Test Report

Residual solvent detection - Company A testing and addition solvent tests.

Analytes tested – Acetone, Chloroform, Diethyl ether, Ethanol, Hexane, Methanol, Toluene
Matrix – Oxcarbazepine and Ropinirole

Dr. M D Allsworth
7th Jun 09
## Contents

Testing Objectives and Overview ........................................................................................................ 3  
The Technology at a glance .................................................................................................................. 4  
Core Technology: Owlstone’s Innovative Microchip solution .............................................................. 5  
Testing Procedure overview ............................................................................................................... 8  
  Basic configuration ............................................................................................................................ 8  
  Alternative configurations .................................................................................................................. 8  
Background Matrix ............................................................................................................................... 9  
Individual solvent responses .............................................................................................................. 10  
  Chloroform .................................................................................................................................... 10  
  Diethyl ether .................................................................................................................................... 12  
  Other solvents – Acetone, Ethanol, Hexane and Toluene ............................................................... 14  
  Methanol ........................................................................................................................................ 15  
Solvent drying ..................................................................................................................................... 16  
  Mix 1 ............................................................................................................................................... 16  
  Mix 2 ............................................................................................................................................... 18  
Future work .......................................................................................................................................... 19  
  Other Solvents and selectivity .......................................................................................................... 19  
  An alternative testing configuration – dissolving samples in water .................................................. 19  
Summary ............................................................................................................................................ 20
Testing Objectives and Overview

The aim of the Company A testing was to demonstrate the Lonestar platforms viability for making real-time quantitative measurements of residual solvent content in pharmaceuticals. Testing on site at Company A’s New York site on the 18th February 2009 focused on two drugs Oxcarbazepine and Ropinirole spiked with various concentration levels of different solvents at the ppm level. Follow up tests were carried out at Owlstone’s Cambridge labs to show a wider range of solvent responses and mixtures of solvents using optimised sampling and FAIMS filter fields.

There are 60+ class 1, 2 and 3 solvents listed by United States Pharmacopeia USP which should not be present above set thresholds in any pharmaceuticals, though in practice the particular solvents used in the drug synthesis are the most likely to be present. Testing therefore focused on the solvents associated with each drug, though all the solvents on the list should be detectable to sub ppm levels. For the two drugs tested these solvents were

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Specific Solvents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ropinirole</td>
<td>methanol, ethanol, diethyl ether, acetone, toluene</td>
</tr>
<tr>
<td>Oxcarbazepine</td>
<td>acetone, n-propanol, toluene</td>
</tr>
</tbody>
</table>

Other representative solvents were selected from USP list and tested, these were – chloroform and hexane to show the response to a halogenated compound and to a simple aliphatic hydrocarbon.
The Technology at a glance

**What makes a chemical different?**
The basic problem of detection is how to detect the chemical of interest in a complex mixture. Owlstone technology identifies chemicals using a property known as ‘mobility’, a measure of how quickly an ion moves through an electric field. The mobility relates to size and mass, and is used to specifically distinguish and identify the chemical of interest.

**Detection with Owlstone FAIMS**
The Owlstone sensor acts as a reprogrammable filter, which separates and identifies chemicals according to their characteristic mobility. The sensor ‘filters’ out the background chemicals that do not have the correct mobility ‘fingerprint’. The power and flexibility of the system is due to the fact that the filter can be easily reprogrammed through software and electrical signals to detect almost any chemical.

**Identification with a chemical ‘fingerprint’**
The Owlstone FAIMS technology rapidly creates a fingerprint for all the chemicals present in a mixture, even at extremely low concentrations. Software is used to analyse the fingerprint to provide the user accurate information on the type, and quantity of chemicals present, to allow them to make the right decisions with a high degree of confidence.

**Chemical ‘Fingerprint’**
The following are a series of chemical fingerprints from internal testing programs. The different fingerprints are unique to the compound and are easily distinguishable and identifiable from each other. Software algorithms are used to classify each fingerprint, resulting in identification with greatly reduced occurrences of false positives.

Benzene (Carcinogen)  
DMMP (Sarin Simulant)  
Acetone (Blood glucose marker)
Core Technology: Owlstone’s Innovative Microchip solution

The heart of the Owlstone detection technology is a microchip sized spectrometer. The device is fabricated using processes similar to those in the semiconductor industry, enabling a highly integrated ‘dime’ sized detector. The parallel, batch fabrication process results in economies of scale, which dramatically reduces unit cost.

