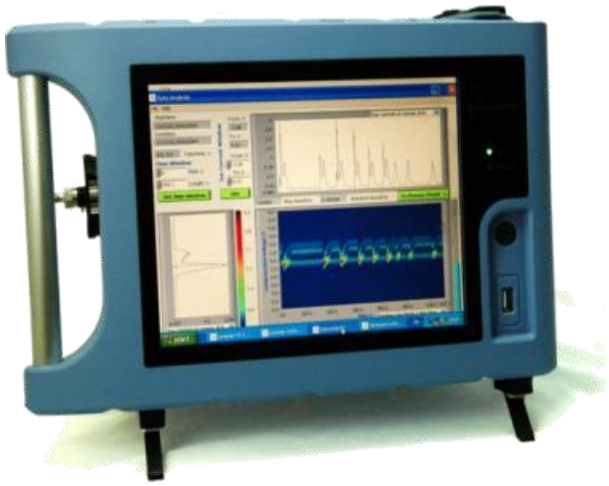
Monitoring Solvent Levels in Pharmaceutical Waste Water Using a LONESTAR™ Portable Analyzer

A case study with 4-nitrophenol, 1,2 Dimethoxyethane, Methyl t-Butyl Ether and Tetrahydrofuran



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Introduction

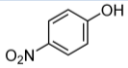
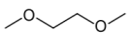
Bacteriological biodegradation is used to treat certain waste water streams however excesses of some solvents will poison the bacteria reducing their efficiency. Consequently there is value in being able to quickly measure concentrations of these critical solvents so they can be pretreated/diluted to protect the bacteria.

It is believed at Owlstone that it is practical to perform a rapid solvent level determination in the waste water background matrix using headspace analysis by Field Asymmetric Ion Mobility Spectrometry (FAIMS). The advantages over other analytical methods are mainly in speed and ease of use. The suitability of a FAIMS based detection for this application is due to a combination of factors, firstly the target analytes and associated volatile background proton affinities are in the right order, high proton affinity for the target analytes and low proton affinity for the background (alcohols, acetates); This means preferential ionization of the target analytes over the background and therefore higher sensitivity to the target analyte (more details on ion affinity are available in appendix A). Secondly the volatility of the target analytes is high enough that a direct headspace of the sample can be taken without any extraction and minimal preparation steps on the waste water (at most a single dilution step e.g. 1ml into 20ml of clean water). Combined this should enable a sample turnaround (time from starting first sample analysis to next sample analysis) of a few minutes.

Objectives

Test the feasibility of using Lonestar for the detection of the chemicals outlined in Table 1 at the stated alert concentrations in known production samples.

Table 1 List of target chemicals and their properties

Name	4-nitrophenol	1,2 Dimethoxyethane	Methyl t-Butyl Ether	Tetrahydrofuran
Abbreviation	PNP	DME	MTBE	THF
CAS	100-02-7	110-71-4	1634-04-4	109-99-9
Alert concentration	1-5ppm$_{w/w}$	30ppm$_{w/w}$	70ppm$_{w/w}$	40ppm$_{w/w}$
Structure				
Molar mass	139.11 g mol	90.12	88.15	72.11
Melting point	114C	-58C	-109C	-108.4C
Boiling point	279C	85C	55.2C	66C
Proton affinity	-	858 KJ mol	841.6 KJ mol	822.1 KJ mol
Solubility at 25C	16g L	Miscible	Immiscible	Miscible
Pka	7.15	-	-	-

The LONESTAR Platform

The LONESTAR is a powerful and adaptable chemical monitor in a portable self contained unit. Incorporating Owlstone's proprietary FAIMS technology, the instrument offers the flexibility to provide rapid alerts and detailed sample analysis. It can be trained to respond to a broad range of chemical scenarios and can be easily integrated with other sensors and third party systems to provide a complete monitoring solution. As a result, the LONESTAR is suitable for a broad variety of applications ranging from process monitoring to lab based R&D.

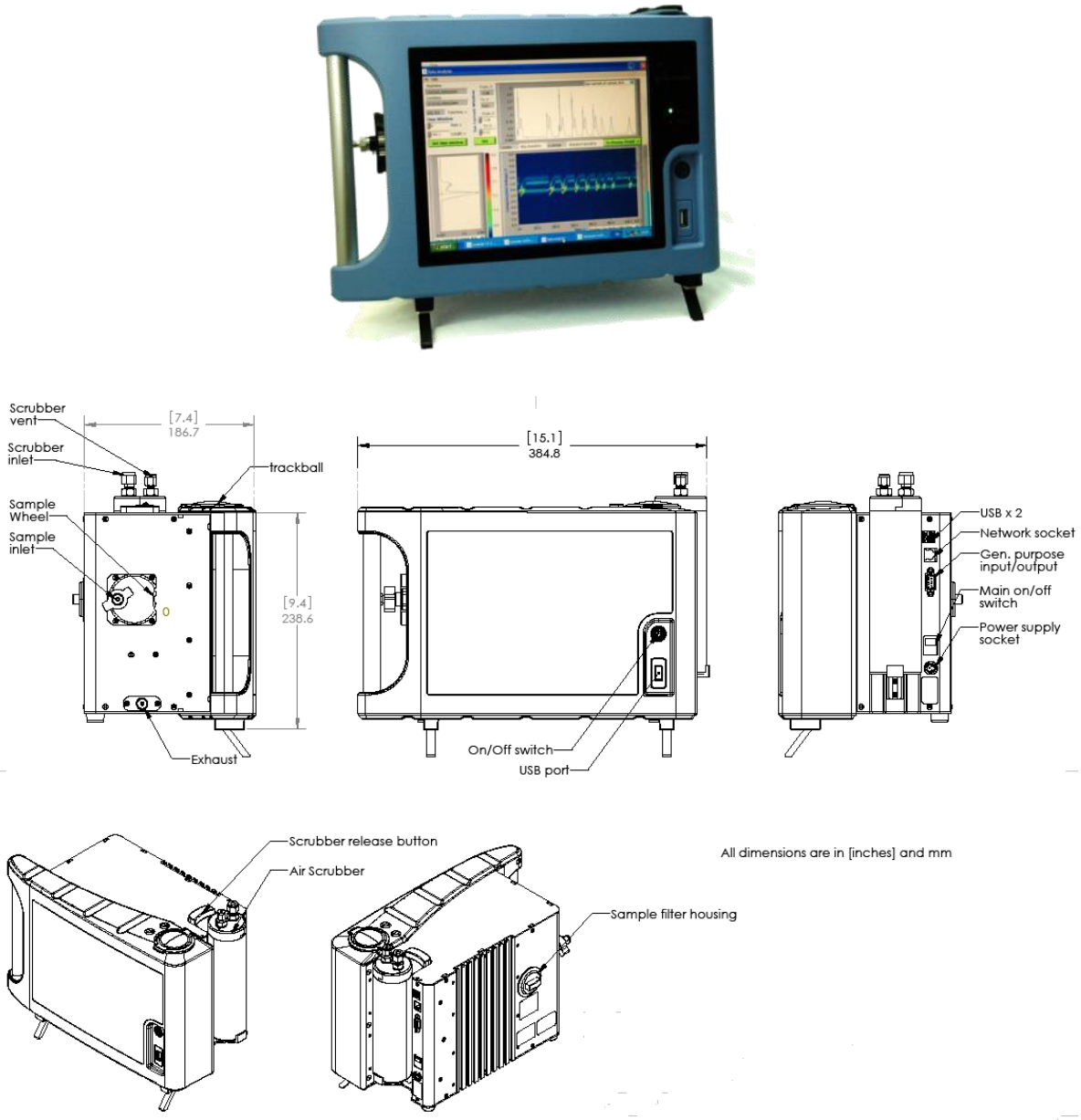


Figure 1 - LONESTAR connection figures.

Experimental Setup

Figure 2 is a schematic of the experimental apparatus used for the waste water study. The Lonestar system is connected to a headspace sampling interface (left) in which sample bottles (VWR trace clean) can be mounted allowing all the environmental conditions at the sample to be controlled. 20ml of the water sample to be analysed is then placed in the bottle, the system flushes air (light blue arrows) through the sample headspace and into the analyser via a particulate filter, an additional make up flow can be added if required to give additional dilution and/or humidity control if this additional flow is humidified.

