique could be devised which could quickly determine specific components. The real-time monitoring of these components could then be used to judge a wine's suitability.

This study specifically investigated whether a FAIMS system could quantify the presence of ethyl acetate in wine. This single compound is recognised as having both a positive and negative presence within wine depending on its concentration level. Since the grade given

to a wine can influence its eventual price [13], any information should be of value to the producer.

6.3 Ethyl acetate in wine

It is accepted that the presence of too much ethyl acetate within wine is not desirable [14-21]. An aroma similar to acetone is present if the concentration of ethyl acetate exceeds a threshold. This threshold is most often quoted as being between 100 - 200 mg/l [17, 19-21] but one source did mention a limit as low as 7.5 mg/l [15]. This large range was further supported by the outcome of an investigation into the threshold levels of ethyl acetate, where participants appeared to fall into one of two groups with different sensitivities to the compound [11]. The former threshold is by far the most widely reported.

It has also been reported that increasing the concentration of ethyl acetate generally has a suppressive effect upon the formation of other compounds responsible for a fruity aroma, even before the sensory threshold has been breached [22]. However, the presence of ethyl acetate within wine is not universally to the detriment of the wine. Others report that a concentration below the sensory threshold can add a depth of body, richness and sweetness to a wine and is therefore sometimes desirable [18, 19]. Another source states that at concentrations between 50 - 80 mg/l ethyl acetate contributes to the hard character of a wine and becomes part of the pleasant bouquet of red wines [23].

Ethyl acetate can be formed in wine through the action of yeast or separately via esterification. Yeasts, present throughout fermentation, normally create the majority of ethyl acetate within a wine. The final concentration created in this way is dependent upon the species of yeast and the initial constituents of the wine [18, 21].

As stated, ethyl acetate can also continue to be formed through the acid-catalysed esterification of ethanol and acetic acid (Equation 6.1) and this is one of the contributing reasons why ethyl acetate is the most abundant ester found within wine. Due to the weak acids found within wine, esterification is not the principal mechanism for the presence of ethyl acetate [24].

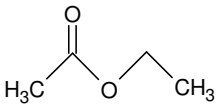
$$CH_3COOH + C_2H_5OH \overset{H^+}{\rightleftharpoons} CH_3COOC_2H_5 + H_2O \qquad 6.1$$

Given that a concentration of ethyl acetate below the human detection threshold has been recognised as a positive constituent of wine, it is desirable that a simple quantification of the compound should be available so its concentration can be ascertained at any stage of wine manufacture. While it is difficult to detect the individual flavours associated with ethyl acetate, they are often some of the first components of a wine to be recognised [10]. Therefore, the ability to monitor the evolution of the compound may enable the ability to produce excellent wine while restricting its presence to below the level where its presence becomes negative.

6.4 Ethyl acetate

Ethyl acetate (systematic name ethyl ethanoate) is the focus of this chapter and a brief summary of the compound's properties are summarised in Table 6.1.

Table 6.1 General properties of ethyl acetate

Properties	
Molecular formula	$C_4H_8O_2$
Molar mass	88.11 g/mol
Density	0.897 g/ml
Boiling point	77.1°C
Structure	

As stated previously, the ^{63}Ni ionisation source of the Owlstone FAIMS unit preferentially ionises compounds with a high proton affinity (Section 1.4). As shown in Table 6.2, ethyl acetate, being an ester, has a greater proton affinity than either water or ethanol (abundant compounds within wine).

Table 6.2 List of proton affinities

Compound	Proton affinity (kJ/mol)
Ethanol	776.4 [25]
Ethyl acetate	835.7 [25]
Water	691 [25]

6.5 Experimental set-up

The experimental set-up used for the detection of ethyl acetate in wine has been previously detailed in Section 3.10. The apparatus did undergo some modification through the study, such as the implementation of a separate GC column and reconfiguration to accommodate a greater pressure of carrier flow for the FAIMS unit. Details of such changes will be highlighted as they occur through the remainder of this chapter.
Solutions were prepared in 10 ml volumetric flasks using proline mechanical pipettes (10 - 100 and 100 - 1000 µl capacity, Fisher brand) and a glass syringe (1 - 10 µl capacity, SGE). Distilled water used in solutions was obtained from a Direct-Q Ultrapure Water System (Millipore) which utilised 0.22 µm filters.

As anticipated, based on relative boiling points and polarity, ethyl acetate was among the first compounds present in wine to elute off the GC column.

6.6 Preliminary work

Before the detection of ethyl acetate was attempted brief optimisation of the settings of the FAIMS unit and GC were undertaken. The dispersion field (DF) settings were held constant throughout testing as running of the FAIMS unit in continuous mode enabled a better time resolution for the discrete elutions from the GC (Section 6.7).

During optimisation, the volume injected was 1 µl (liner capacity: 3 ml), from a solution of 10 ml of distilled water spiked with 1 µl ethyl acetate (≥ 99%, Fisher Scientific). The injection was into the apparatus operating with an ambient pressure carrier flow to the FAIMS unit.

Please note, throughout this chapter, when the carrier flow is referred to as being at ambient pressure it means that the carrier flow was exhausted at ambient pressure. When the carrier flow is referred to as being at a different pressure it means that a restriction was placed in-line, on the exhaust of the FAIMS unit (Section 3.4.1.6) elevating the pressure to the stated value.

6.6.1 Optimisation of DF and carrier flow

The desired DF value was one that provided adequate separation of ion responses without large losses due to diffusion. The DF was initially coarsely adjusted by 10% of full scale but then smaller steps (1%) were used to discover that the most appropriate level was 38% of the full DF strength obtainable. A DF field lower than 38% resulted in a greater intensity of ion response, but the ion signal resulting from the ethyl acetate could not be inferred, due to mixing with other ion responses, such as those from reactant ions and column bleed. With a DF field greater than 38% the ion intensity from ethyl acetate enjoyed better resolution from the other ion responses present but the ion intensity began to be lost.

The carrier flow to the FAIMS unit is another way of increasing the ion intensity detected, the greater the flow the greater the resultant signal. Increased flow, however, results in a greater broadening of the ion response and has a detrimental effect upon the resolution. It was found empirically that the loss of resolution through increased flow was not as severe as through reducing the DF. Therefore increasing the flow, at a DF of 38%, still enabled the resolution of ethyl acetate. This meant that carrier flow was constrained by the operational limit of the apparatus and not any analytical constraints. The flow rate range tested was 1000 - 3000 ml/min, initially in increments of 500 ml/min and then 50 ml/min.

The maximum flow that could be provided from the mass flow controller used was 3000 ml/min. However, flow to the FAIMS sensor was finally set at 2750 ml/min. A lower flow than the maximum possible was selected so that the equipment was not at an absolute limit and allowed some range either side of the standard operating value should a suitable situation through investigation arise.

6.6.2 GC column temperature and splitless injection

The temperature of the GC column was set isothermally at 70°C, to imitate the conditions used by a published method for the separation of compounds within alcoholic beverages [26]. A more complicated thermal scheme was not implemented due to the constraints imposed by the available software (also, the intention was to provide a simple and widely employable method). The temperature of the injector was also isothermal and set at 235°C to ensure all the analyte was made gaseous and that no sample could condense.

Injection of the sample into the GC was through a splitless injection. The liner used had a capacity of 3 ml so a vapourised injection of 1 µl could be easily accommodated. Without the high flow available through a split injection, a column flow of 1.5 ml/min, at a head pressure of 14 psi (gauge), was used (pressure of the injector will be stated in psi to better differentiate from the carrier flow pressure in the FAIMS unit, stated in kPa). Following optimisation the injection volume employed was reduced to 0.5 µl. With the relatively low injector flow, compared to splitless injection, a high concentration of compounds in the liner would have led to a larger bandwidth of constituents on the column. The change helped improve the resolution between compounds as it led to a decrease in the eventual tailing of compounds from the GC.

Additionally, having a large temperature difference between the injector and column encouraged sample to condense at the front of the column aiding chromatography by focussing the analyte into a narrower bandwidth. The transfer line between the GC oven and FAIMS unit was held at 120°C, elevated above the main GC column temperature to ensure migration in to the FAIMS unit.

This preliminary optimisation was not exhaustive but did provide a consistent and uncomplicated system to operate and maintain. Those familiar with GC will recognise that there are a great number of ways with which the chromatography could be improved within this study. Further GC optimisation was not pursued for three reasons - (1) the operation of the FAIMS unit is of greatest interest to this investigation; (2) a fully optimised GC method would provide very tight compound elutions from the column, resulting in a shorter available time to accomplish compensation voltage (CV) sweeps. In contrast to the use of traditional GC detectors, achieving multiple CV sweeps across an eluting peak required that the chromatography had to be degraded from its optimal performance; (3) the application of interest involves the FAIMS system being used outside the laboratory. Utilising a simple isothermal temperature profile, splitless injection and direct injection of sample would better demonstrate the possibility of translation for use in the field.

6.7 Data collection

Compounds typically elute from the GC column over a period of seconds. In comparison a single CV sweep, for positive and negative ions, takes approximately one second. Therefore a major consideration was to obtain several data points across an analyte peak to better characterise a response and to ensure that the response recorded was truly

representative (*e.g.* response has not been 'clipped' through sampling). A DF sweep across an elution would further enhance the data returned; however, due to sampling speeds, the synchronisation required and the inability to collect multiple data points from a peak under identical conditions, it was not pursued through this investigation. The FAIMS unit was therefore held at a continuous DF strength to maximise the number of data points from an elution. The particular DF strength used was selected through the optimisation process summarised in Section 6.6.1.

