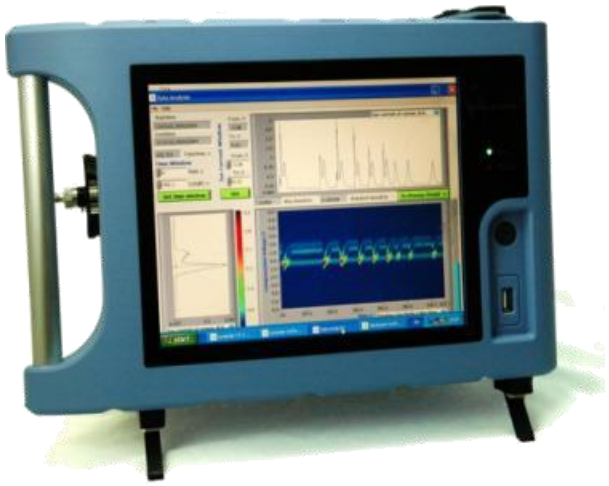
Testing for Contaminants in Milk Using a LONESTAR™ Portable Analyzer

Analysis of 2,4-dichlorophenol, 2,4-dibromophenol, lactic acid, triethylamine and benzoic acid in whole milk



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Summary

The aim of this investigation was to ascertain if a Lonestar chemical monitor could be used to detect 2,4-dichlorophenol (DCP) and other common milk contaminants in whole milk. It was found that the Lonestar unit could to detect DCP at 1 ppb levels in whole milk. It was also found that other contaminants 2,4-dibromophenol, triethylamine and benzoic acid were detectable along with lactic acid, a biomarker that correlates with the age of milk.

Introduction

During the production process, trace contaminants contained in fresh milk can become concentrated in processed dairy products such as cheese, butter, yoghurt and cream. This can result in wasted batches of food product with 'off flavors'. An example of one such trace contaminant is the common pesticide breakdown product 2,4-dichlorophenol (DCP). DCP and other compounds are found in trace amounts in the grass based feed of dairy cattle. Chlorinated phenols have a very strong 'medicinal' flavor and are detectable by the human palette in extremely small quantities.

Due to the low initial concentration of the compounds in un-processed milk, detection can be difficult without skilled personnel performing expensive and time consuming analytical techniques such as high performance liquid chromatography-mass spectrometry (HPLC-MS) or gas chromatography-mass spectrometry (GC-MS). This manuscript details testing that shows the Lonestar platform can provide an alternative highly sensitive, simple and fast solution to detecting DCP and other common contaminants in milk.

The LONESTAR™ Portable Analyzer

The LONESTAR is an analytically powerful, portable analyzer that can be operated by nonspecialist users. Incorporating Owlstone's proprietary FAIMS technology (see Appendix A), the instrument combines high sensitivity and selectivity. New methods can be developed using Owlstone's EasySpec software, making the LONESTAR suitable for a broad range of applications in the food and beverage industry.

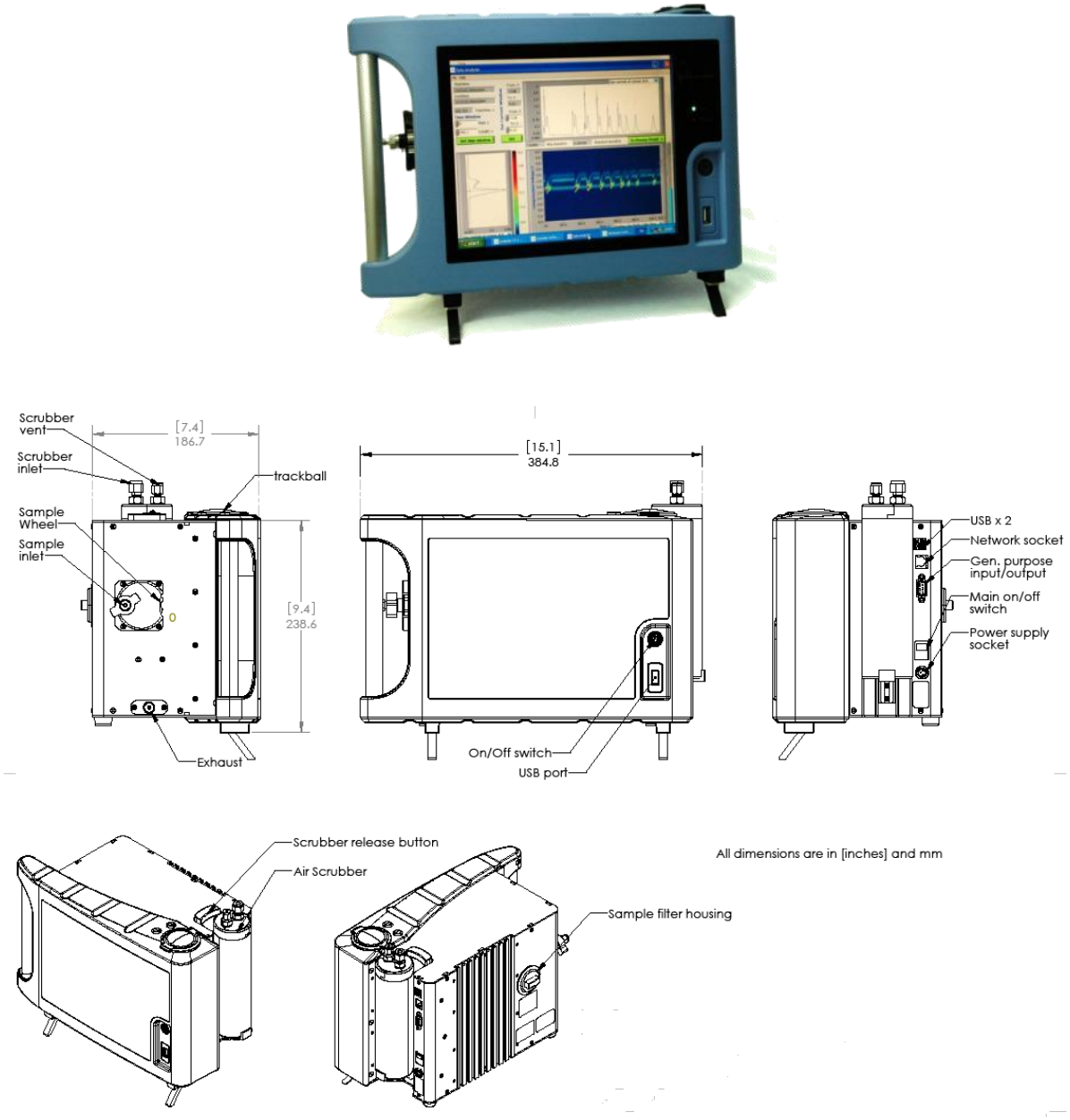

Figure 1 LONESTAR connection figures

Equipment Set up

The Lonestar instrument setup is shown in Figure 2. For this investigation the Lonestar unit was run under a positive pressure. Increasing the pressure of the input flow increases the density of the gas at the chip. For a given voltage applied across the channel, a higher gas density will result in a lower dispersion field (DF).