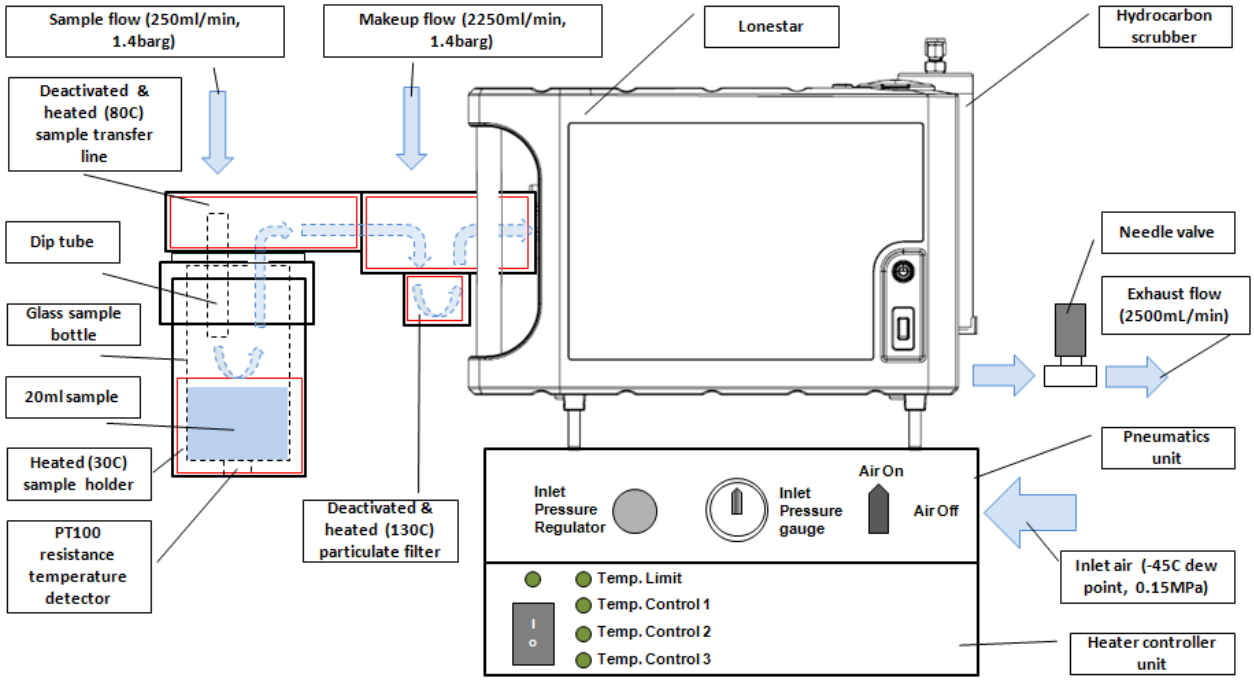


Figure 2 – waste water experimental schematic

Approach

The initial sample list is summarised below in Table 2, it was constructed to demonstrate the system responses as the concentrations in the production samples approached the desired alarm levels. To achieve this the provided production sample were spiked with each solvent in turn at two levels below the alarm level, the detection of these two concentrations in a production sample matrix should show whether a full calibration would be successful for the desired concentration range. Interferent solvents were also spiked into the matrix at 100ppm to show that they would not affect the quantification of the solvents of interest. The even number injections not listed are blank sample bottles being checked before use, ensuring no carryover of chemical between sample runs.

Table 2 - Waster water samples run and their corresponding spike

experiment	Injection	waste water sample	Sample spikes							
			PNP	DME	MTBE	THF	MeOH	EtOH	EtOAc	IPA
1	13	1								
2	15	2								
3	17	1	1ppm							
4	19	1	4ppm							
5	21	1		10ppm						
6	23	1		20ppm						
7	25	1			25ppm					
8	27	1			50ppm					
9	29	1				10ppm				
10	31	1				20ppm				
11*	33	1		10ppm		10ppm	100pm			
12*	35	1		10ppm		10ppm		100ppm		
13*	37	1		10ppm		10ppm			100ppm	
14*	39	1		10ppm		10ppm				100ppm
15*	41	1				10ppm				
16*	43	1				20ppm				

*additional experiment at different pressure 22/12/11 sample refrigerated for intervening time (see THF results for details)

The initial injections (1-11) not listed were used to perform a quick optimisation of the system for sample preparation and sampling conditions. The setup used is listed in the table below. The 20:1 dilution in water (Deionised, Millipore, conductivity > 18Ω.m) was to quickly bring the concentrations closer to the linear range of the instrument, this could also be achieved using an instrument split but with the limited time available for method development dilutions were used.

Table 3 – Lonestar parameters

Lonestar setup	
Sample dilution	1ml in 20ml water
Pressure	1.4 bar_g
Sample flow	250 ml/min
Total flow	2500 ml/min
Sample temperature	30 °C
Frequency	26 MHz
Inline humidity sensor	6% RH
Number of lines	36
Start & End DF	30-100%
Averages	1
Sensor temperature	70 °C

Sample 1 and 2 arrived frozen and were defrosted the day before. Analyses before they were frozen showed the following concentration breakdown in $ppm_{w/w}$.

Table 4 – Results from waste water samples before they were shipped

Analyte	Sample 1 (Nov 21 sample) concentration / $ppm_{w/w}$	Sample 2 (Apr 19 sample re-analysed Nov 21) concentration / $ppm_{w/w}$
PNP	0.74	0.08
MTBE	1.38	22.71
DME	0.66	1.71
THF	1.84	1.72
MeOH	0.28	28.93
EtOH	17.90	12.78
IPA	0.91	1.73
t-BuOH	3.03	4.57
EtOAc	141.59	152
IPAc	0.50	1.47

N-Methylmorph	1.25	4.02

After the initial tests it was seen that the PNP was not being resolved sufficiently for quantification at 1ppm so the sampling setting were re-optimised and the following tests run:

The new tests were run with the following parameters changed (Table 5) from the original setup, averaging was increased to increase the signal to noise ratio and the number of lines in the matrix was reduced so the time to collect the data was similar, the sample dilution was removed and sample flow increased to maximise the transfer of analyte to the sensor, this resulted in saturation of the positive ion mode response but boosted the negative ion response to allow PNP quantification.

Table 5 - Amended Lonestar parameters for PNP

Lonestar setup	
Sample dilution	None, 20ml of sample run neat
Sample flow	2500 ml/min
Number of lines	2
Start & End DF	95-96%
Averages	9

Results

PNP spikes

The initial runs on the PNP could not resolve the PNP peaks at the required concentrations so additional tests were run at different conditions; these tests were run without any sample dilution (20ml of sample loaded directly into the sample bottle). Initially a large spike of 40ppm of PNP was run to confirm peak trajectory, this can be seen below Figure 3.

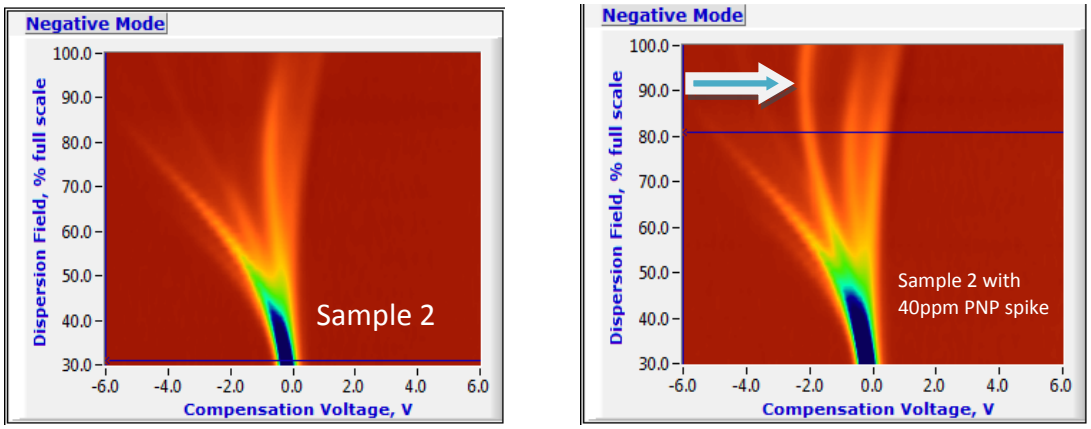


Figure 3 – Negative mode response of waste water sample 2 without (left) and with (right) a 40ppm PNP spike