The full response from the GC-FAIMS system was therefore dependent upon the retention time of the compounds through the GC, the CV of the ion response and the ion intensity recorded by the FAIMS unit. An example of the data returned is given in Figure 6.2

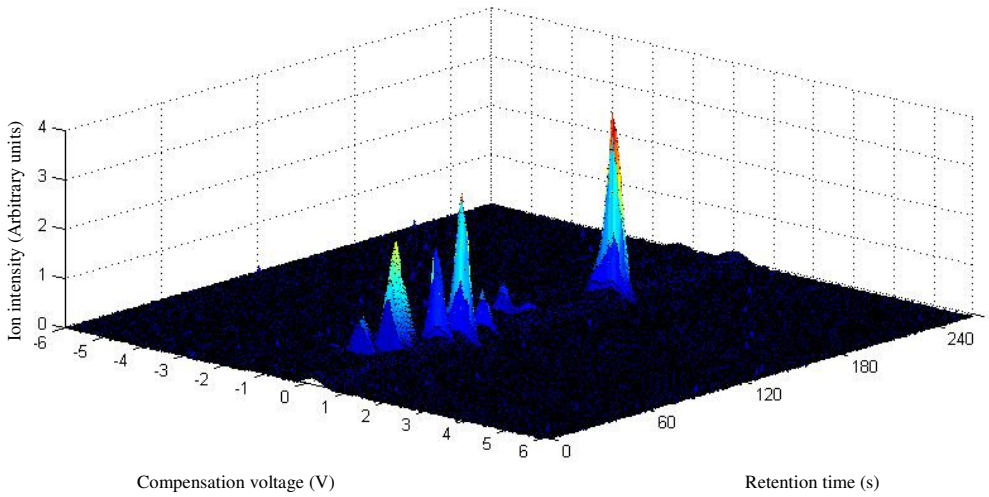


Figure 6.2 Surface plot of data obtained over 267 seconds from a 0.5 µl injection of wine into the GC-FAIMS system in the positive mode.

The large amount of information contained within each analytical run was reduced before further investigation. Each three dimensional plot was assessed to isolate the CV value that described the maximum response from the analyte of interest. With this characteristic property known the data could be re-plotted as a conventional two dimensional chromatogram at the characteristic CV value. Integrating the areas of the peaks obtained

from these reduced spectra enabled the response of the selected analyte to be determined. Although much of the original data set was ignored during this process the characteristic CV position was recorded so an element of the additional FAIMS separation was retained. The area of the ion response was found through a constructed Matlab program which removed the baseline response and obtained the area through a Monte-Carlo method [27].

A demonstration of how the FAIMS sensor, when used as a GC detector, could improve sensitivity and selectivity of specific compounds is presented in Figure 6.3. The chromatograms provided are from CV values that provided a maximum intensity for the first and second peaks from the full ion response as given in Figure 6.2.

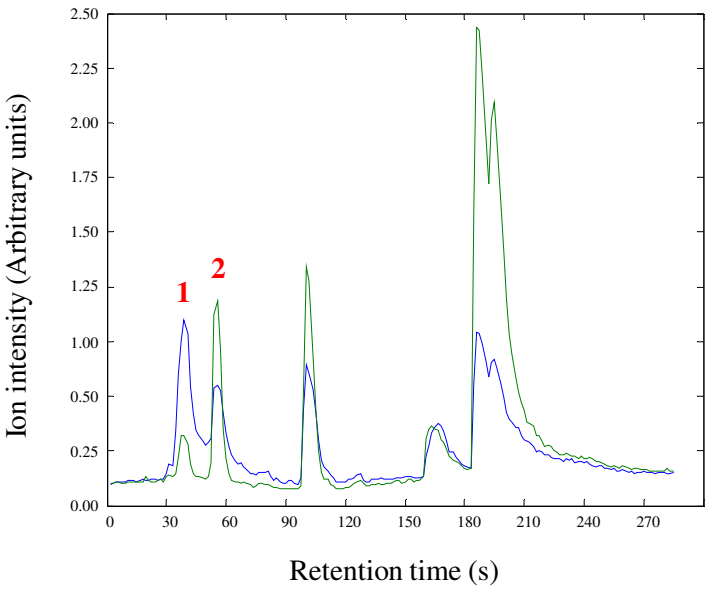


Figure 6.3 Two plots of ion response versus retention time at specific CV values of a full GC-FAIMS response are shown. The CV values isolated were selected as being characteristic of the peak ion response of the first peak (blue) and second peak (green).

It can be seen from Figure 6.3 that by selecting an appropriate CV value, to isolate ion response, a clearer analyte response can be achieved. For example if the second peak represented the analyte of interest the CV value characteristic of its peak response not only

results in a greater ion intensity but also baseline resolution. This extra specificity is a result of the orthogonal separation between the GC and FAIMS.

While data throughout this chapter was predominantly collected through the method described above, full CV spectra at a single retention time were also occasionally explored. Where data is described in such a manner it will be explicitly stated, otherwise all data is a result of observing the ion intensity against the retention time of the GC at a specific CV.

It is also important to comment on the reactant ion population as compounds elute from the column. Normally a constant population of reactant ions is initially present and they get converted to product ions as material passes through the ionisation region, thereby reducing their population. Compounds may have a small concentration within a solution but because the GC focuses the elution of compounds within a short space of time they will have a much higher concentration within the FAIMS sensor. It is therefore the case that as material elutes from the column it is possible to lose the entire population of reactant ions. This saturation of the reactant ions can lead to an underestimate of the population of analyte. To mitigate against this, the injection volume was reduced from 1.0 to 0.5 µl and the area of a response was recorded as opposed to its amplitude. Another consequence was that the high analyte concentration during elution encouraged the formation of dimers. Following the maximum of analyte elution the reactant ions return.

6.8 Initial testing

Following optimisation, testing was undertaken to evaluate the capability of the GC-FAIMS system to detect ethyl acetate. The objectives of these initial tests were to characterise the typical response to ethyl acetate and whether its limit of detection (within a

simple solvent), was below the threshold of human perception. The operational settings of the GC-FAIMS system during these initial tests is given in Table 6.3.

Table 6.3 Operational settings of initial testing for the detection of ethyl acetate

Operational parameter	Quantity
GC column	MTX-5 (15 m × 0.25 mm × 1 μm) (5% diphenyl)
GC temperature profile	70°C, isothermal
GC carrier gas	Nitrogen
Liner capacity	3 ml
Column pressure	14 psi
Injector temperature	235°C
Injector	Splitless
Injection volume	0.5 μl
Transfer line temperature	120°C
FAIMS sensor carrier flow	2750 ml/min air
DF field strength of FAIMS unit	38% of maximum
Pressure of FAIMS carrier flow	One atmosphere
Syringe cleaning	3 washes in methanol prior to any injection

The column used within the GC was a MTX-5 (Thames Restek UK Ltd.); it was selected for its low column bleed [28]. Such a column is typically employed for general purpose applications in solvent impurities and semi volatiles [29]. The column is noted as being flexible, rugged and inert. The low polarity of the column also meant the system was better suited to dealing with steam, resulting from samples where water was the solvent.

6.8.1 Distilled water spiked with ethyl acetate

The first solution that was put through the GC-FAIMS system was distilled water which had been spiked with various concentrations of ethyl acetate. Distilled water was used as the solvent since wine is predominantly composed of water (~ 88%). A stock solution was made which had an ethyl acetate concentration of 358.8 mg/l (well above the human perception threshold). This stock was then serially diluted to produce a concentration range over two orders of magnitude and which had a final ethyl acetate concentration of 5.6 mg/l. All injections were repeated in triplicate and the error presented in Figure 6.4 is a single standard deviation of those triplicate readings.

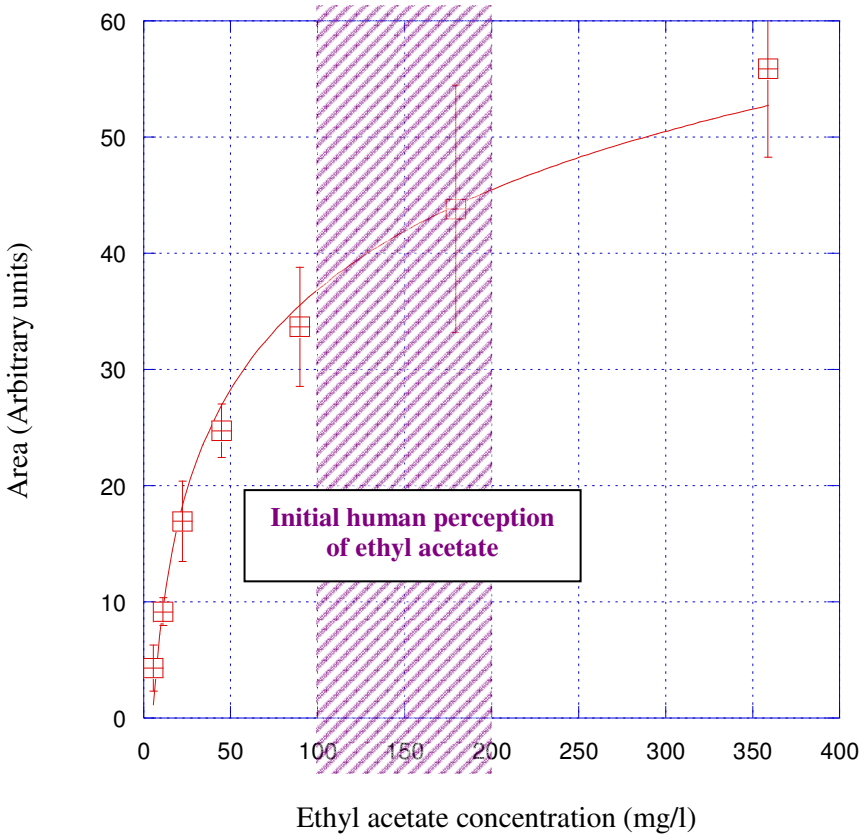


Figure 6.4 The response obtained in the GC-FAIMS system from spiked samples of distilled water with various concentrations of ethyl acetate.