The pressure regulator controls the supply of carrier gas into the sample bottle and ultimately controls and sets the pressure within the Lonestar. It was set to maintain a pressure of 0.9 bar. A rotameter connected to the exhaust port of the Lonestar unit controlled the flow of air through the Lonestar, rather than the unit's in built flow controller. This was set to a flow of 2.5 ml min^{-1} whilst care was taken to maintain the pressure regulator's 0.9 bar setpoint.

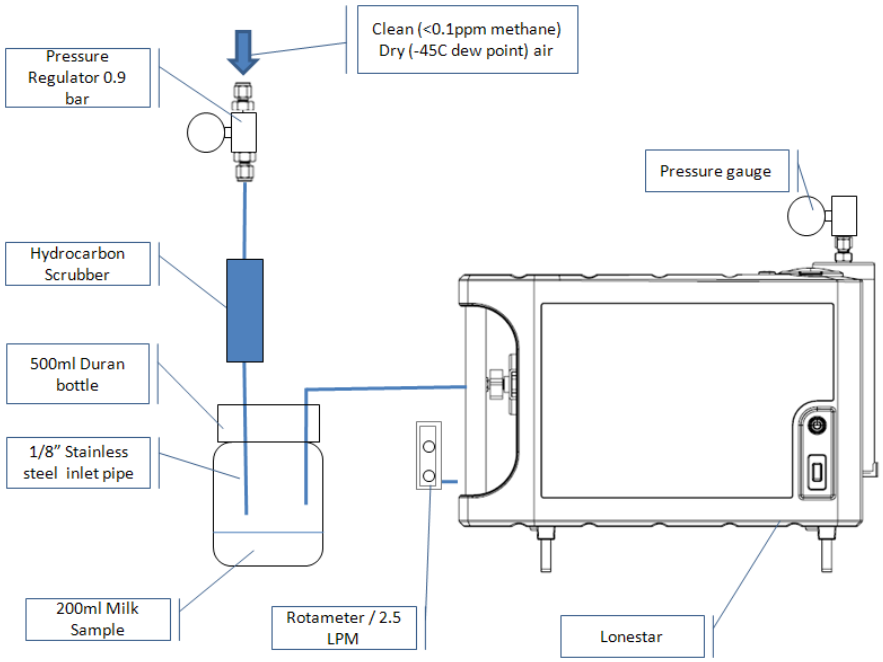

Figure 2 Schematic of the sampling apparatus

The Duran bottle inlet tube was cut to length so it was approximately 5 mm above the surface of the milk sample. This ensured that the surface of the liquid was disturbed sufficiently by the flow of carrier gas to help volatilize the dissolved DCP. The tube must be kept above the surface of the milk to prevent the sample being forced into the Lonestar unit's internal sample line. Using this Lonestar configuration, headspace (the gas above the liquid sample) samples were taken from 500 ml bottles containing 200 ml of whole milk both with and without 1 ppb of DCP. Figure 3 shows a screen shot of the Lonestar settings screen.

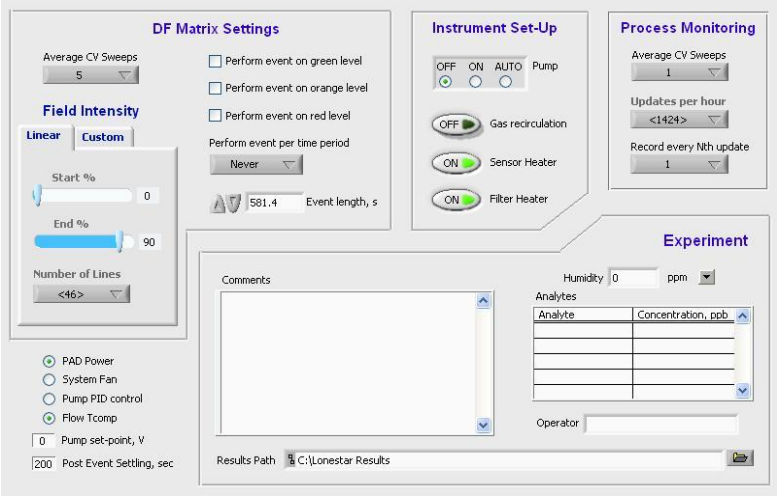

Figure 3 The Lonestar settings screen for the positive pressure experiments.

DCP in milk testing procedure

Sample preparation

DCP in water standard solution

For a 100 ppm solution, 0.01 g DCP was weighed out in a fume cupboard and then added to 100 ml of purified water. The mixture was heated and sonicated for 20 minutes to completely dissolve the DCP. A 1 ml measure of the 100 ppm standard was added to 99 ml of purified water to produce a 1 ppm standard.

1 ppb DCP in milk standard

Prior to the addition of DCP, the milk was prepared based on the technique described by Mardones et al. (2008)[1]. Sodium chloride (8 g) was dissolved in fresh whole milk (200 ml). To make the 1 ppb DCP in milk standard, 0.2 ml of the 1 ppm DCP in water standard (see above) was added to the 200 ml sodium chloride and whole milk mixture.

A 'blank' solution of sodium chloride dissolved in whole milk of the same concentration of that described above was also prepared. This was sampled using the Lonestar unit to provide a background spectrum.

[1] Mardones, C., et al. "Determination of halophenolic wood preservant traces in milk using headspace solid-phase microextraction and gas chromatography-mass spectrometry." Journal of Chromatography A, 1215 (2008) pg1-7.

DCP results and discussion

Figure 4 shows the spectra obtained from an instrumental blank (i.e. a clean, empty 500 ml bottle). This clearly shows the reactive ion peak (RIP) on both the positive and negative DF matrices.