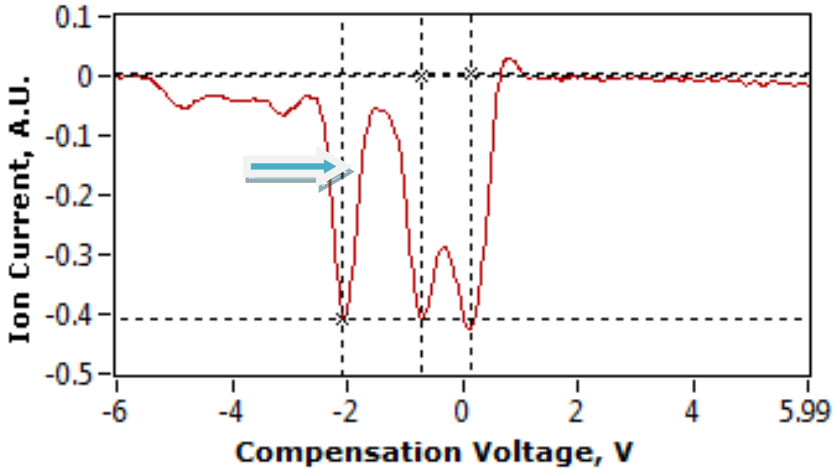


Figure 4 – 40ppm PNP spike into waste water sample 2, with the PNP peak seen at -2V CV (arrow)

Sample 1 and sample 2 were rerun with 9 averages to increase the signal to noise only a short section of the matrix between 95-96% was analysed to keep the sampling time short, a 4ppm spikes of PNP was injected into sample 1 and was also run and the peak height is plotted in Figure 5 and Figure 6 .

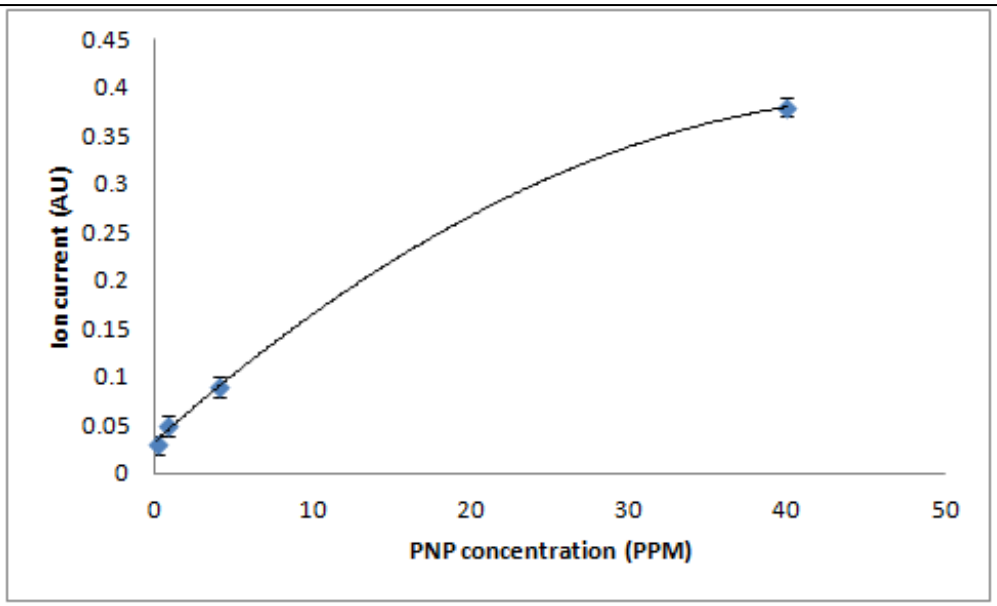


Figure 5 – PNP in waste water samples with 4 and 40ppm PNP spikes with 9 averages at 95% dispersion field

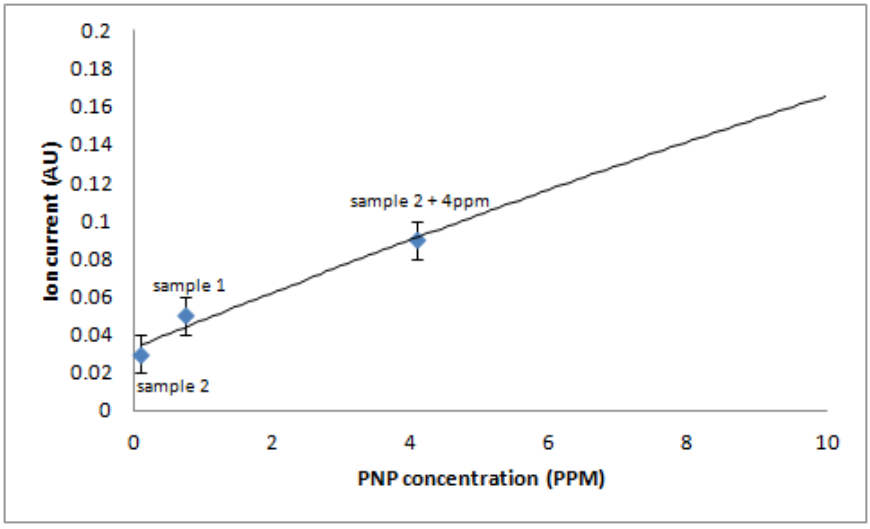


Figure 6 – Expanded view of lower PNP responses

With different sampling conditions the PNP response can be resolved for the two samples and the spikes, however using these conditions the positive mode responses of some of the solvents are saturated so simultaneous quantification of all the analytes is not possible directly. To quantify all four analytes of interest in the same sample would require it to be run with two different inline splits consecutively or the sample rerun with two different dilutions.

The non-zero intercept in Figure 6 indicates that the Lonestar is reading higher concentrations than the supplied analyses, taking the scale of the 4ppm spike the concentration in both matrixes appears to be about 1.4ppm higher than the original analyses, this would need to be investigated further but could be for a number of reasons.

DME spikes

10ppm and 20ppm spikes of DME were injected into sample 1 (residual 0.66ppm). Figure 7 shows the response in the production matrix where the peak height is altered by the background peak however due to the characteristic instability of the DME ion at high electric fields (Figure 8) it is possible to correct for a background peak which is present at higher electric fields by a simple subtraction. Figure 9 illustrates the different decays as the electric field increases which enables the subtraction. Figure 10 shows the corrected values.

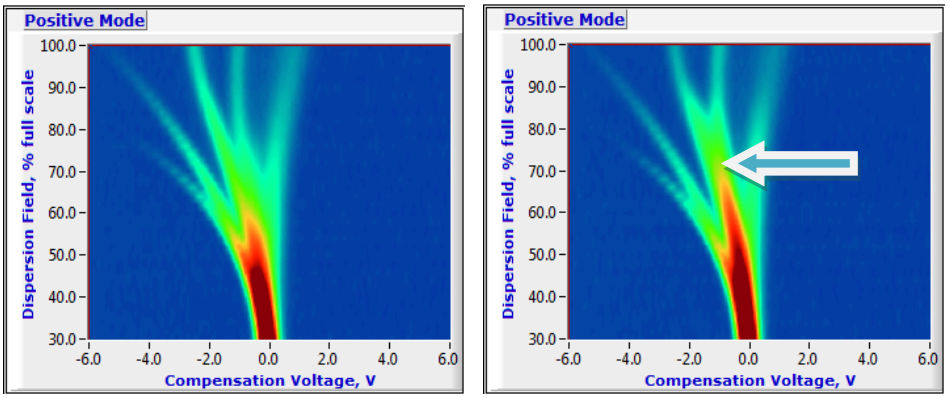


Figure 7 – Waste sample 1 blank left and DME 10ppm spike (right), this DME is identified with the arrow

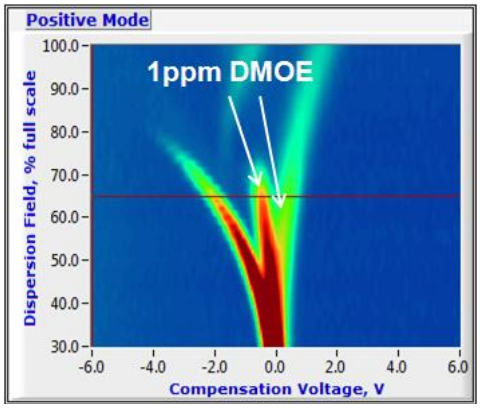


Figure 8 – 1ppm DME response taken in a previous study, exhibiting unstable ions at high electric fields (above 80% df), this is a distinguishing feature allowing it to be separated from the background