The trend line in Figure 6.4 is logarithmic and has been added to help trace the response.

The form of the ion response, across the analyte concentration shown in Figure 6.4, is typical of that found within an ion mobility device, as the ion response is dependent upon the abundance of reactant ions. As analyte concentration increases more reactant ions are converted to product ions and the reactant ion population eventually decreases. This decrease in the reservoir of reactant ions means it becomes more difficult for remaining analyte to form product ions resulting in a non-linear response across a large analyte concentration range. Other ion mobility studies within the literature demonstrate similar behaviour; *e.g.* work undertaken with an ion mobility spectrometer by Smith *et al*. [30].

The clear response of the system down to a level, by an order of magnitude, below the typically recognised human perception threshold of ethyl acetate was encouraging with respect to the detection of ethyl acetate within wine.

From Figure 6.4, the errors reported increase with the concentration analyte. This was attributed to the formation of ions being dependent upon the random interactions in the ionisation region. Such interactions can result in a reactant ion colliding with an analyte molecule and forming a product ion. When the analyte concentration is low the variation possible within the population, between successful and unsuccessfully created product ions, is more limited compared to when there is a large analyte concentration. The errors from triplicate readings are expected to increase with respect to analyte concentration until the point that 50% of the sample population is analyte.

The reproducibility of the response (and hence the apparatus) was also investigated by repeating the experiment two weeks after the initial run using newly made solutions. Data from this follow up investigation and that of the initial work is presented within Figure 6.5.

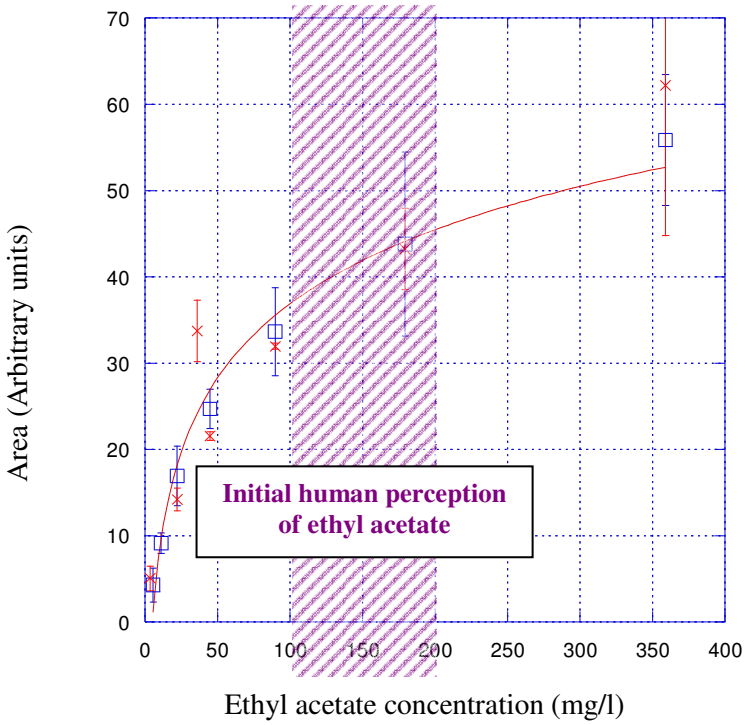


Figure 6.5 Responses obtained from samples of distilled water spiked with various concentrations of ethyl acetate from investigations undertaken two weeks apart (blue squares and red crosses).

The results (with the exception of one outlier) suggest that the set-up and experimental procedure were reproducible within the errors specified. The errors presented are a single standard deviation of repeated triplicate readings.

6.8.2 Wine spiked with ethyl acetate

To investigate further the ability to detect and measure the presence of ethyl acetate within wine a simple and cheap white wine (Co-Op, Chilean) was purchased and used as the solvent. White wine is known to have the lowest level of ubiquitous ethyl acetate compared to either red or rosé wine [15, 22]. Also, it was assumed that the background signal would be less complicated than red wine, with its associated rich flavour and body. As ethyl acetate was known to be present within the solvent a background abundance had to be characterised, which was later subtracted from the spiked sample responses. This background signal was obtained through repeating triplicate ethyl acetate responses from

0.5 µl injections of the wine into the GC-FAIMS system (average response of 12.6 A.U (3 s.f.) and standard deviation of 1.2).

The spiked wine samples were diluted from a single stock, as previously when distilled water was the solvent. The apparatus and data collection were also run in an identical manner. The responses obtained from triplicate injections, following subtraction of the ethyl acetate background, are displayed in Figure 6.6.

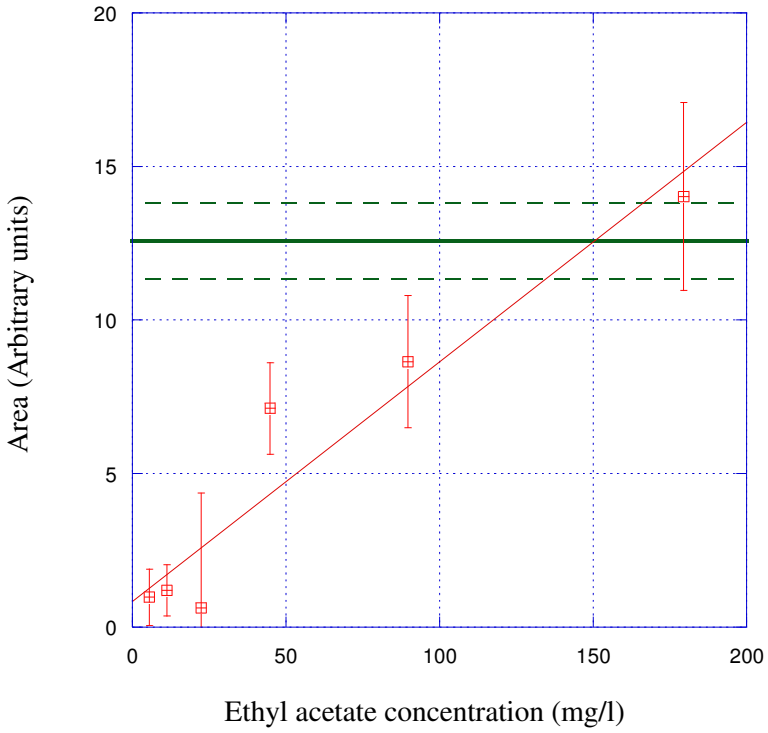


Figure 6.6 The response obtained in the GC-FAIMS system from samples of wine spiked with different concentrations of ethyl acetate minus the response from un-spiked wine (red crossed squares). The average response (solid green) and limits from a single deviation of triplicate readings (dashed green) from un-spiked wine is also given.

The trend line in Figure 6.6 is linear and has been added to help trace the response.

In this scenario the response from ethyl acetate was greatly diminished compared to those obtained when distilled water was used as the solvent. Where Figure 6.4 displayed behaviour typical of an abundance of analyte, Figure 6.6 behaves as if a small concentration of analyte is present [30].

The retention times and CV positions of the ion responses associated with ethyl acetate were unchanged in the more complex solvent. This indicates that the ions observed with distilled water were identical to those in wine. The formation of these ions was, however, greatly attenuated. It was hypothesised that this attenuation was a result of the action of additional constituents present in the wine.

Additionally, the observed level of ethyl acetate within the un-spiked wine suggested that it could be regarded as spoilt. The wine stock had been opened prior to acquiring this data so it is possible that exposure to oxygen allowed bacteria present to form acetic acid and increase the concentration of ethyl acetate within the wine through esterification (Equation 6.1). It is therefore likely that the initial detected concentration of ethyl acetate, if this data was collected immediately following the opening of the wine, would have been below the human perception threshold.

6.9 Reason for reduction in signal

To understand the reasons for the attenuation of the ethyl acetate signal the compounds responsible for separate ion responses had to be confirmed. This had previously been accomplished for ethyl acetate as samples composed exclusively of ethyl acetate in distilled water had been made and could be compared to the results from wine. Ethanol, as a major constituent of wine was also tested in the same way; the outcome is given in Figure 6.7. The solutions of ethanol (≥ 99.8%, Fisher Scientific) and ethyl acetate were made up to have similar concentrations to those found within wine. The ion responses were also modified to start from zero to aid comparison.