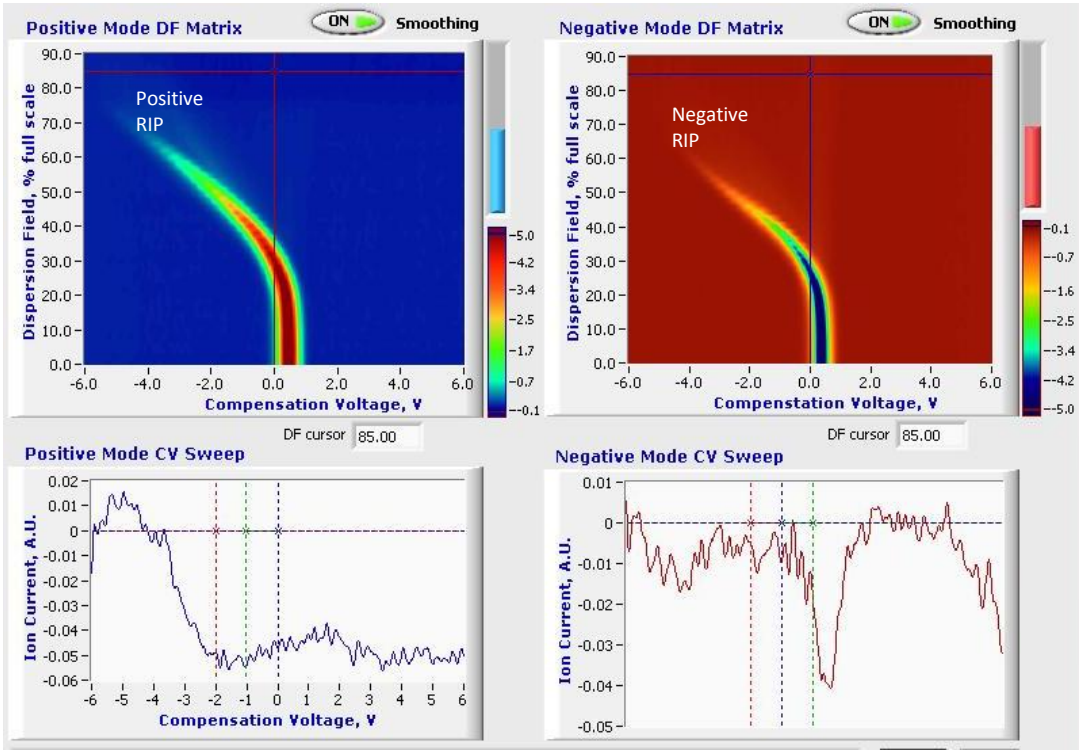

Figure 4 Lonestar blank showing the hydronium ions in the positive reactive ion peak (RIP) and the Hydrated O_2 ions in the negative mode RIP.

Figure 5 and Figure 6 show the instrumental response to the whole milk blank headspace and the headspace of the whole milk with 8 g NaCl respectively.

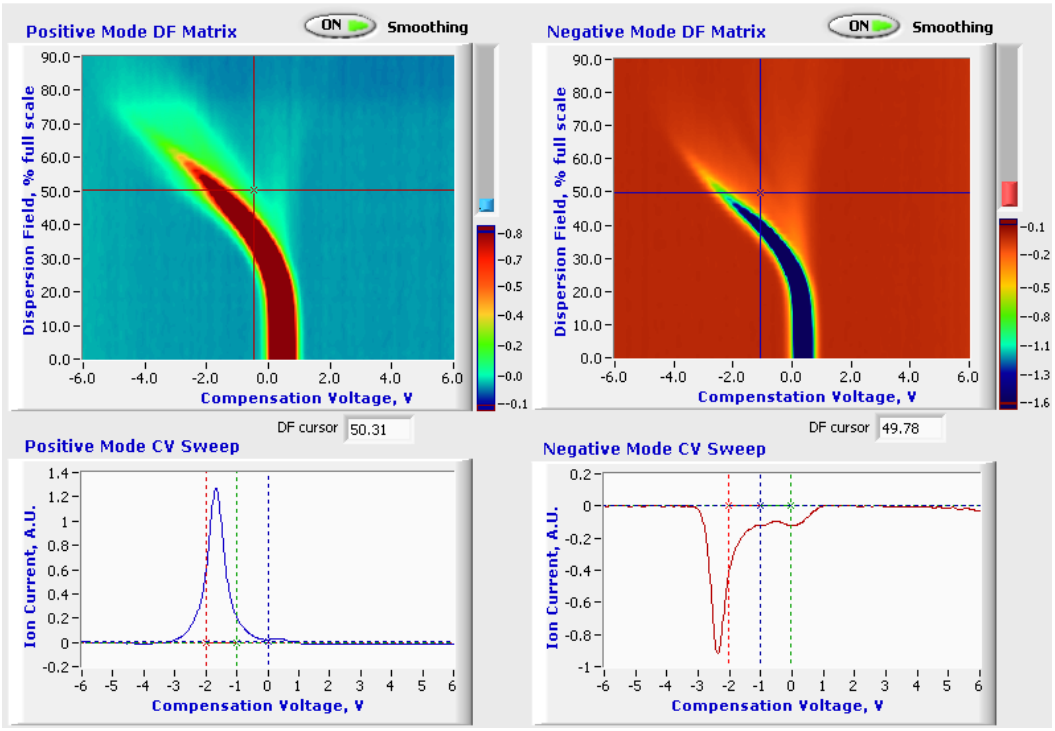

Figure 5 Whole milk blank response

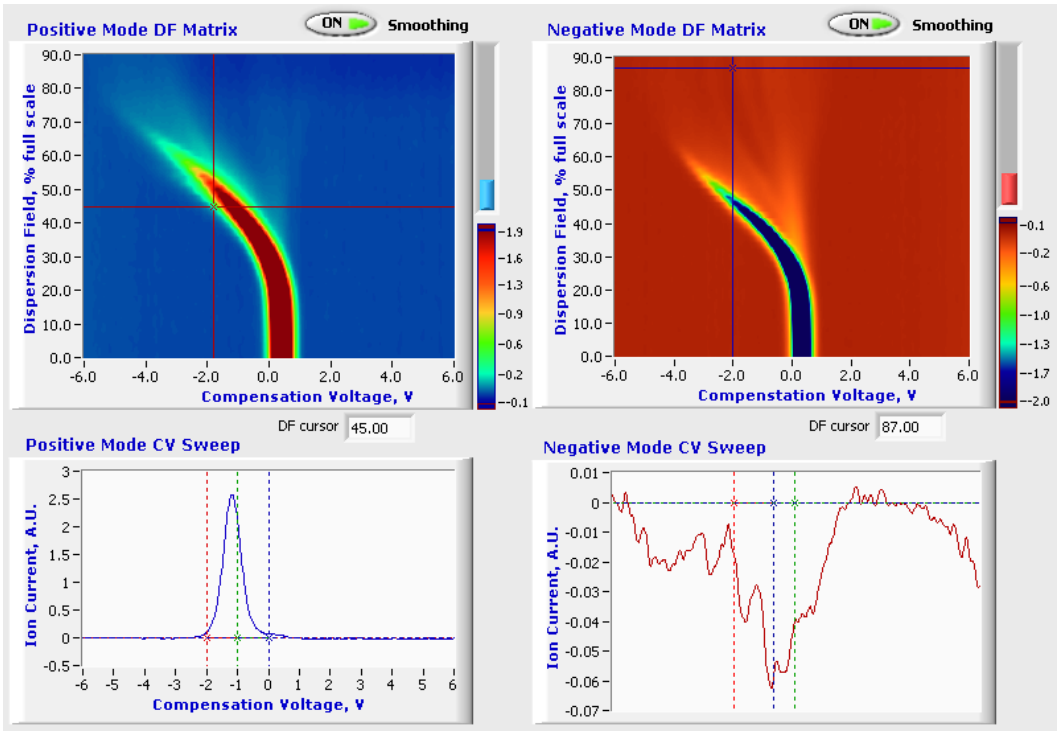

Figure 6 Whole milk blank and 8 g NaCl

The Lonestar response from the 1 ppb DCP in milk standard (with NaCl) can be seen as peaks in Figure 7 that were not visible in the milk with NaCl blank shown in Figure 6. The DCP peaks can

be seen more clearly in the graph view below the –ve DF sweep matrix. The DF cursor is set to 87% and the peaks appear at -0.8 CV and -0.5 CV.