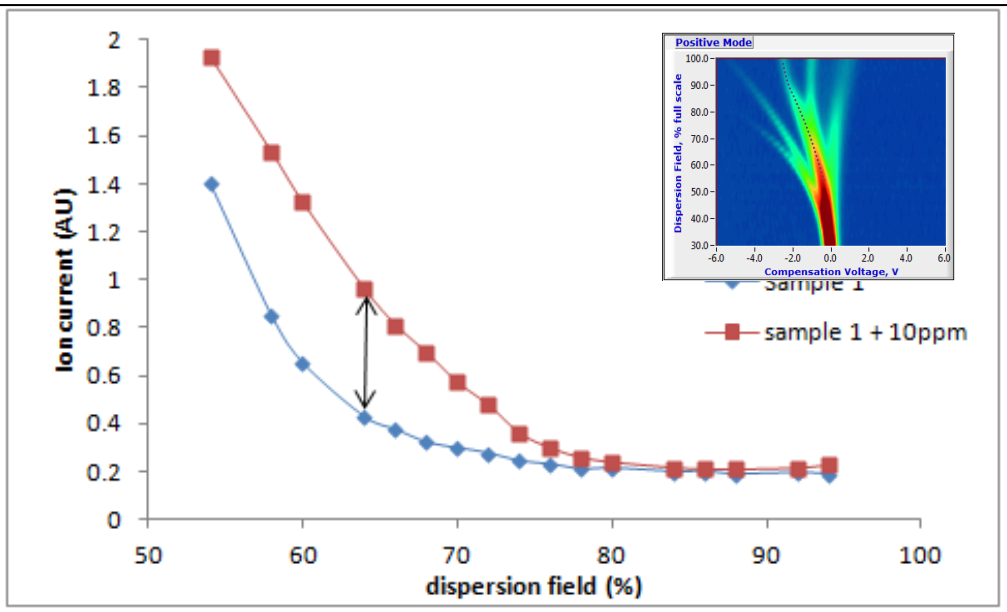


Figure 9 – using ion stability to differentiate DME, plot of dotted line on insert

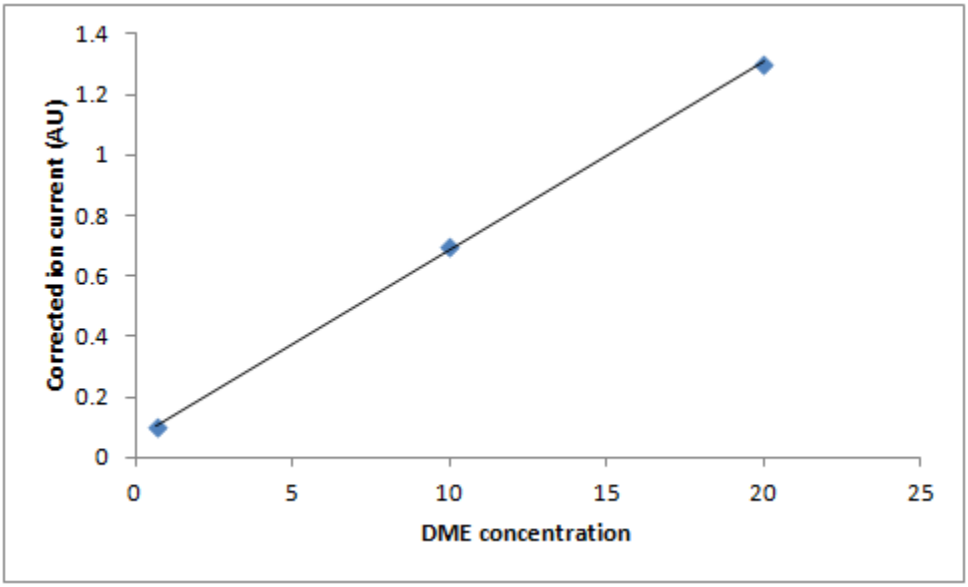


Figure 10 - DME spikes of 10 and 20ppm with the corrected ion current

MTBE spikes

25ppm and 50ppm spikes of MTBE were injected into sample 1 (residual 1.38ppm), this was with the 20:1 dilution (1ml of sample in 20ml of deionised water) at 2.5lpm at 1.4bar, the peak can be seen fully resolved in Figure 11 and Figure 12 shows the peak height of the two spiked responses, the plot does not go through zero implying that there is a higher residual MTBE concentration than the original analysis suggested.

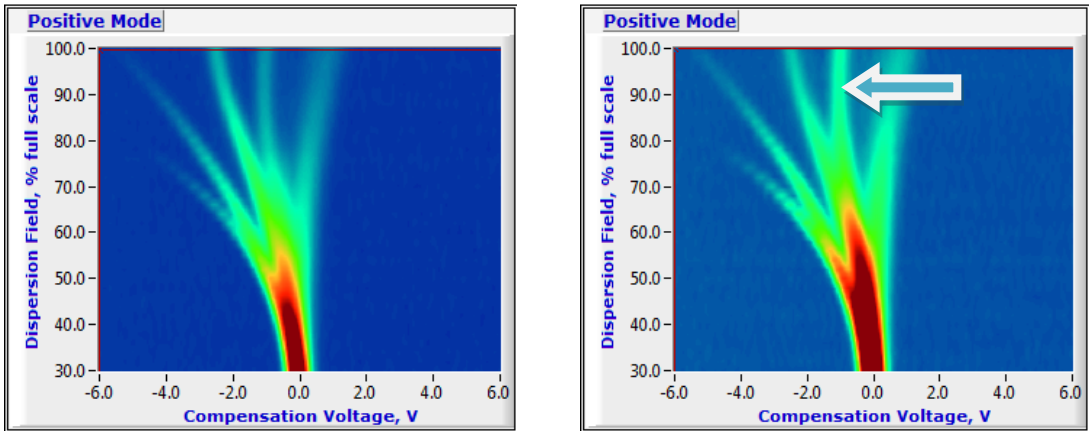


Figure 11– Sample 1 (left), 25ppm MTBE spike (right)

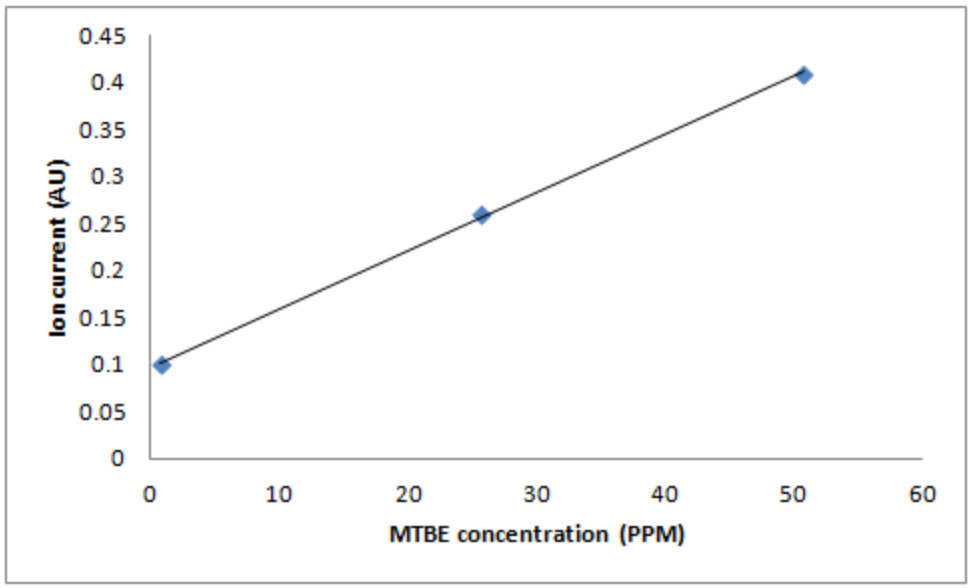


Figure 12– MTBE response with 25 and 50ppm spikes into sample 1

THF spikes

The initial experiment with THF had co-elution with another peak. By tweaking the pressure down slightly to 0.85bar$_g$ (changing the number of collisions so the differential mobility of the ion and increasing the effective electric field) the THF peak was resolved from the interfering peak (see Figure 13). THF was then be quantified, spike responses match the expected concentration in the sample when extrapolated (Figure 14).