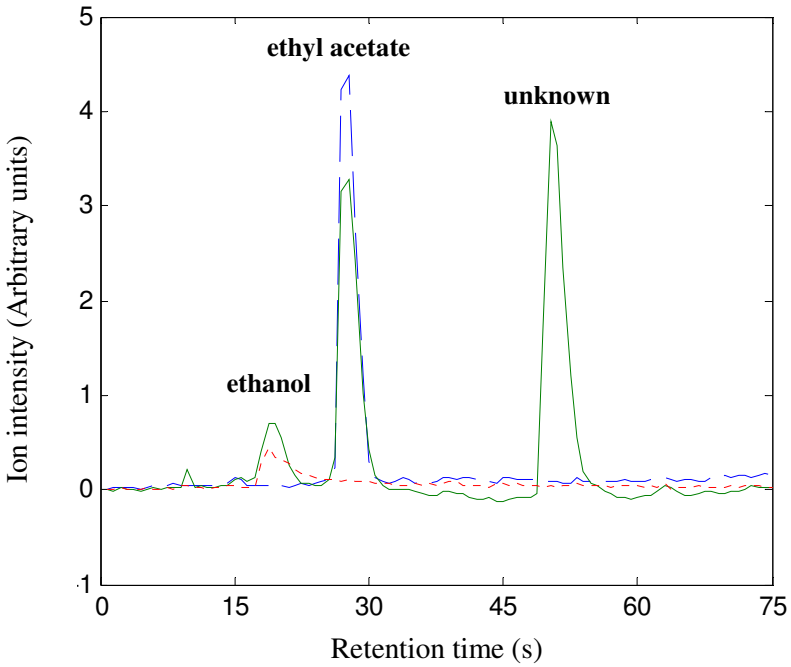


Figure 6.7 Signal isolated at peak response of ethyl acetate for solutions of ethyl acetate in distilled water (blue dashed), ethanol in distilled water (red dashed) and wine (green solid). Labels are the inferred compounds responsible for the signal.

From Figure 6.7 it was inferred which ion responses from wine result from ethanol and ethyl acetate.

Since the initial response of ethyl acetate within distilled water was so successful (Section 6.8.1) and because water is known to be the most abundant compound within wine, it was incorrectly assumed that the remaining constituents found within wine would have a minimal effect. Ethanol is however extremely abundant within wine (typically ~ 12% by volume) and should therefore be treated as a co-solvent. Ethanol, unlike water and most constituents, elutes from the GC before ethyl acetate. From considering the output of the GC-FAIMS unit, when considered from the CV resulting in the maximum ethyl acetate signal, the response attributable to ethanol is typically an order of magnitude below that of ethyl acetate, despite its abundance. This outcome is understood through appreciating the selectivity of the ionisation (Table 6.2) and that the CV value used for isolating the ethyl acetate is not optimum for detecting ethanol. This means that the ion response of ethanol

and ethyl acetate is not mixed within the output from the GC-FAIMS but it is still possible for the two compounds to be present alongside one another in the ionisation region of the FAIMS sensor, with ethanol in higher abundance.

Given the above, it was hypothesised that the presence of ethanol within the ionisation region, in a large enough concentration, would prevent the full ionisation of the ethyl acetate. To investigate whether this was true solutions of distilled water and 12% by volume ethanol were made up and spiked with ethyl acetate, to the same concentrations as in the initial studies. Results from the detection of ethyl acetate in distilled water, wine and distilled water and 12% ethanol are plotted together in Figure 6.8.

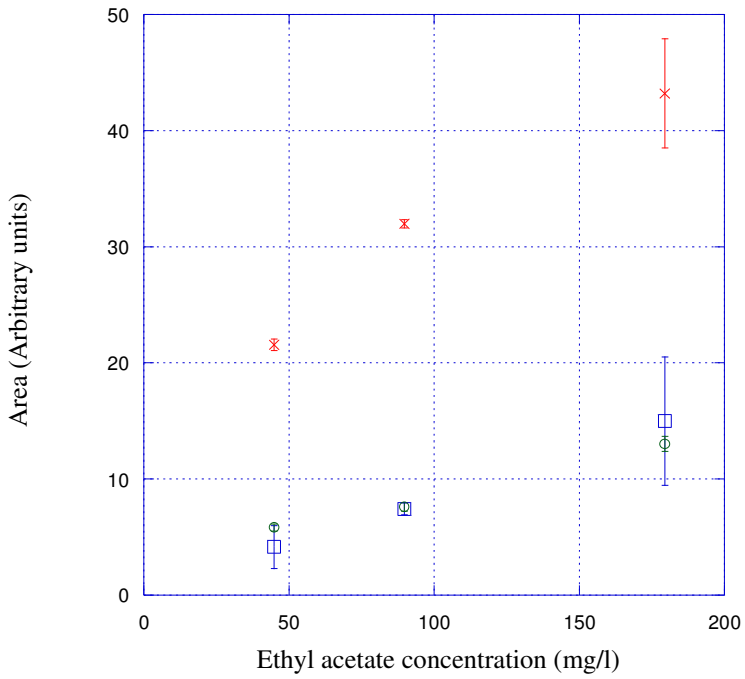


Figure 6.8 Ethyl acetate spiked solutions with various solvents; Distilled water (red), distilled water and 12% ethanol (blue) and wine (green).

What the above responses reveal is that the presence of ethanol greatly diminishes the sensitivity of the GC-FAIMS system to ethyl acetate. Furthermore it appears that the presence of ethanol is the dominant reason for the attenuation of sensitivity as areas at the three concentrations tested all equalled one another within a single standard deviation.

It transpires that the detection of ethyl acetate within alcoholic beverages is also complicated by the presence of ethanol in investigations using metal oxide semiconductor detectors [31-33]. It is obvious that more must be done to separate not only the ethanol and ethyl acetate within the ion response but also within the ionisation region of the FAIMS sensor.

The most straight forward way of completing this was through increasing the time between elution of the ethanol and ethyl acetate from the GC column. This was accomplished through the substitution of the low polarity MXT-5 column with one with a greater polarity. Ethanol, water and ethyl acetate are polar compounds that separate best on a column which better matches their polarity. In addition, extra column length and a thicker stationary phase will further increase the time that compounds remain in contact with the stationary phase, again increasing the separation between the compounds. The disadvantage of changing the column for one with the characteristics stated is that the analysis time will be extended since all the compounds will take longer to elute. A BP-624 (30 m × 0.25 mm × 1.4 μm, SGE) column was installed into the SRI GC but all other settings were maintained.

A stock solution of ethyl acetate spiked into wine was serially diluted to produce solutions with the same ethyl acetate concentration used previously (Section 6.8.1). Injections were made at each dilution and the responses for ethyl acetate using the polar column are presented in Figure 6.9, along with responses obtained using the non-polar column.

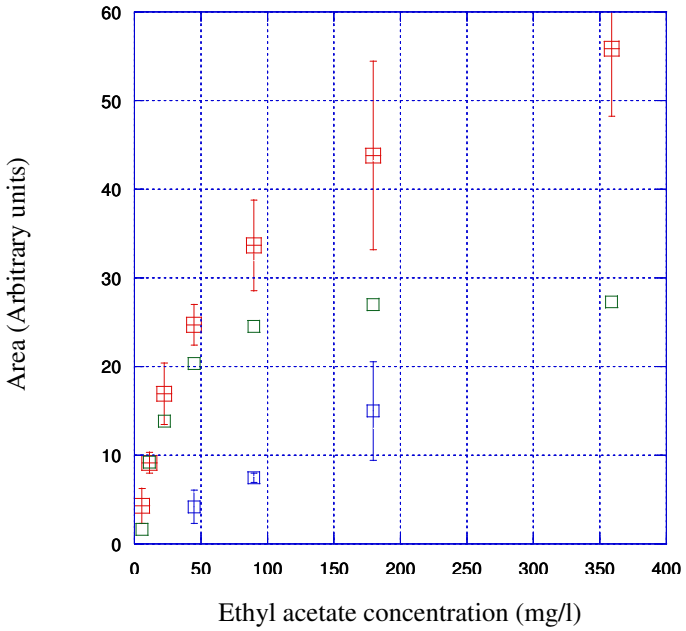


Figure 6.9 The response obtained for ethyl acetate within distilled water (red), and wine (blue + green). The red and blue data was obtained with a non-polar column and the green data was obtained with a polar column.

The errors presented in Figure 6.9 are a single standard deviation of repeated triplicates. The investigation with the polar column was not repeated in triplicate and there is no error reported.

The data presented is further evidence that the attenuation of the sensitivity of the system is attributable to ethanol. As anticipated the polar column resulted in greater temporal separation of ethanol from ethyl acetate, which meant that during the period the ethyl acetate eluted a smaller concentration of co-eluting ethanol was present within the ionisation region. More of the reactant ions are therefore available to go on to form the product ions with ethyl acetate. However, ethanol has not been eradicated from the ionisation region at the time that ethyl acetate elutes, as demonstrated from the plateau of response as the concentration of ethyl acetate increases. At low ethyl acetate

concentrations (< 50 μg) the response obtained from the polar column compares favourably to the response obtained when using a solvent of only distilled water. At low concentrations it is proposed that there are enough available reactant ions to combine with all of the high proton affinity analyte. The change of column has therefore recovered lost sensitivity at low ethyl acetate concentrations; however, the system does not have the same dynamic range. At higher concentrations competition with ethanol has again become important and has resulted in suppression of the potential response.

The retention time of ethyl acetate, within the polar column was approximately three times longer than within the non-polar column. In addition to this direct impact upon analysis time the clean down time between injections was also increased, as it took longer for all constituents present to elute. With a complex solution, such as wine, the clean down time became so long that it necessitated the column oven temperature to be increased to 200°C, to increase the rate of elution from the column following the detection of analytes of interest. The thermal environment was again stabilised at the required experimental conditions before another injection. The total cycle time using the polar column was approximately one hour, roughly three times as long compared to investigations with the non-polar column.

6.10 Further optimisation of the analysis of ethyl acetate

The work thus far had demonstrated that ethyl acetate could be detected with the GC-FAIMS system at concentrations that were applicable to the study of ethyl acetate within wine. It was also found that the high abundance of ethanol in the wine had a major effect upon the response for ethyl acetate but it could be recovered by ensuring a sufficient reservoir of reactant ions remained to create the product ions. This was achieved by

increasing the temporal resolution of ethyl acetate and ethanol through the selection of a more appropriate GC column phase.