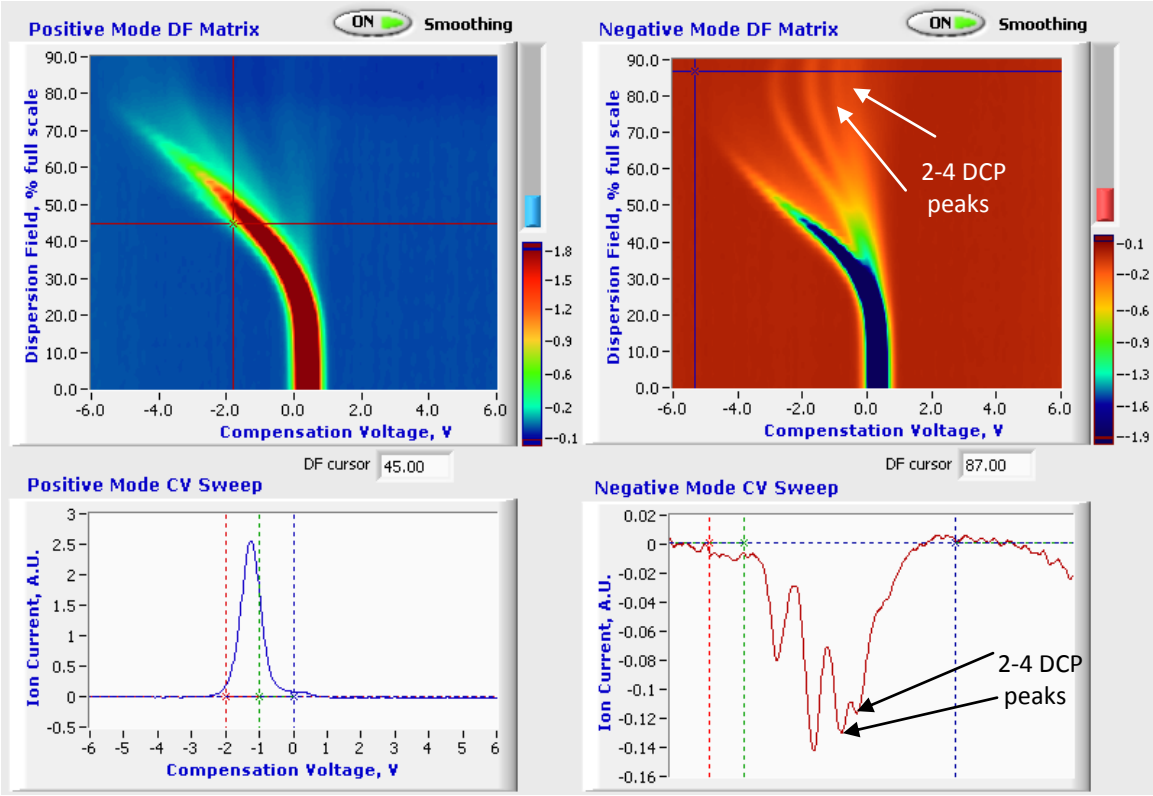

Figure 7 Lonestar sample spectra for 1 ppb 2,4-dichlorophenol (DCP) in whole milk and NaCl solution.

Detecting other chemical species

As well as DCP, the response of the Lonestar unit to other important chemicals in milk was also tested using the same method. These included a 200 ppm concentration of lactic acid (Figure 8), 10 ppb of dibromophenol (DBP) (Figure 9) and triethlyamine (TEA) (Figure 10) all in whole milk.

Lactic acid

Lactic acid (also known as milk acid) is a cause of milk spoilage (or souring) and is produced in ageing milk via fermentation by bacteria including *lactobacillus*. Comparison of the whole milk blank spectra (Figure 5) and the spectra for whole milk containing 200 ppm lactic acid show that Lonestar was able to clearly identify the lactic acid peak using 85% DF. This indicates that Lonestar could potentially be used to monitor the condition of fresh milk prior to its use or during storage.

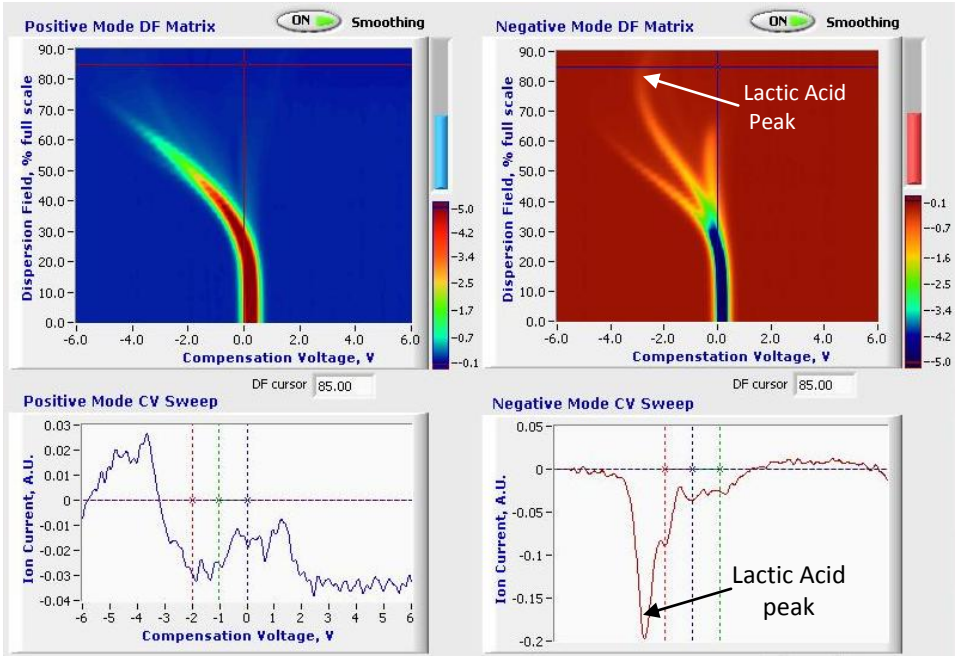

Figure 8 Whole milk with 200 ppm lactic acid

2,4-dibromophenol

DBP is a very similar compound to DCP and is a source of iodoform-like off flavours in foodstuffs including some seafoods such as prawns. It was found that DCB was detectable by Lonestar at 10 ppb concentrations (Figure 9), at 87% DF..