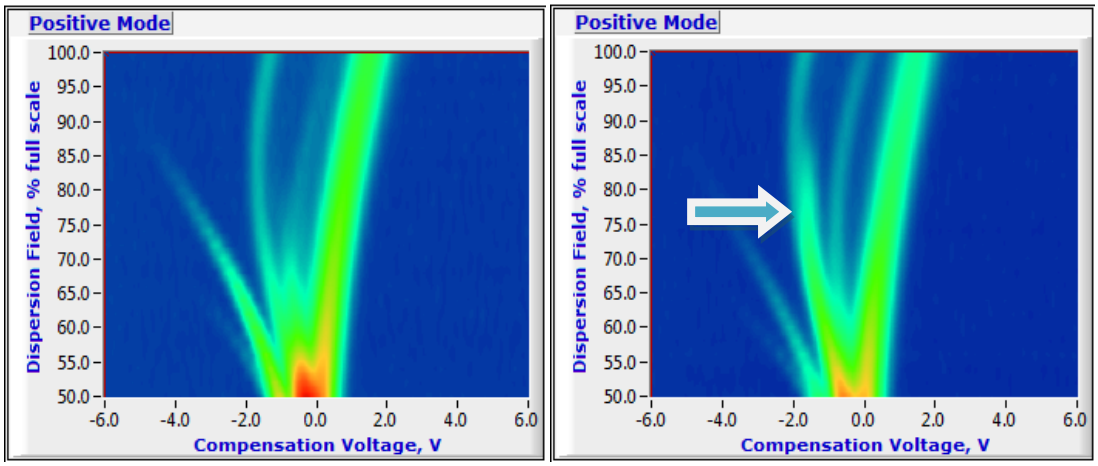


Figure 13 - Sample 1 (left), 20ppm THF spike (right)

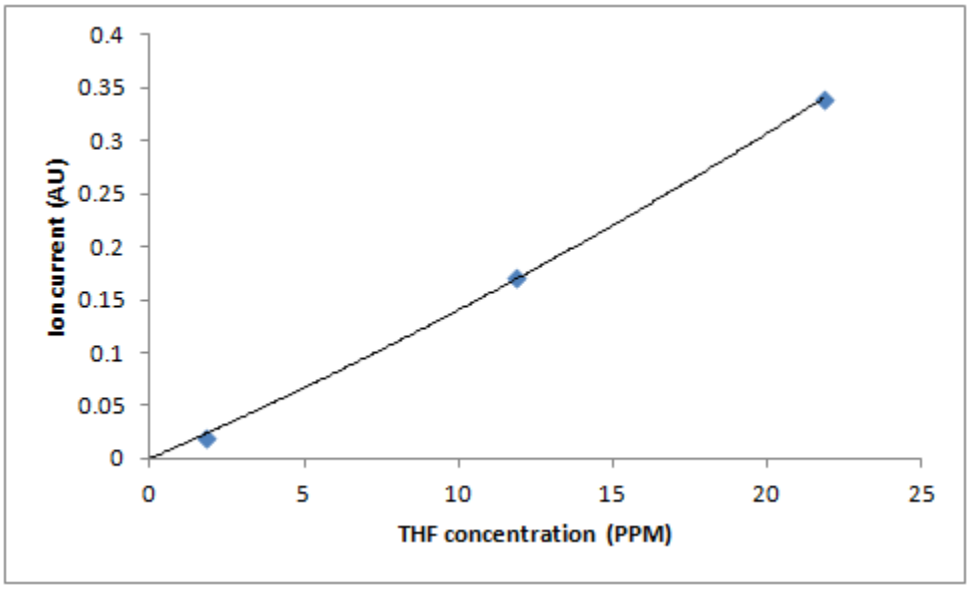


Figure 14 - THF response with 10 and 20ppm spikes into sample 1

Changes in matrix/interfering compounds

Potential interfering compounds were also tested with single large spikes of 100ppm, the tested analytes were

- Methanol
- Ethanol
- Ethyl acetate
- Iso propyl alcohol

Peak heights of the positive mode responders were compared with the background spikes, Table 6 shows that there was no significant effect from these spikes (within experimental error), this is mainly because these chemicals are all fairly low affinity so the ions tend to lose their charge to the deliberate excess of water in the background. In order to quantify these chemicals an analysis would need to be carried out dry to remove the water suppression effect. Measurements were carried out at the adjusted pressure as all three positive mode analytes can be quantified under these conditions

Table 6 - Peak heights of the target compounds with 100ppm spikes of interfering compounds

Analytes	MTBE	THF	DME
No interference	0.251	0.202	0.44
Methanol	0.24	0.194	0.48
Ethanol	0.256	0.185	0.44
Ethyl acetate	0.255	0.192	0.49
IPA	0.247	0.199	0.52
Variation	3%	3%	7%

Conclusion

The testing carried out demonstrates detection of all four analytes of interest at concentrations slightly below the required alarm levels. No complex sample preparation is required and analyses currently takes 5 minutes and there is scope to reduce this time. The data collected suggests a calibration for quantification should be practical for all four analytes.

Changes in the background chemicals studied had little effect on the analysis though there are three significant unidentified peaks (two in the negative ion mode and one in the positive mode).

Three issues have been identified from the testing which require further investigation if a full method is to be developed

- PNP sensitivity is lower compared to the other analytes so requires a different dilution to quantitate at the required concentration levels, this will either require a second run with a neat sample or a longer analysis with a switchable inline split.
- Both PNP and MTBE measurements show systematically higher values in the production samples than the provided analysis. The offsets may be due to different measurement techniques but further investigation would be needed.
- The dynamic range (dilution stages) still needs further optimisation as with the solvent concentrations in the production samples at below 4% of alarm levels for many of the analytes it is likely that the error will be large (20%+) at the low levels if optimised for the alarm level, a suitable trade off needs to be chosen.

Appendix A: FAIMS Technology at a Glance

Field asymmetric ion mobility spectrometry (FAIMS), also known as differential mobility spectrometry (DMS), is a gas detection technology that separates and identifies chemical ions based on their mobility under a varying electric field at atmospheric pressure. Figure 15 is a schematic illustrating the operating principles of FAIMS.

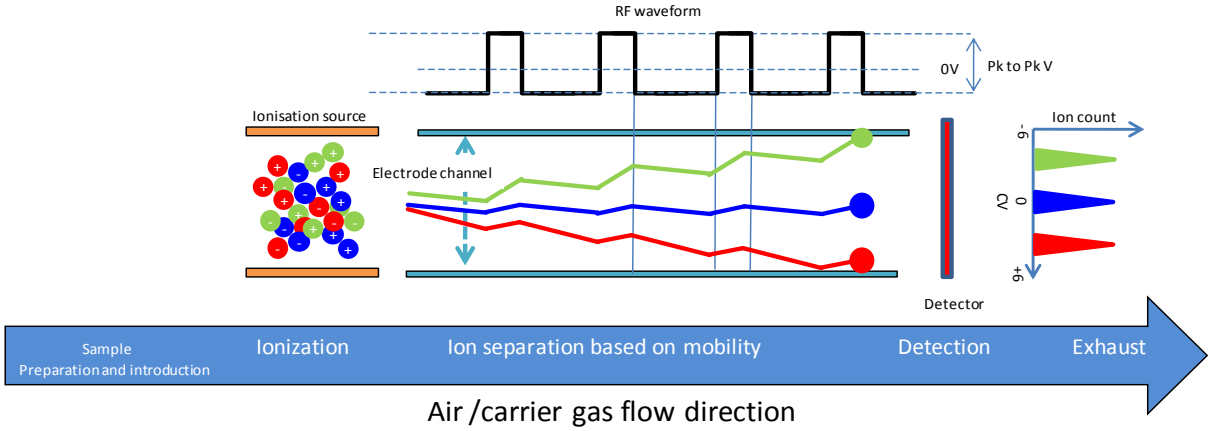


Figure 15 FAIMS schematic. The sample in the vapour phase is introduced via a carrier gas to the ionisation region, where the components are ionised via a charge transfer process or by direct ionisation, dependent on the ionisation source used. It is important to note that both positive and negative ions are formed. The ion cloud enters the electrode channel, where an RF waveform is applied to create a varying electric field under which the ions follow different trajectories dependent on the ions' intrinsic mobility parameter. A DC voltage (compensation voltage, CV) is swept across the electrode channel shifting the trajectories so different ions reach the detector, which simultaneously detects both positive and negative ions. The number of ions detected is proportional to the concentration of the chemical in the sample

Sample preparation and introduction

FAIMS can be used to detect volatiles in aqueous, solid and gaseous matrices and can consequently be used for a wide variety of applications. The user requirements and sample matrix for each application define the sample preparation and introduction steps required. There are a wide variety of sample preparation, extraction and processing techniques each with their own advantages and disadvantages. It is not the scope of this overview to list them all, only to highlight that the success of the chosen application will depend heavily on this critical step, which can only be defined by the user requirements.