Previous investigations (Chapter 5) have indicated that modifying the pressure of the carrier flow to the FAIMS unit had beneficial effects. A study was devised to investigate whether the same effects were witnessed for the detection of ethyl acetate. The apparatus had to be modified to accommodate operation at an increased pressure. Details of these modifications are provided in Section 3.10.1.

6.11 Elevated pressure of carrier flow

It was hypothesised that increasing the pressure of the carrier flow to the FAIMS unit could be engineered to result in an increase in the population of reactant ions; so that even in the presence of relatively large amounts of ethanol the reactant ions would not become saturated and result in a reduction in the response of the FAIMS system.

Increasing the pressure normally results in a decrease in separation of different ion species within the FAIMS separation region (Section 2.5.3, Appendix E). This is a result of a reduction in the energy available to the ions, described by a decrease in the ratio *E*/*N*. As reported previously, this loss of separation can be countered through the phenomenon known as clustering (Sections 2.3.5 and 5.5.2) which, as long as the humidity is large enough, is promoted through greater pressures. Alternatively, dilution of the water in the carrier flow using a high pressure can mitigate against the detrimental effects of the high polarity water.

Another finding, from the work undertaken within Chapter 5, was that increasing the number density of the carrier gas did not result in a large increase in the formation of reactant ions (Section 3.5.1). This would suggest that the course of action proposed would have little direct effect upon the availability of reactant ions. However, reducing the *E*/*N* ratio experienced by the ions not only decreases separation but increases ion response as less ions are lost due to diffusion to the side walls of the FAIMS sensor (Section 2.9). It was anticipated that the loss of separation by both a lower *E*/*N* ratio and increased interaction due to an increased pressure will be countered by increased clustering. The result should then be an increased sensitivity, with a comparable separation of compounds as observed at ambient pressure.

6.11.1 Equivalent *E*/*N* under an elevated pressure

As the studies in Chapter 5 were not conducted using a gas chromatography unit, the first priority was to confirm whether an increase in clustering would be observed. Specifically, it needed to be determined whether an increase in carrier pressure at a constant *E*/*N* ratio, resulted in an increase in ion species separation. This was accomplished by running the elevated pressure set-up at an *E*/*N* ratio consistent with the reference found with a DF of 38% of the maximum, at ambient pressure. This required the management of the imposed electric field strength, in the same way as detailed within Section 5.2.3. The GC column was changed back to the MXT-5 to decrease analysis time and to ensure ethanol was present in the ionisation region when ethyl acetate eluted.

0.5 µl of a stock solution of 89.7 mg/l of ethyl acetate in distilled water was injected into the GC-FAIMS system at various pressures of carrier flow. Each injection was repeated three times to obtain an average response. The peak of response from ethyl acetate was

again isolated from the full data set but instead of isolating the specific CV position (Section 6.7) the retention time of elution was found. This provided an individual CV sweep on which peak fitting was undertaken. The positions of these peaks were recorded to evaluate how changing the pressure had affected the CV position of ethyl acetate.

The peak fitting resulted in two distinct responses, which were attributed to the monomer and dimer of the ethyl acetate. Figure 6.10 displays both ion species alongside one another but with separate axes, so that the non-linear response of the dimer can easily be observed. The CV displacement was determined as the separation of the peak position of the ion response from the peak position of ions when no field was applied.

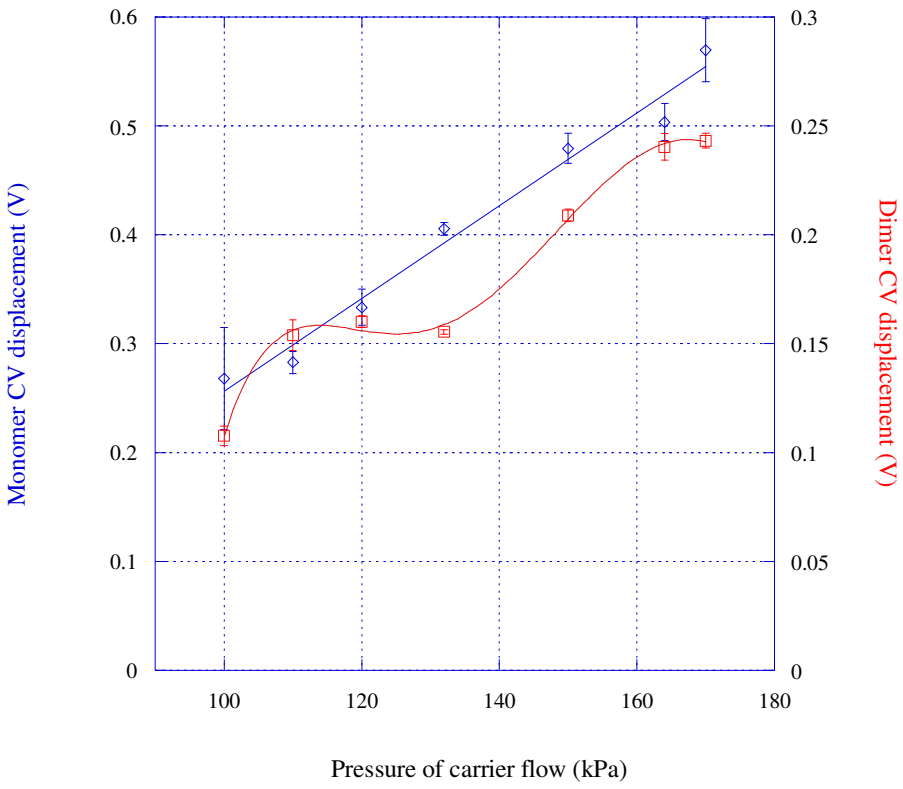


Figure 6.10 Compensation voltages of peak response of Ethyl Acetate from the GC-FAIMS system over a range of pressures. Monomer (blue diamonds) and dimer (red squares) responses are displayed alongside one another. System maintained at a constant *E/N*.

The lines added to Figure 6.10 are fitted by least squares (monomer) and a smoothing function (dimer) and are included to aid in tracing the response. Zero CV displacement is the CV of ion responses when no electric field is applied.

As pressure increased, the monomer required a greater negative compensation voltage to allow passage of the molecular ions through to detection, while the dimer required a greater positive compensation voltage. This confirmed observations from the first experiment in this study. The increase in monomer separation observed is in keeping with what would be expected if clustering was occurring while the effect on the dimer could be explained by benefits obtained through dilution.

The monomer ion response was influenced more by the increase in pressure than the dimer. This was probably due to a larger inherent mobility coefficient and an easier association with any water molecules present (*e.g.* the second analyte molecule not influencing the charge centre of the molecular ion). The response of the monomer ion species, with respect to increasing pressure, is also linear. From the work undertaken with DMMP (Chapter 5), a linear increase of CV position with increasing pressure was observed only at humidities greater than that present within the air supplied to the apparatus in this study. However, due to the abundance of distilled water within all test solutions, and an isothermal column temperature of 70°C, humidity is expected to be constantly present in the ionisation region of the FAIMS sensor, permitting clustering to occur.

The CV displacement of the dimer shows a non-linear response across the pressure range studied. It initially increases, appears to level and then begins to increase again followed by a possible second plateau. The trend may be an artefact of the peak fitting, but since the

monomer does not display a complimentary response and the two ion responses were mixed, the trend appears to be real. It is possible that the 'humps' in the CV displacement of the ethyl acetate dimer are a result of additional solvation that becomes more likely at greater carrier flow pressures.

Due to sample introduction into the FAIMS sensor being from a GC, with respect to this investigation, the response of the dimer is more critical than the monomer. As previously discussed, the high concentration of analyte entering the ionisation region within a short time frame encourages dimer formation. Additionally, ethanol appears in a similar CV position to the ethyl acetate monomer (presented in Section 6.11.3) making resolution between the two difficult. The dimer response, however, is typically separated enough for relatively easy resolution and detection.

What is obvious from Figure 6.10 is that an increase in pressure results in an increase in ion separation for both the ethyl acetate monomer and dimer. This indicates that separation of ion species can be maintained at reduced applied electric field strengths by increasing the pressure. This should allow for a greater reservoir of reactive ions, which potentially will lead to a more comparative response for ethyl acetate within either a solvent of pure distilled water or with a 12% ethanol added co-solvent.

6.11.2 Equivalent DF using an elevated pressure carrier flow

Once again ethyl acetate (89.7 mg/l) was injected via a splitless injector into the GC-FAIMS system, in triplicate, over a range of pressures. Two different solutions were once again used. One held the ethyl acetate in distilled water and the second held the analyte in a mixed solvent of distilled water and ethanol (12%). Previously, where *E*/*N* was held

constant the electric field strengths were amended in relation to the pressure. In this investigation the dispersion field strength was held at 38% of the maximum possible (as within the previous investigation using a carrier flow at ambient pressure) while the pressure of the neutral carrier gas was increased. The pressure across the column was maintained at 14 psi, as it had been throughout all investigations with the GC. Data was obtained in the manner detailed within Section 6.7 and only the ethyl acetate dimer was recorded. The data obtained from the two solutions is presented within Figure 6.11.