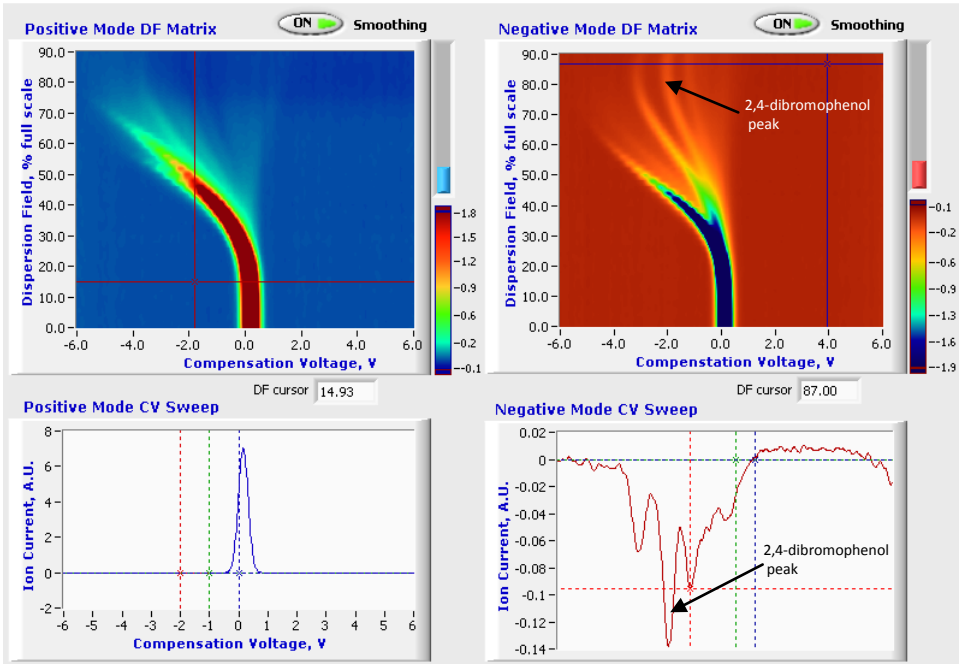

Figure 9 A 10 ppb concentration of 2,4-dibromophenol in whole milk.

Triethylamine

TEA has a fishy, ammonia-like odour and was found to be detectable by Lonestar in the positive mode at 64% DF.

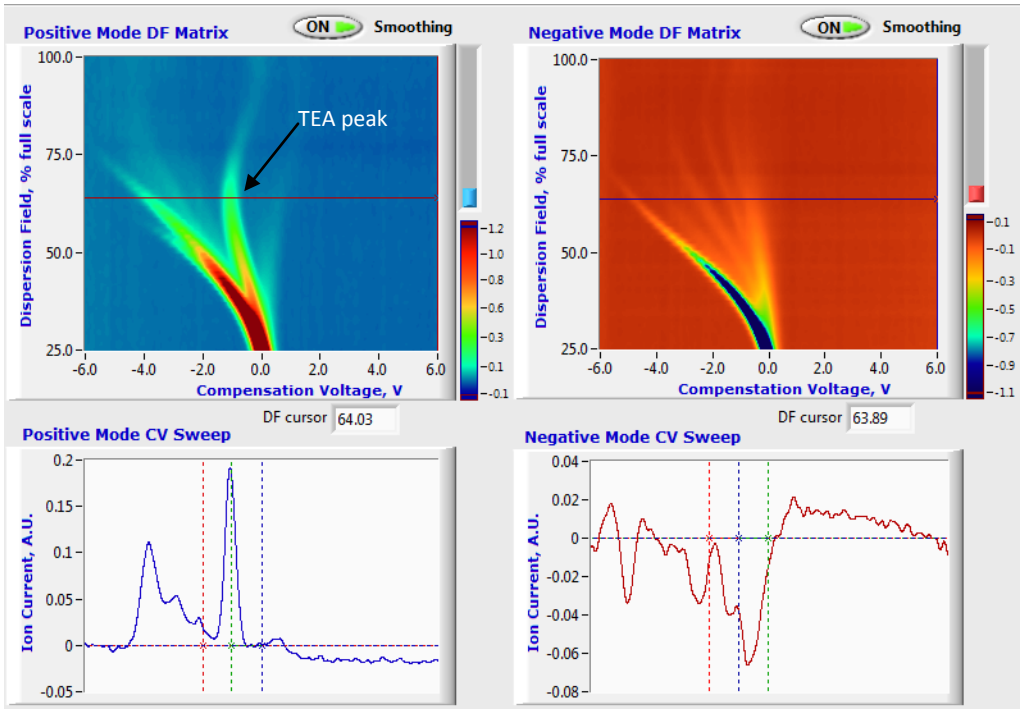

Figure 10 Triethylamine in milk

Benzoic acid

Benzoic acid is produced in milk by microbial action and is sometimes added as an adulterant to increase shelf life [2]. Figure 11 and Figure 12 show Lonestar spectra of a milk blank sample and milk containing 0.2% benzoic acid. The benzoic acid was clearly visible as a peak in the negative mode spectra.