There are two mechanisms of introducing the sample into the FAIMS unit: discrete sampling and continuous sampling. With discrete sampling, a defined volume of the sample is collected by weighing, by volumetric measurement via a syringe, or by passing vapor through an adsorbent for pre-concentration, before it is introduced into the FAIMS unit. An example of this would be attaching a container to the instrument containing a fixed volume of the sample. A carrier gas (usually clean dry air) is used to transfer the sample to the ionization region. Continuous sampling is where the resultant gaseous sample is continuously purged into the

FAIMS unit and either is diluted by the carrier gas or acts as the carrier gas itself. For example, continuously drawing air from the top of a process vat.

The one key requirement for all the sample preparation and introduction techniques is the ability to reproducibly generate and introduce a headspace (vapour) concentration of the target analytes that exceeds the lower limits of detection of the FAIMS device.

Carrier Gas

The requirement for a flow of air through the system is twofold: Firstly to drive the ions through the electrode channel to the detector plate and secondly, to initiate the ionization process necessary for detection.

As exhibited in Figure 16, the transmission factor (proportion of ions that make it to the detector) increases with increasing flow. The higher the transmission factor, the higher the sensitivity. Higher flow gives a larger full width half maximum (FWHM) of the peaks but also decreases the resolution of the FAIMS unit (see Figure 17).

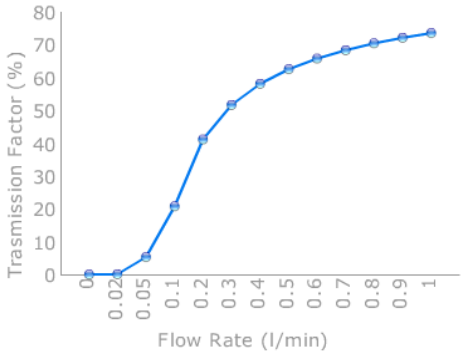


Figure 16 Flow rate vs. ion transmission factor

The air/carrier gas determines the baseline reading of the instrument. Therefore, for optimal operation it is desirable for the carrier to be free of all impurities (< 0.1 ppm methane) and the humidity to be kept constant. It can be supplied either from a pump or compressor, allowing for negative and positive pressure operating modes.

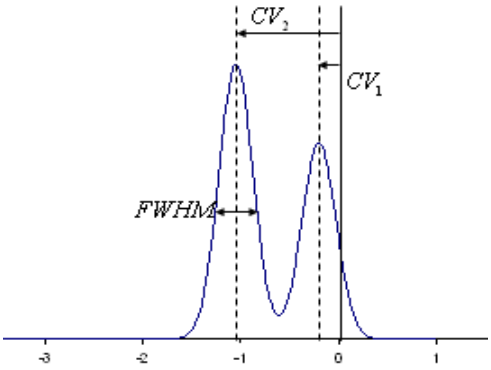


Figure 17 FWHM of ion species at set CV

Ionisation Source

There are three main vapor phase ion sources in use for atmospheric pressure ionization; radioactive nickel-63 (Ni-63), corona discharge (CD) and ultra-violet radiation (UV). A comparison of ionization sources is presented in Table 7.

Ionisation Source	Mechanism	Chemical Selectivity
Ni^{63} (beta emitter) creates a positive / negative RIP	Charge transfer	Proton / electron affinity
UV (Photons)	Direct ionisation	First ionisation potential
Corona discharge (plasma) creates a positive / negative RIP	Charge transfer	Proton / electron affinity

Table 7 FAIMS ionization source comparison

Ni-63 undergoes beta decay, generating energetic electrons, whereas CD ionization strips electrons from the surface of a metallic structure under the influence of a strong electric field. The generated electrons from the metallic surface or Ni-63 interact with the carrier gas (air) to form stable +ve and -ve intermediate ions which give rise to reactive ion peaks (RIP) in the positive and negative FAIMS spectra (Figure 18). These RIP ions then transfer their charge to neutral molecules through collisions. For this reason, both Ni-63 and CD are referred to as indirect ionization methods.

For the positive ion formation:

$N_2 + e^- \rightarrow \mathbf{N_2^+} + e^-$ (primary) + e^- (secondary)
$\mathbf{N_2^+} + 2N_2 \rightarrow \mathbf{N_4^+} + N_2$
$N_4^+ + H_2O \rightarrow 2N_2 + \mathbf{H_2O^+}$
$H_2O^+ + H_2O \rightarrow \mathbf{H_3O^+} + OH$
$H_3O^+ + H_2O + N_2 \leftrightarrow \boldsymbol{H^+(H_2O)_2} + N_2$
$H^+(H_2O)_2 + H_2O + N_2 \leftrightarrow \boldsymbol{H^+(H_2O)_3} + N_2$

For the negative ion formation:

$O_2 + e^- \rightarrow \mathbf{O_2^-}$
$B + H_2O + \mathbf{O_2^-} \leftrightarrow \boldsymbol{O_2^-(H_2O)} + B$
$B + H_2O + O_2^-(H_2O) \leftrightarrow \boldsymbol{O_2^-(H_2O)_2} + B$

The water based clusters (hydronium ions) in the positive mode (blue) and hydrated oxygen ions in the negative mode (red), are stable ions which form the RIPs. When an analyte (M) enters the RIP ion cloud, it can replace one or dependent on the analyte, two water molecules to form a monomer ion or dimer ion respectively, reducing the number of ions present in the RIP.

Monomer Dimer

$$H^+(H_2O)_3 + M + N_2 \leftrightarrow M\boldsymbol{H^+(H_2O)_2} + N_2 + H_2O \leftrightarrow M_2\boldsymbol{H^+(H_2O)_1} + N_2 + H_2O$$

Dimer ion formation is dependent on the analyte's affinity to charge and its concentration. This is illustrated in Figure 18A using dimethyl methylphsphonate (DMMP). Plot A shows that the RIP decreases with an increase in DMMP concentration as more of the charge is transferred over to the DMMP. In addition the monomer ion decreases as dimer formation becomes more favourable at the higher concentrations. This is shown more clearly in Figure 18B, which plots the peak ion current of both the monomer and dimer at different concentration levels.

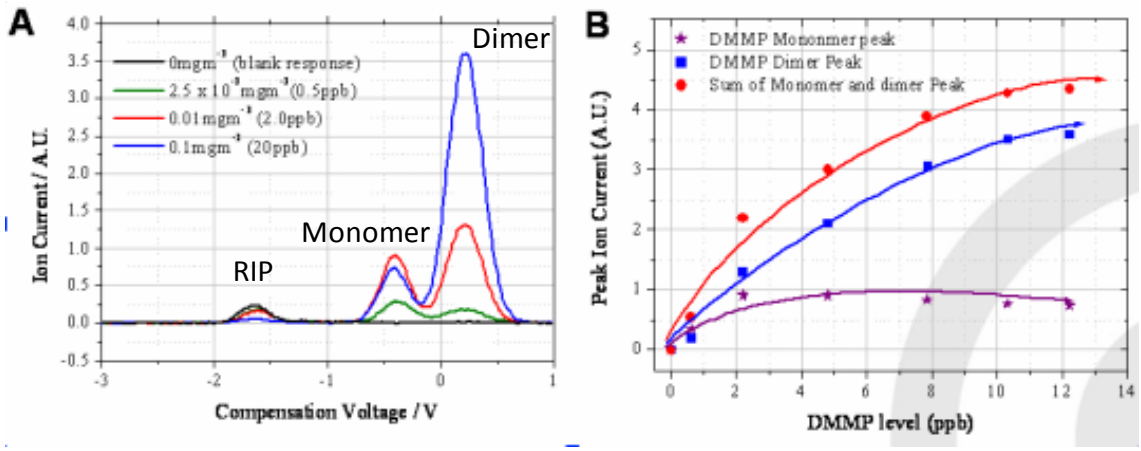


Figure 18 DMMP Monomer and dimer formation at different concentrations

The likelihood of ionization is governed by the analyte's affinity towards protons and electrons (Table 8 and Table 9 respectively).

In complex mixtures where more than one chemical is present, competition for the available charge occurs, resulting in preferential ionisation of the compounds within the sample. Thus the chemicals with high proton or electron affinities will ionize more readily than those with a low proton or electron affinity. Therefore the concentration of water within the ionization region will have a direct effect on certain analytes whose proton / electron affinities are lower.