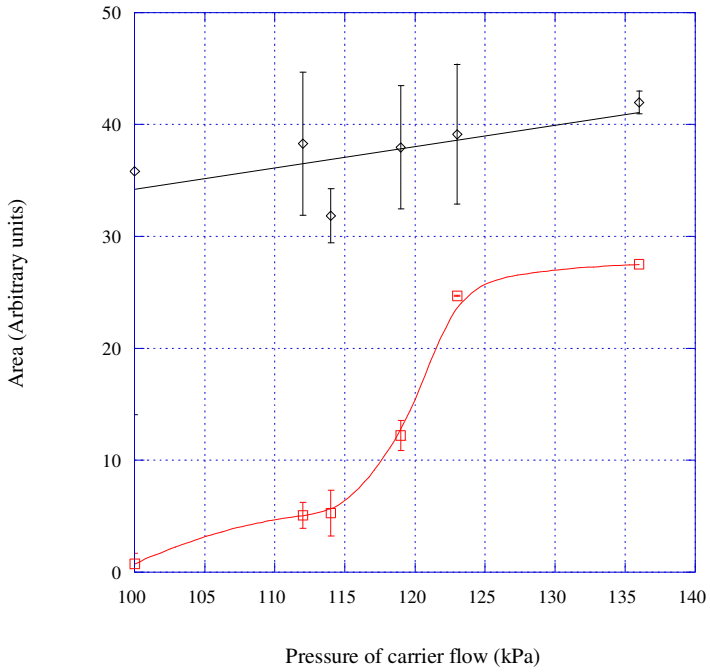


Figure 6.11 Area of ion response of ethyl acetate from a solvent of distilled water (black) and distilled water and 12% ethanol (red).

The lines in Figure 6.11 were added through linear least square fit (distilled water solvent) and a smoothing function (distilled water and 12% ethanol solvent) to help trace the response. The error bars presented are a single standard deviation of the repeated triplicate readings.

The linear trend obtained from the solution with pure distilled water was expected. The line of best fit indicated that a modest increase in ion response was observed and will have

resulted from the trade-off between increased availability of reactive ions and increased losses through diffusion.

The ion intensity of the second solution is more complex. It appears that increasing the pressure has resulted in the increase of ethyl acetate response anticipated and desired. It also appears that response for ethyl acetate will never reach the same levels as when ethanol is not present. This is to be also expected because since ethanol will always play some part in the ion chemistry, if present. Increasing the pressure further may dilute the constituents so that, eventually, a decrease in ethyl acetate response is observed.

An additional effect upon the chromatography is that the elution of compounds takes longer. Since the pressure across the column is held constant this result was attributed to being able to observe lower concentrations of the compounds. Figure 6.12 shows two traces, the first has a carrier flow held at a pressure of 112 kPa (absolute) and the second is held at a pressure of 136 kPa. Injections from the two solutions used above are shown at both pressures; the same DF was applied throughout (38% of maximum possible).

a) b)

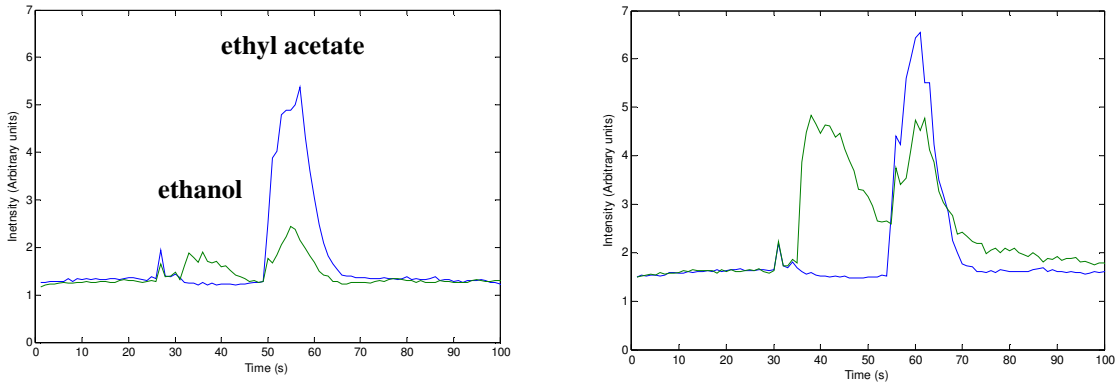


Figure 6.12 Responses obtained from the GC-FAIMS system at a gauge pressure of a) 120 kPa and b) 136 kPa. Ethyl acetate in solvents of distilled water (blue) and distilled water and 12% ethanol (green) are shown alongside one another.

Figure 6.12 a) shows that there is little direct interference of ethanol (first elutes ~ 31 seconds) with the ethyl acetate response (first elutes ~ 50 s) but there is still a reduction in the intensity of the ethyl acetate response in the presence of ethanol. Increasing the pressure of the carrier flow further, as presented in Figure 6.12 b), shows that the ethyl acetate response has increased when ethanol is present. However, the ethanol response has also grown to an extent that the two ion responses no longer exhibit baseline resolution. This demonstrates that while increasing the pressure of the carrier flow to the FAIMS unit is beneficial for sensitivity it will also degrade resolution between ethyl acetate and ethanol.

Given the mixed ion responses observed at high carrier flow pressures it is worth considering the likely identity of those ion responses. While it is likely that the ethyl acetate, having a greater proton affinity, will preferentially react with the available reactive ions it should not be assumed that the ion response will be absent of product ions resulting from ethanol, owing to its abundance. Additionally, because of a larger population of reactive ions, following an increase in carrier flow pressure, both ethyl acetate and ethanol are potentially ionised, providing a more complicated signal to interpret. This is an interesting scenario that could be investigated through coupling with a mass spectrometer, however, this was beyond the scope of this study.

6.11.3 Increased losses attributable to diffusion

It was also observed that a higher pressure of carrier flow and the presence of ethanol led to reduced tailing from the ion responses. Figure 6.13 shows two plots, where each plot has two ion responses displayed. In both cases the ion responses result from a high concentration of ethyl acetate (~ 300 µg/l) and are compensated to maintain a constant *E*/*N*

environment (equivalent to using an ambient pressure of carrier flow). The blue lines describe the use of distilled water while the ion responses described by the green lines were obtained using a mixed solvent of ethanol (12%) in distilled water. The CV isolated for each ion response was where ethyl acetates maximum response occurred. Figure 6.13 a) was obtained with an ambient pressure of carrier flow supplied to the FAIMS unit while Figure 6.13 b) used a carrier flow pressure of 177 kPa (absolute).

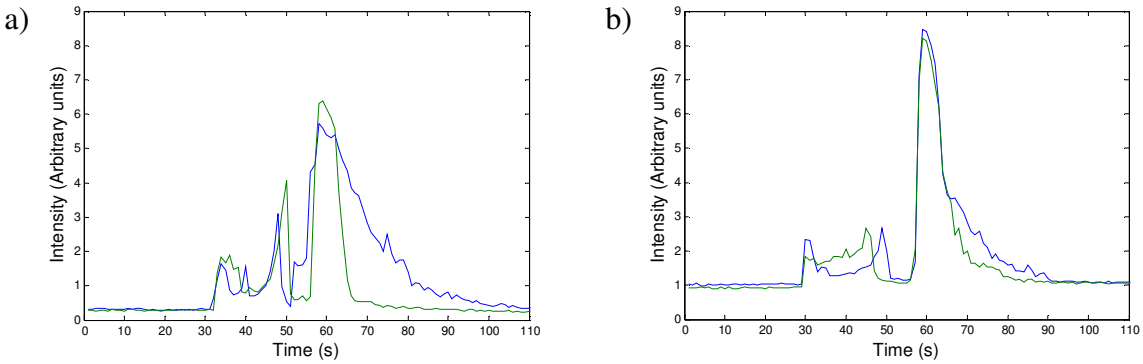


Figure 6.13 Responses obtained for high concentrations of ethyl acetate within distilled water (blue) and a solvent of distilled water and 12% ethanol (green), a) was undertaken with a carrier flow at ambient while b) used a carrier flow of 177 kPa.

The use of high concentrations of ethyl acetate emphasised what had been witnessed in previous injections. From Figure 6.13 it is apparent that both increasing the pressure of the carrier flow and the presence of ethanol reduces tailing of the ion responses. It is proposed that the constituents of the analyte tail are sensitive to losses attributable to diffusion. As pressure of the carrier flow increases there will be an increase in the interactions experienced by ions within the separation region of the FAIMS sensor. This will result in a greater proportion coming into contact with the sensor walls and becoming neutralised. Also, introducing ethanol, a species that has a higher molecular weight (MW) than air, will further increase interactions within the separation region. It does appear that the bulk of the main response from analyte elution appears to be relatively unaffected by the increased losses due to diffusion. It is not proposed that this main response is unaffected by the increased losses, only that the high concentration of ethyl acetate at the peak of elution has

resulted in, despite the increased losses to the sensor walls, the compound being in such abundance that saturation of the reactant ions still occurs.

A further demonstration of the reduction in tailing is depicted through contour maps, given in Figure 6.14 and Figure 6.15, of the ethyl acetate and ethanol response at different pressures of carrier flow. These plots, which focus on the elution of ethyl acetate, provide information spanning not only the retention time of the GC but also the compensation voltage of the FAIMS separation. Features discernable in Figure 6.14 include tailing following compound elution and the presence of the ethyl acetate monomer and why it is not observed in the presence of ethanol.