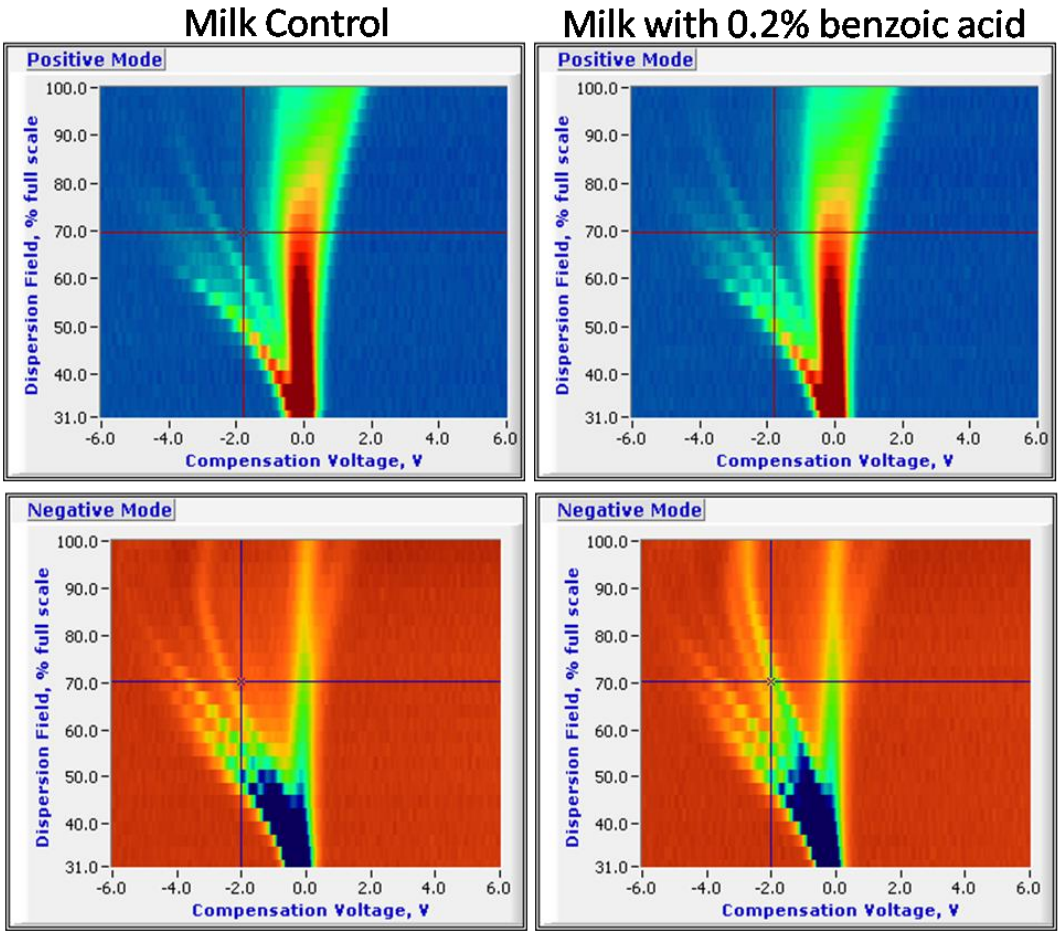

Figure 11 Lonestar spectra comparing a whole milk blank (right hand panels) with milk containing 0.2% benzoic acid.

[2] Qi, P. et al., "Assessment of benzoic acid levels in milk in China", Food Control, 20 (2009) p.414–418

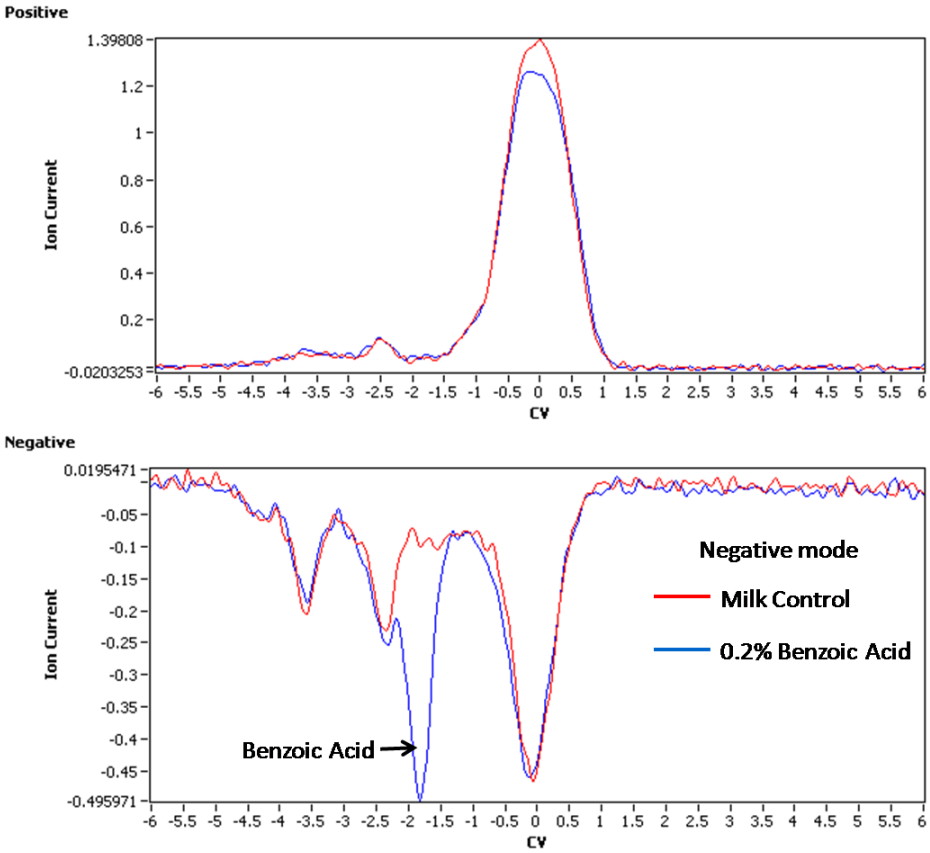

Figure 12 Positive (top) and negative (bottom) mode CV sweeps for a milk blank and for milk containing 0.2% benzoic acid.

Conclusions

The Lonestar platform was able to rapidly detect 2,4-dichlorophenol (DCP) in whole milk samples at 1 ppb when using a positive pressure and having added sodium chloride to milk samples. The Lonestar platform was also found to be able to detect a series of other common milk impurities. Lactic acid in whole milk was detectable at 200 ppm concentrations and 2,4-dibromophenol could be resolved at 10 ppb. A 0.2% concentration of benzoic acid was clearly detectable as was triethylamine. Further work is required to find the limits of detection for these compounds.

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 13 is a schematic illustrating the operating principles of FAIMS.

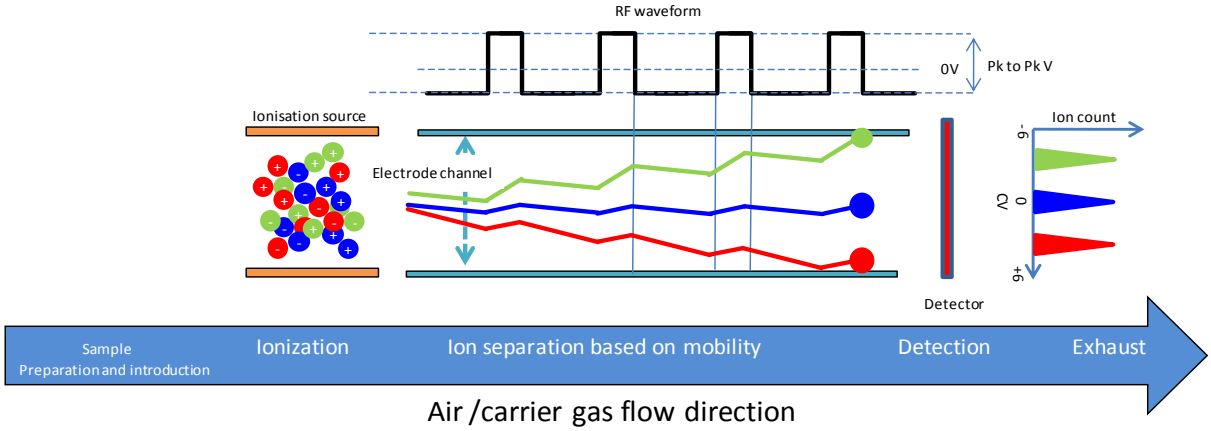

Figure 13 FAIMS schematic. The sample in the vapour phase is introduced via a carrier gas to the ionisation region, where the components are ionised via a charge transfer process or by direct ionisation, dependent on the ionisation source used. It is important to note that both positive and negative 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 14, 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 15).