Chemical Family	Example	Proton affinity
Aromatic amines	Pyridine	930 kJ/mole
Amines	Methyl amine	899 kJ/mole
Phosphorous Compounds	TEP	891 kJ/mole
Sulfoxides	DMS	884 kJ/mole
Ketones	2- pentanone	832 kJ/mole
Esters	Methly Acetate	822 kJ/mole
Alkenes	1-Hexene	805 kJ/mole
Alcohols	Butanol	789 kJ/mole
Aromatics	Benzene	750 kJ/mole
Water		691 kJ/mole
Alkanes	Methane	544 kJ/mole

Table 8 Overview of the proton affinity of different chemical families

Chemical Family	Electron affinity
Nitrogen Dioxide	3.91eV
Chlorine	3.61eV
Organomercurials	
Pesticides	
Nitro compounds	
Halogenated compounds	
Oxygen	0.45eV
Aliphatic alcohols	
Ketones	

Table 9 Relative electron affinities of several families of compounds

The UV ionization source is a direct ionization method whereby photons are emitted at energies of 9.6, 10.2, 10.6, 11.2, and 11.8 eV and can only ionize chemical species with a first ionization potential of less than the emitted energy. Important points to note are that there is no positive mode RIP present when using a UV ionization source and also that UV ionization is very selective towards certain compounds.

Mobility

Ions in air under an electric field will move at a constant velocity proportional to the electric field. The proportionality constant is referred to as mobility. As shown in Figure 19, when the ions enter the electrode channel, the applied RF voltages create oscillating regions of high ($+V_{HF}$) and low ($-V_{HF}$) electric fields as the ions move through the channel. The difference in the ion's mobility at the high and low electric field regimes dictates the ion's trajectory through the channel. This phenomenon is known as differential mobility.

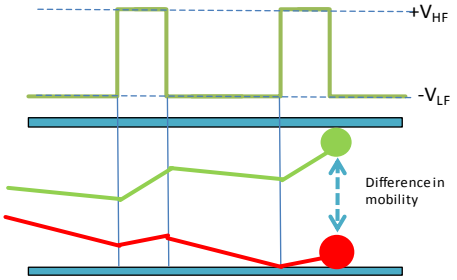


Figure 19 Schematic of a FAIMS channel showing the difference in ion trajectories caused by the different mobilities they experience at high and low electric fields

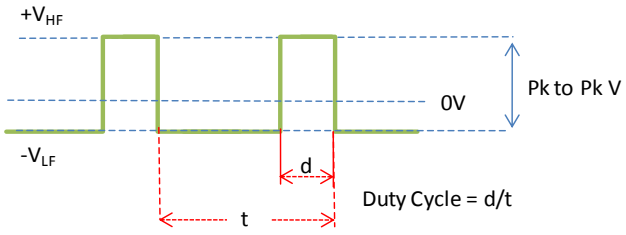


Figure 20 Schematic of the ideal RF waveform, showing the duty cycle and peak to peak voltage (Pk to Pk V)

The physical parameters of a chemical ion that affect its differential mobility are its collisional cross section and its ability to form clusters within the high/low regions. The environmental factors within the electrode channel affecting the ion's differential mobility are electric field, humidity, temperature and gas density (i.e. pressure).

The electric field in the high/low regions is supplied by the applied RF voltage waveform (Figure 20). The duty cycle is the proportion of time spent within each region per cycle. Increasing the peak-to-peak voltage increases/decreases the electric field experienced in the high/low field regions and therefore influences the velocity of the ion accordingly. It is this parameter that has the greatest influence on the differential mobility exhibited by the ion.

It has been shown that humidity has a direct effect on the differential mobility of certain chemicals, by increasing/decreasing the collision cross section of the ion within the respective low/high field regions. The addition and subtraction of water molecules to analyte ions is referred to as clustering and de-clustering. Increased humidity also increases the number of water molecules involved in a cluster ($MH^+(H_2O)_2$) formed in the ionisation region. When this cluster experiences the high field in between the electrodes the water molecules are forced away from the cluster reducing the size (MH^+) (de-clustering). As the low field regime returns so do the water molecules to the cluster, thus increasing the ion's size (clustering) and giving the ion a larger differential mobility. Gas density and temperature can also affect the ion's mobility by changing the number of ion-molecule collisions and changing the stability of the clusters, influencing the amount of clustering and de-clustering.

Changes in the electrode channel's environmental parameters will change the mobility exhibited by the ions. Therefore it is advantageous to keep the gas density, temperature and humidity constant when building detection algorithms based on an ion's mobility as these factors would need to be corrected for. However, it should be kept in mind that these parameters can also be optimized to gain greater resolution of the target analyte from the background matrix, during the method development process.

Detection and Identification

As ions with different mobilities travel down the electrode channel, some will have trajectories that will result in ion annihilation against the electrodes, whereas others will pass through to hit the detector. To filter the ions of different mobilities onto the detector plate a compensation voltage (CV) is scanned between the top and bottom electrode (see Figure 21). This process realigns the trajectories of the ions to hit the detector and enables a CV spectrum to be produced.

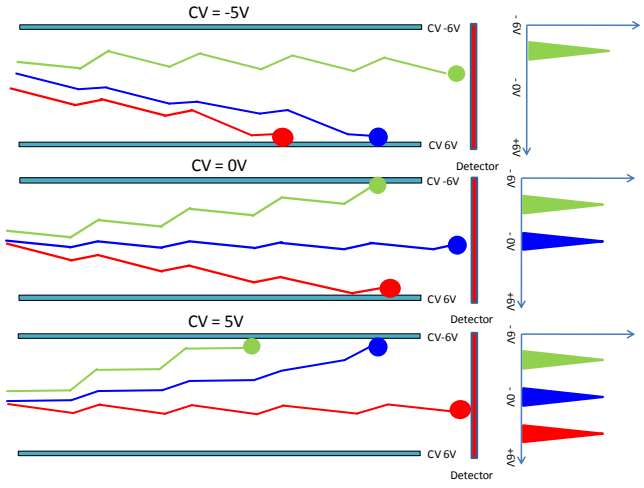


Figure 21 Schematic of the ion trajectories at different compensation voltages and the resultant FAIMS spectrum

The ion's mobility is thus expressed as a compensation voltage at a set electric field. Figure 22 shows an example CV spectrum of a complex sample where a de-convolution technique has been employed to characterize each of the compounds.

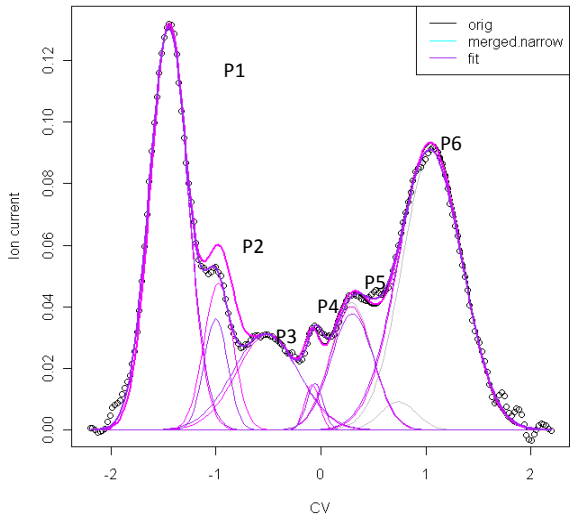


Figure 22 Example CV spectra. Six different chemical species with different mobilities are filtered through the electrode channel by scanning the CV value

Changing the applied RF peak-to-peak voltage (electric field) has a proportional effect on the ion's mobility. If this is increased after each CV spectrum, a dispersion field matrix is constructed. Figure 23 shows two examples of how this is represented; both are negative mode dispersion field (DF) sweeps of the same chemical. The term DF is sometimes used instead of electric field. It is expressed as a percentage of the maximum peak-to-peak voltage used on the RF waveform. The plot on the left is a waterfall image where each individual CV scan is represented by compensation voltage (x-axis), ion current (y-axis) and electric field (z-axis). The plot on the right is the one that is more frequently used and is referred to as a 2D color plot. The compensation voltage and electric field are on the x, and y axes and the ion current is represented by the color contours.