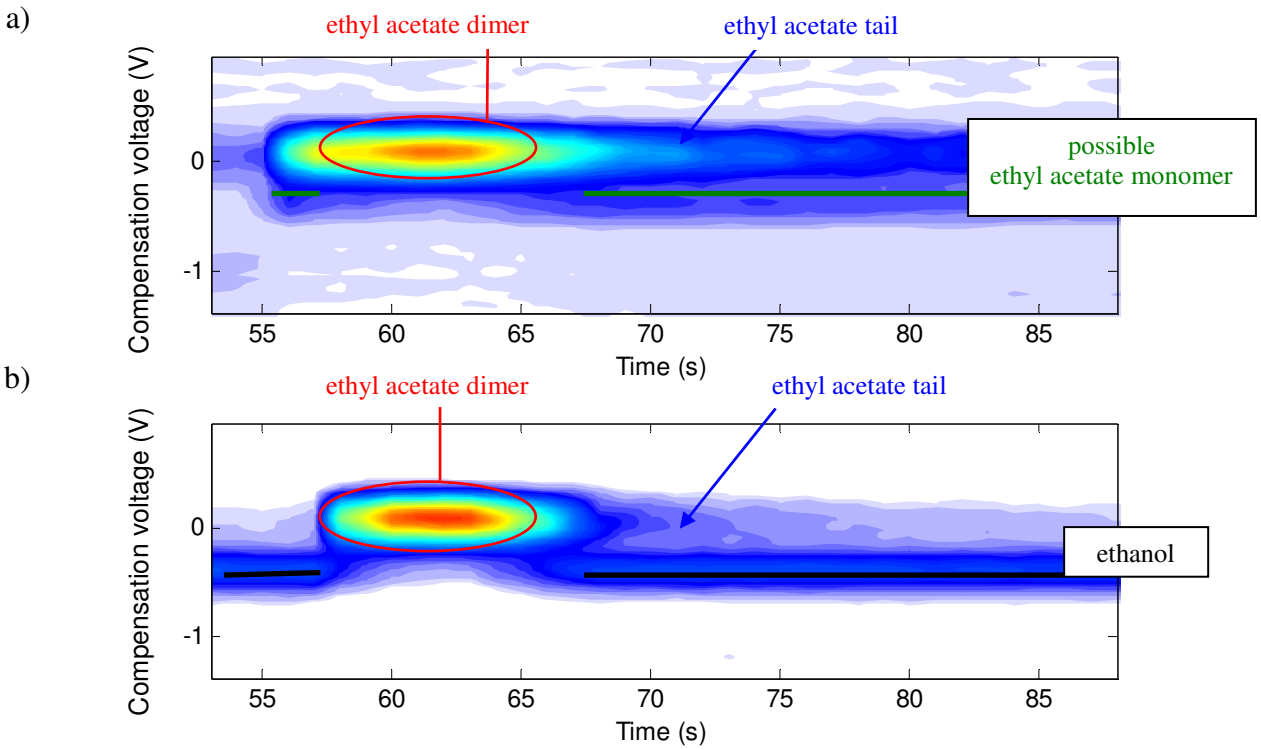


Figure 6.14 Reponses obtained from the GC-FAIMS system operating with an ambient pressure of carrier flow to FAIMS unit. a) Injection of distilled water spiked with ethyl acetate b) Injection of ethyl acetate within a solvent of distilled water and ethanol (12%).

In Figure 6.14 a) a prominent tail is observed following the maximum response of ethyl acetate. A monomer response from ethyl acetate is also suggested by the data but at the maximum time of elution it appears to be lost. This was attributed to the high abundance of

analyte at that point saturating the reactant ions and making formation of the dimer more likely.

In Figure 6.14 b) the response from ethanol, which eluted earlier from the GC column than ethyl acetate, can be seen trailing through the ion response. The only break in its presence is across the maximum response of the ethyl acetate dimer as the higher proton affinity species is at peak concentration, competing for and winning the reactant ions. The tailing from the ethyl acetate is also discernibly reduced in the presence of ethanol, as expected following discussions earlier in this section.

Figure 6.15 was obtained in a similar way to Figure 6.14, except the carrier flow pressure was increased to 177 kPa. Greater separation of ion species is apparent, which makes the features observed previously more obvious.

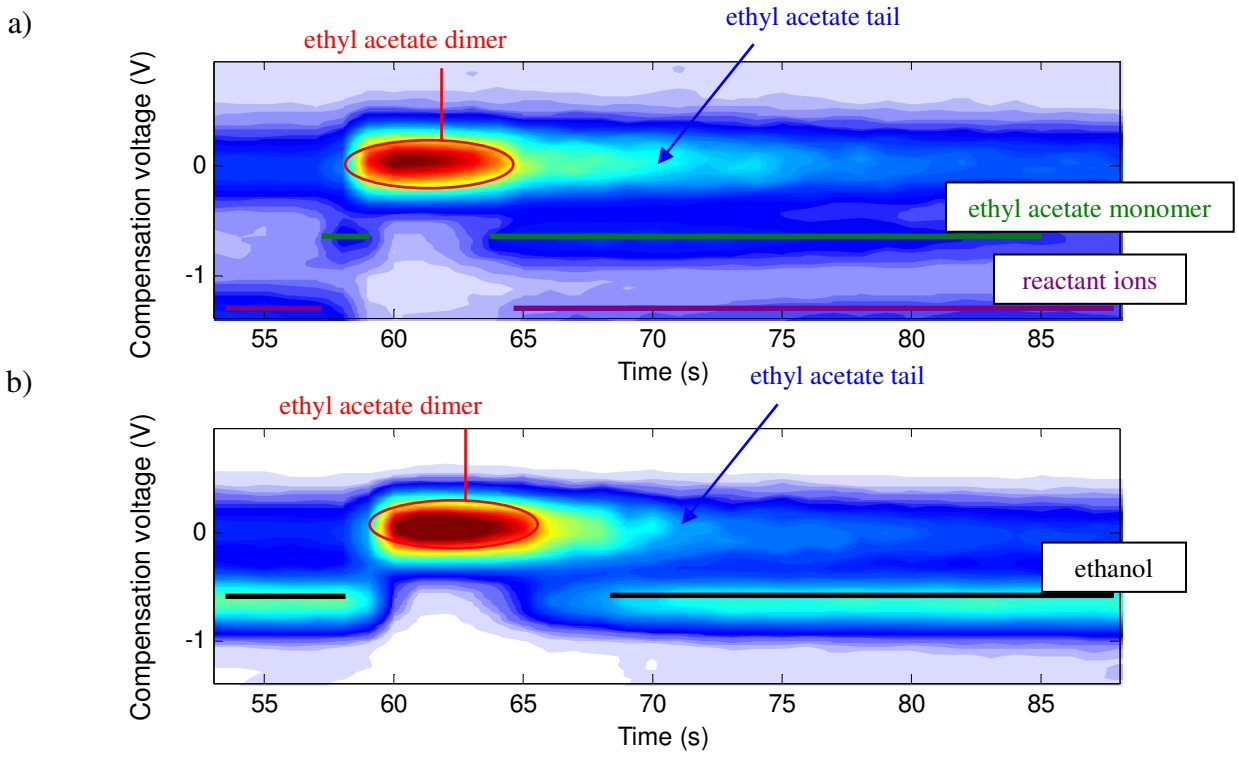


Figure 6.15 Reponses obtained from the GC-FAIMS system operating with a carrier flow pressure of 177 kPa (absolute) to the FAIMS unit. a) Injection of distilled water spiked with ethyl acetate b) Injection of ethyl acetate within a solvent of distilled water and ethanol (12%).

The top tile of Figure 6.15 a) shows what is now more clearly visible, the monomer species of ethyl acetate, beginning just prior to the dimer response. The monomer is lost through the peak of ethyl acetate elution as the analyte is in such abundance that all monomer ions are converted to dimer. In Figure 6.15 b) it can be seen that the ethanol response is also lost through the maximum ethyl acetate elution, as it was with a lower pressure of carrier flow. Also, despite the increased separation between ion species, the ethanol response still overlays that of the ethyl acetate monomer. This is proposed as the reason that the ethyl acetate monomer has not been easily observed previously. Again, the tailing that is present in a solvent of distilled water can be clearly seen; equally the effect of the presence of ethanol upon the tailing is dramatic.

6.11.4 Detection of ethyl acetate using high pressure carrier flow

It has been demonstrated that increasing the pressure of the carrier flow to the FAIMS unit, while maintaining the DF strength, increases separation by increasing the population of reactant ions. This is arises through the promotion of clustering and/or dilution of humidity (Section 6.11.2). It remains to apply the methodology to a range of ethyl acetate concentrations in the presence of 12% ethanol.

A stock solution of 12% ethanol in distilled water was spiked with ethyl acetate so that the final concentration was 89.7 µg/l. This solution was serially diluted to provide a range of concentrations that were injected into the GC-FAIMS system with a carrier flow pressure of 122 kPa (absolute). A second stock solution with the same concentration of ethyl acetate was also made up in distilled water for comparison. The results obtained for these dilutions are plotted in Figure 6.16 alongside the ethyl acetate response (as found in Section 6.8.1). The CV separation used to isolate the ion responses is representative of the maximum ion

response from each injection. Injections were completed in triplicate and the standard deviation of those injections is provided as the error. A carrier flow pressure of 122 kPa was selected so that increased response of ethyl acetate would occur while still maintaining baseline resolution from ethanol, with regard to retention time (Section 6.11.2).