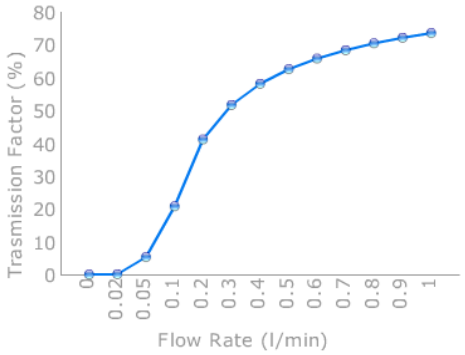

Figure 14 Flow rate vs. ion transmission factor

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.

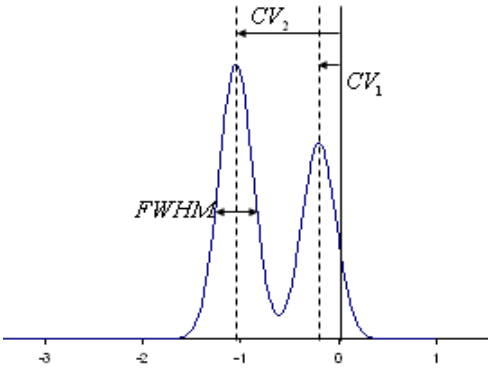

Figure 15 FWHM of ion species at set CV

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 1.

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 1 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 16). 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 16A 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 16B, which plots the peak ion current of both the monomer and dimer at different concentration levels.

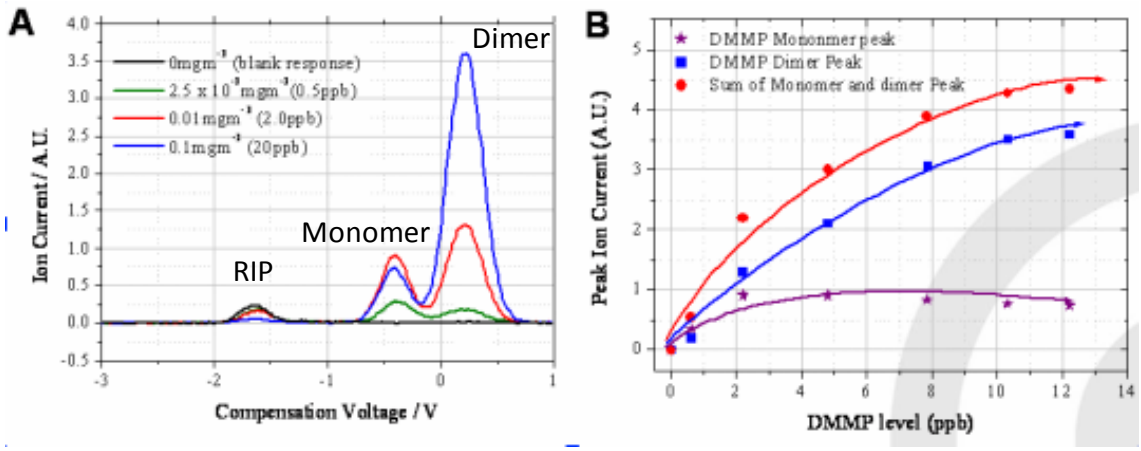

Figure 16 DMMP Monomer and dimer formation at different concentrations

The likelihood of ionization is governed by the analyte's affinity towards protons and electrons (Table 2 and Table 3 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 2 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 3 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 17, 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.

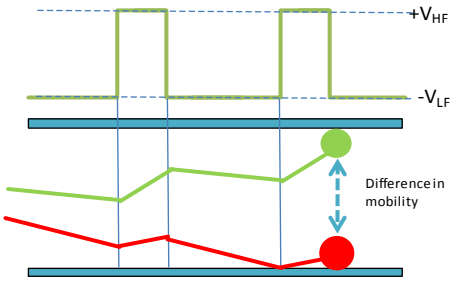

Figure 17 Schematic of a FAIMS channel showing the difference in ion trajectories caused by the different mobilities they experience at high and low electric fields

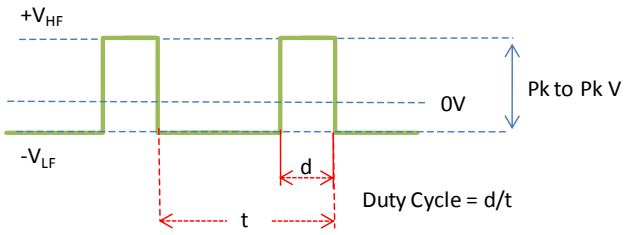

Figure 18 Schematic of the ideal RF waveform, showing the duty cycle and peak to peak voltage (Pk to Pk V)

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 18). 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 19). This process realigns the trajectories of the ions to hit the detector and enables a CV spectrum to be produced.

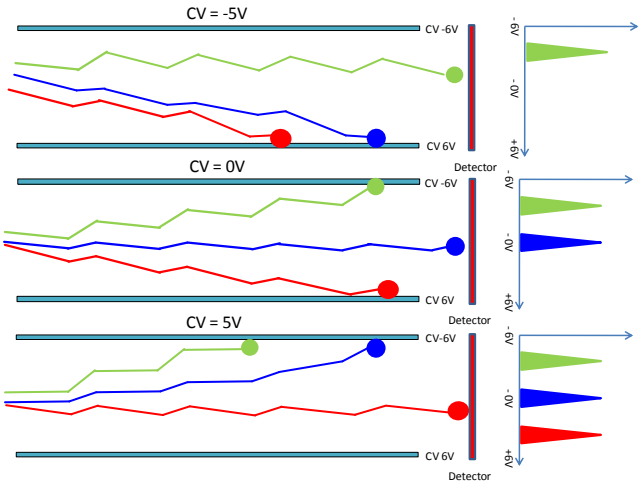

Figure 19 Schematic of the ion trajectories at different compensation voltages and the resultant FAIMS spectrum

The ion's mobility is thus expressed as a compensation voltage at a set electric field. Figure 20 shows an example CV spectrum of a complex sample where a de-convolution technique has been employed to characterize each of the compounds.

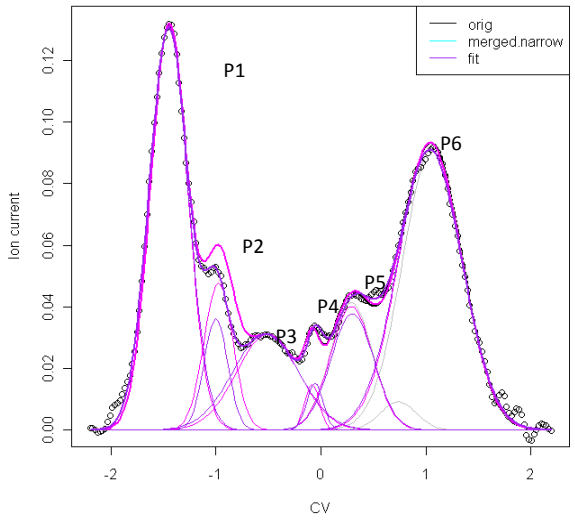

Figure 20 Example CV spectra. Six different chemical species with different mobilities are filtered through the electrode channel by scanning the CV value

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 21 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.