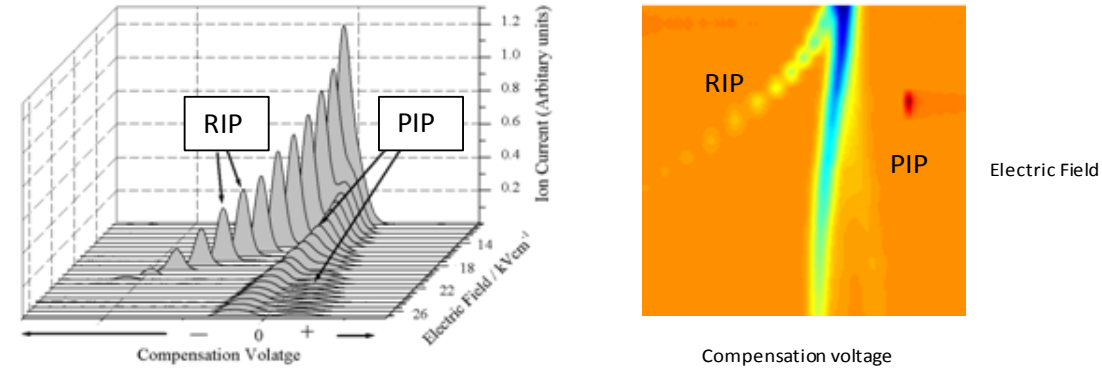


Figure 23 Two different examples of FAIMS dispersion field matrices with the same reactive ion peaks (RIP) and product ion peaks (PIP). In the waterfall plot on the left, the z axis is the ion current; this is replaced in the right, more frequently used, colorplot by color contours

With these data rich DF matrices a chemical fingerprint is formed, in which identification parameters for different chemical species can be extracted, processed and stored. Figure 24 shows one example: here the CV value at the peak maximum at each of the different electric field settings has been extracted and plotted, to be later used as a reference to identify the same chemicals. In Figure 25 a new sample spectrum has been compared to the reference spectrum and clear differences in both spectra can be seen.

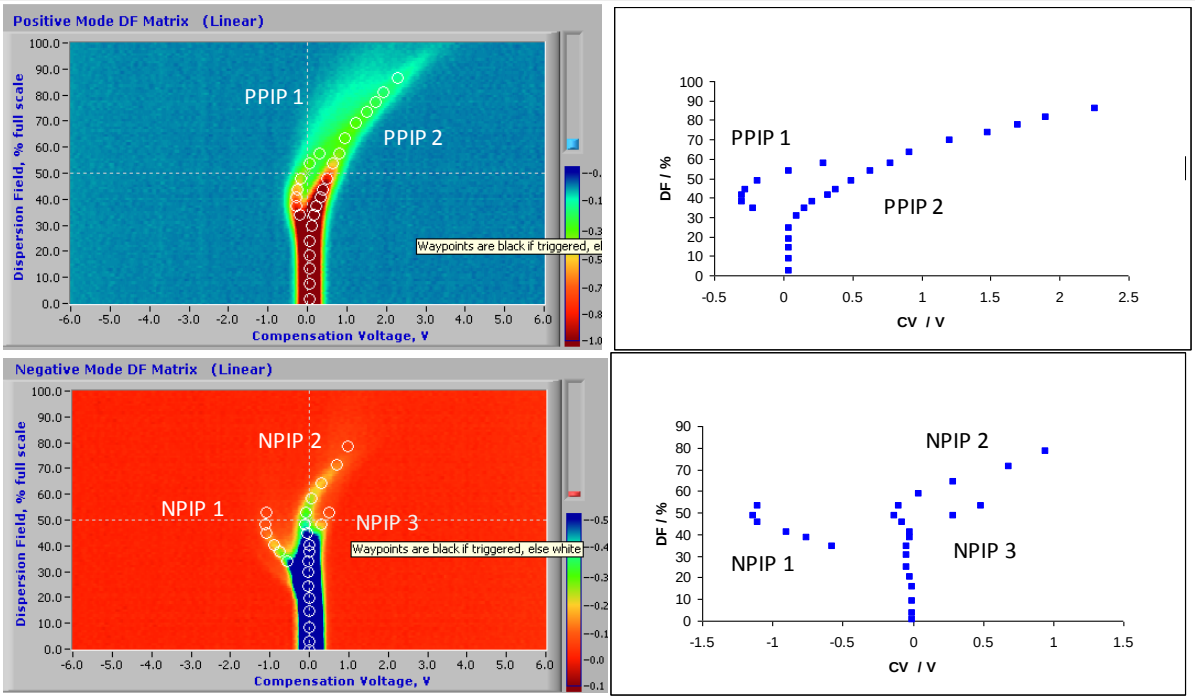


Figure 24 On the left are examples of positive (blue) and negative (red) mode DF matrices recorded at the same time while a sample was introduced into the FAIMS detector. The sample contained 5 chemical species, which showed as two positive product ion peaks (PPIP) and three negative product ion peaks (NPIP). On the right, the CV at the PIP's peak maximum is plotted against % dispersion field to be stored as a spectral reference for subsequent samples.

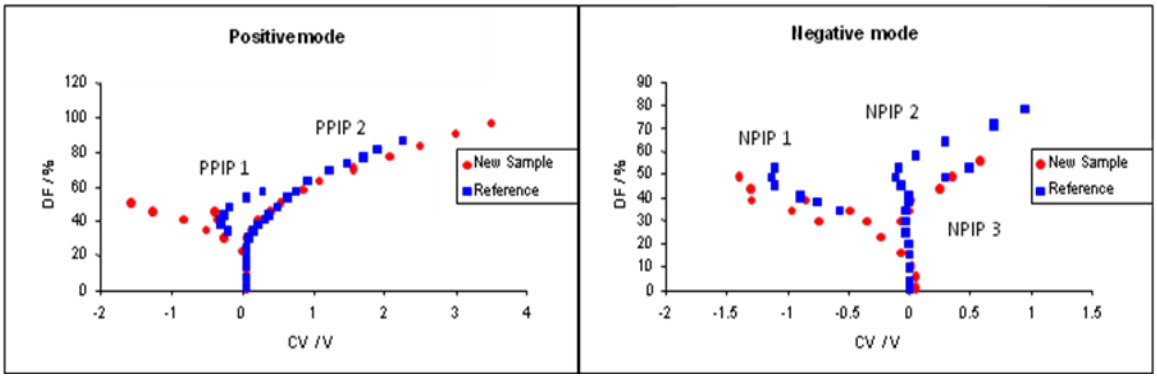


Figure 25 Comparison of two new DF plots with the reference from Figure 10. It can be seen that in both positive and negative modes there are differences between the reference product ion peaks and the new samples

Appendix B

Generating Calibration Standards with OVG-4™

Calibration standards can be generated using permeation tubes and Owlstone's OVG-4 Calibration Gas Generator. The permeation tubes are gravimetrically calibrated to NIST traceable standards. For this study an acetone permeation source was used as a confidence check to ensure the LONESTAR was operating within defined parameters.

BENEFITS

- High number of available analyte compounds, including solids and liquids as well as gases
- Easy generation of multi-component mixtures using combinations of tubes
- Cost savings by elimination of multiple expensive gas cylinders
- Reduced risk of exposure to dangerous chemicals due to small quantities used
- Fast and easy sample replacement
- Elimination of hazards associated with high pressure cylinders
- Quick and easy to set up and generate blended gas mixtures
- Adjustable concentration levels from ppm to ppb
- High accuracy and precision, even at the lowest concentrations
- Superior long term stability and repeatability*
- Portable, with compact footprint
- Easily integrated with the Owlstone Humidity Generator (OHG) for realistic environmental testing

**Owlstone offers an optional service for regular validation and instrument calibration*

The Owlstone OVG-4 is a system for generating NIST traceable chemical and calibration gas standards. It is easy to use, cost-effective and compact and produces a very pure, accurate and repeatable output.

The very precise control of concentration levels is achieved using permeation tube technology, eliminating the need for multiple gas cylinders and thus reducing costs, saving space and removing a safety hazard. Complex gas mixtures can be accurately generated through the use of multiple tubes.

By swapping out permeation tubes the OVG-4 can be used to generate over 500 calibration standards to test and calibrate almost any gas sensor, instrument or analyzer, including FTIR, NDIR, Raman, IMS, GC, GC/MS.

Current customers include – SELEX GALILEO, US Army, US Air Force, US Defense Threat Reduction Agency, Home Office Scientific Development Branch, DSTL, Commissariat à l'Énergie Atomique, EADS, United Technologies, Alphasense, Xtralis, LGC, Genzyme, IEE, Institut de la Corrosion, Rutherford Appleton Laboratory, University Cambridge, Cranfield among others.

Adding Precision Humidity with the Owlstone OHG-4™ Humidity Generator

The OVG-4 can be integrated with the OHG-4 Humidity Generator to create realistic humidified test atmospheres for more realistic testing. Within this study the hygrometer was used to provide accurate inline humidity monitoring of the sample introduction line.

The OVG alongside the OHG for precision humidity generation and control