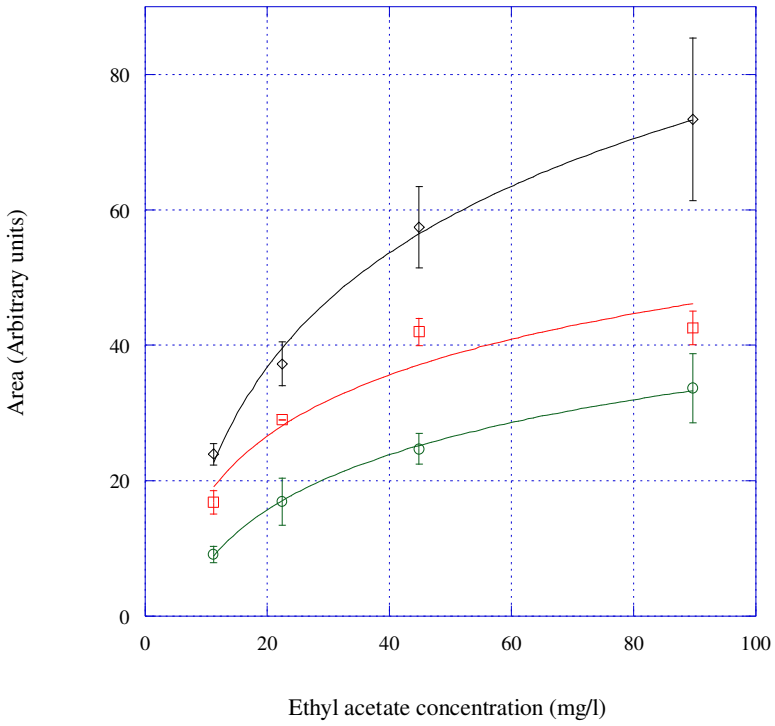


Figure 6.16 The response from ethyl acetate at various concentrations; at a carrier flow pressure of 122 kPa in distilled water (black diamonds), solvent of ethanol (12%) in distilled water (red squares) and at an ambient carrier flow pressure in a solvent of distilled water (green circles).

The lines of best fit in Figure 6.16 are logarithmic and have been added as an aid to trace response.

It can be seen that the response for ethyl acetate, in the presence of ethanol, has been increased above what was previously achievable with an ambient pressure of carrier flow without the presence of ethanol. This is a rewarding result and is further confirmation that the recovery of the reactant ion population is key in enabling good sensitivity. Even at the elevated pressure, at higher ethyl acetate concentrations the saturation of reactant ions again appears to occur in the presence of ethanol. It therefore appears that the signal from

ethyl acetate will always be attenuated in some way by ethanol, through the competition for reactant ions but also the increased losses attributable to diffusion.

Figure 6.17 displays the heights of peaks for which the areas were given in Figure 6.16. Again, the error reported is the standard deviation of triplicate injections.

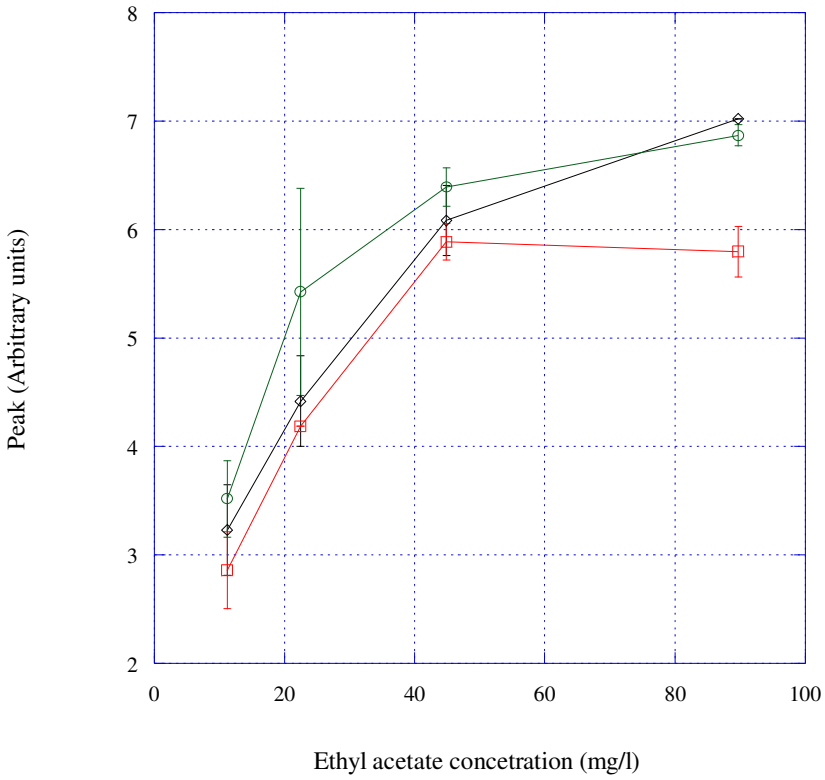


Figure 6.17 Peak intensity of ethyl acetate response at various concentrations at a carrier flow pressure of 122 kPa in a solvent of distilled water (black diamonds) and distilled water and 12% ethanol (red squares) and at an ambient carrier flow pressure and solvent of distilled water (green circles).

Similar to the case where a large concentration of ethyl acetate in the presence of ethanol exhibited the same peak intensity but less total ion response (Section 6.11.3) the peaks of the ion response are in close agreement at the elevated pressure with and without ethanol present. This suggests that increased sensitivity to lower concentrations either side of the peak elution is the main reason for the observed difference in total ion response between solvents with and without ethanol at increased carrier flow pressure.

With respect to the application under study it is clear that the sensitivity to ethyl acetate at levels below the human sensory threshold can be achieved, even in the presence of ethanol in the quantities experienced within wine. There is also good reason to believe that detection limits of ethyl acetate within wine are achievable at least to an order of magnitude below the sensory threshold presenting the opportunity to better observe and manage the evolution of this multifarious compound.

6.12 Conclusion

It was shown that the Owlstone FAIMS sensor can be coupled with a GC so that successful introduction of analyte is possible from the GC to the FAIMS sensor. The utilisation of a GC as an in-line separation stage, prior to the FAIMS detector, improved the discrimination for ethyl acetate, primarily by ensuring a limited number of ion species were present within the FAIMS sensor at any one time. The results from the GC-FAIMS system characterised response in terms of both retention time and compensation voltage.

The successful detection of ethyl acetate using the GC-FAIMS system was then demonstrated within distilled water and wine. From this work, an attenuation of the response for ethyl acetate in wine was suggested as attributable to the presence of ethanol. Further investigation, through changing the non-polar GC column, for one with a greater polarity, demonstrated that increasing the temporal resolution of ethanol and ethyl acetate improved response. This result supported the hypothesis that the presence of ethanol in the ionisation region at the time ethyl acetate eluted from the GC was the cause for the loss of sensitivity. However, it could not be confirmed whether the mechanism for this detrimental effect was purely attributable to competition for the reactant ions or some other means (*e.g.* removal of charge).

A method to decrease analyte losses due to diffusion and increasing the reservoir of reactant ions while maintaining the separation of constituents was formulated and tested. Specifically, this method required increasing the carrier flow to the FAIMS unit to encourage greater CV separation between ion species. The dispersion field applied across the separation region was then held constant, which would normally result in a higher signal intensity but loss of separation, by the FAIMS sensor. However, owing to the increased CV separation it could be preserved while still benefiting from the increased sensitivity. This approach eventually resulted in the successful detection of ethyl acetate at levels below the human sensory threshold despite the presence of ethanol in abundances similar to within wine.

Also, throughout the testing detailed in this chapter, whenever possible, randomised sampling was performed. Undertaking the sampling in this way reduced the likelihood of systematic errors going undetected and adversely affecting the obtained results. Unfortunately randomised sampling was not always feasible, a specific case being if the carrier flow pressure had to be modified between the injections. This was because the reproducibility of returning the pressure to a previous value could result in an error that would otherwise be negated if randomised sampling was suspended. Instead, in these cases the triplicate readings were inspected to ensure there were not consistent trends within the repeated readings. If this work was to be revisited it would be of interest to reconsider this problem and arrive at a solution that permitted randomised sampling between separate carrier flow pressures with an appreciably reduced error.

The work within this chapter has shown that FAIMS is capable of being a sensitive and versatile GC detector even when operating with a complex background. This versatility was implemented without affecting the operation of the coupled GC or total analysis time.

This is in contrast to when the GC column used a more suitable polarity to improve sensitivity. Additional effects through the increase of pressure such as the reduction in tailing were also presented.

Optimisation through the initial stages of this chapter focussed on providing a stable and fit-for-purpose GC-FAIMS system. Contrary to normal GC optimisation, the elution time of compounds was desired not to occur in as short as time as possible; this was to allow more than a single FAIMS CV sweep to be taken across an elution profile. To take full advantage of the potential optimisation of a hyphenated GC system the operation of the FAIMS unit itself would have to be further adapted for the scenario. This would principally be addressed by decreasing the time required to obtain a CV sweep. There is an unavoidable trade-off with sensitivity but the increased temporal resolution may enable greater specification of detected compounds, therefore justifying further investigation. The detection of ethyl acetate throughout this study was made a great deal easier because it had the greatest proton affinity out of the compounds present. This meant that a detectable response would often be evident following interaction with reactant ions, despite a relatively low abundance. In many ways this investigation took advantage of several benefits associated with the FAIMS technology. The separation of compounds through ion mobility was applied to GC separation resulting in simplified CV spectra along with increased selectivity to the full system. Targeting a high proton affinity candidate leant itself to the ionisation source employed within the FAIMS unit and also exploited potential clustering within the separation region (which meant that a greater reservoir of ions could be established). The FAIMS technology certainly has its limitations but can be successful in challenging scenarios if features are correctly managed.

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