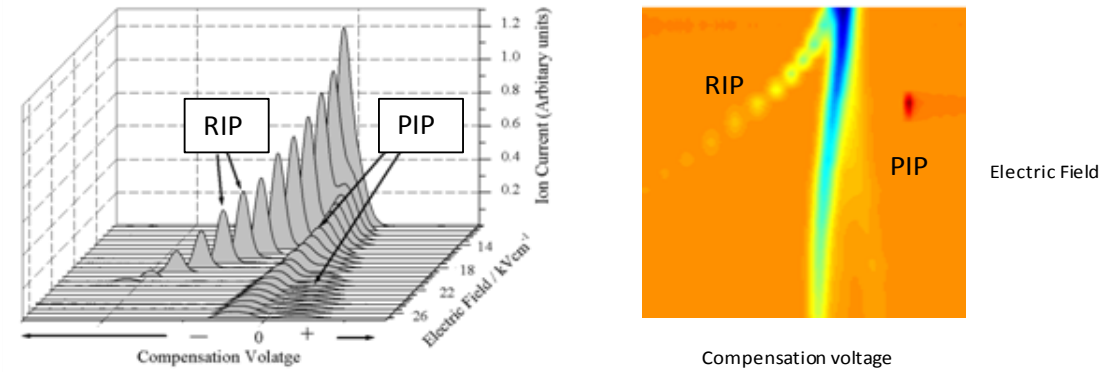

Figure 21 Two different examples of FAIMS dispersion field matrices with the same reactive ion peaks (RIP) and product ion peaks (PIP). In the waterfall plot on the left, the z axis is the ion current; this is replaced in the right, more frequently used, colorplot by color contours

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 22 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 23 a new sample spectrum has been compared to the reference spectrum and clear differences in both spectra can be seen.

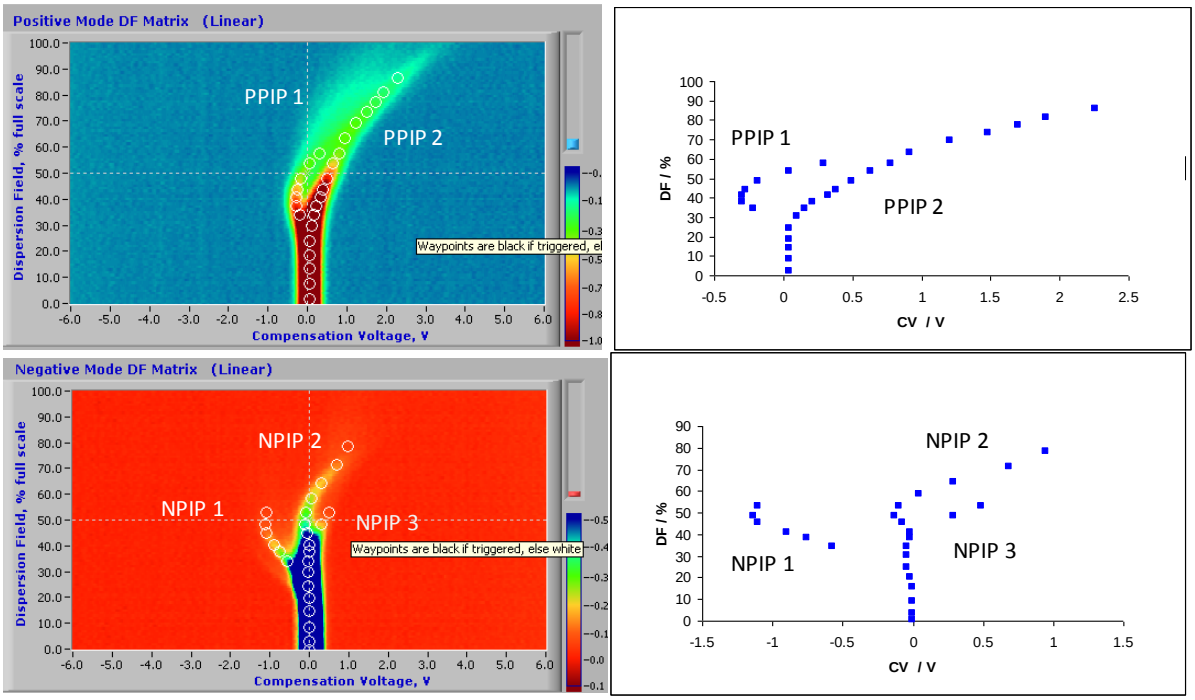

Figure 22 On the left are examples of positive (blue) and negative (red) mode DF matrices recorded at the same time while a sample was introduced into the FAIMS detector. The sample contained 5 chemical species, which showed as two positive product ion peaks (PPIP) and three negative product ion peaks (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.

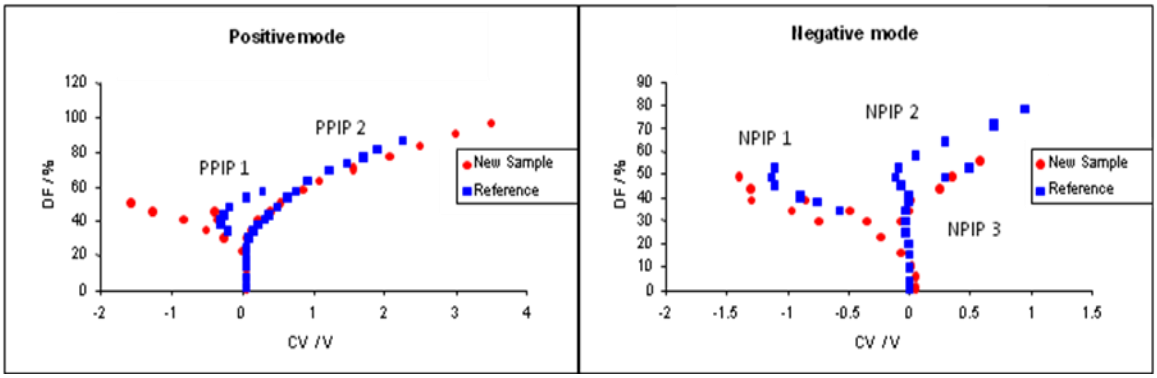

Figure 23 Comparison of two new DF plots with the reference from Figure 10. It can be seen that in both positive and negative modes there are differences between the reference product ion peaks and the new samples