Chemicals enter the detector and are ionised, which means they can be moved by an electric field. Ionisation detection methods are among the most sensitive available, easily achieving detection limits as low as one part per billion. This allows the detection of chemicals at very low thresholds, a key requirement across the whole range of detection applications.

Ions are then drawn into the sensor for analysis. The proprietary Owlstone design incorporates a drive electric field to ‘pump’ ions through the device. Conventional FAIMS technologies use mechanical pumps which are power hungry and bulky. The Owlstone design is integrated to greatly reduce size and complexity. The power consumption is reduced by a factor of a thousand, which means the detector can be used in battery portable applications that demand long operational life.

As ions move through the device an electric field is applied, which filters the ion of interest from the background mixture. Ions with the correct mobility ‘fingerprint’ pass through the device and are detected. Conventional FAIMS technologies have a single filter gap, which requires very high voltages and reduces sensitivity. The Owlstone design has a series of filter electrodes connected in parallel, which results in a significant increase in sensitivity and a further ability to detect chemicals at very low concentrations.
Using microfabrication techniques it is possible to make the parallel gap filter structure extremely small. In addition to making the device small it also reduces the voltage required to operate the sensor, thus simplifying the system in its entirety. It becomes possible to fit all the electronics on another microchip, reducing the size, cost and power consumption. At these lower voltages we can generate ‘cleaner’ signals which improves resolution and reduces the false positive rate.

The sensor quickly generates a ‘fingerprint’ for all the chemicals present, enabling simultaneous identification and characterisation while minimising false positives. The Owlstone FAIMS technology has numerous electrical parameters which can be independently controlled by software, enabling dynamic programming of parameters such as sensitivity and selectivity, even after the system has been deployed.

It is necessary to heat FAIMS sensors to reduce deleterious effects due to changes in environmental conditions, minimise decontamination time and improve detection of ‘sticky’ substances such as explosives. Heating conventional FAIMS technologies requires a lot of energy, which reduces battery life. The Owlstone technology includes an ‘intrinsic’ heater. Due to the small size of the sensor it heats up rapidly with low power consumption making it more suitable for portable applications.

When connected into an instrument the ‘total detection subsystem’ is low cost and miniaturised. It is possible to add a wireless module for remote deployment. By changing the software and drive signals it is possible to update or reprogram the sensor according to the requirements of the application.
The Lonestar Platform

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, Lonestar is suitable for a broad variety of applications ranging from process monitoring to lab based R&D.
Testing Procedure overview

Testing at Company A’s facilities was carried out using the basic configuration described below. Further optimisation of the sampling method and internal filter fields in follow up work in the Cambridge labs gave better separation for the lighter solvents so data from this testing is included to demonstrate the range of options available for solvent detection. To produce spiked samples 10g of sample drug in a powdered form was weighed out and placed in the vial then µl injections of the solvent to be tested were added to the vial to give high concentration samples.

Basic configuration
In order to make a gas phase measurement of the residual solvent content the vial is connected to the Lonestar inlet. The headspace of the vial is then flushed with clean air from the exhaust of the Lonestar. Due to the sensitivity of the instrument most of the sample gas is flushed directly out of an attached vent with only a small proportion being drawn in by the Lonestar.

Alternative configurations
Selectivity can be improved and maintenance requirements can be reduced if a compressed air line is available, the system can then be run at a slight overpressure (which can help with selectivity) and the internal pump is no longer required. Some of the testing presented here is carried out with apparatus configured in this manner.
It is also possible to set up the Lonestar monitoring solvent drying process directly onto a vat if a real time indication of solvent level is required.

**Background Matrix**

Two drugs were tested using the Lonestar in its basic sampling configuration - Ropinirole (4-[2-(dipropylamino)ethyl]-1,3-dihydro-2H-indol-2-one) and Oxcarbazepine (10,11-Dihydro-10-oxo-5H-dibenz(b,f)azepine-5-carboxamide).

Results showed that the two drugs had a low enough volatility that room temperature headspace sampling gives no response on the Lonestar.
Ropinirole response (negative ions left, positive ions right) – due to low volatility there are too few ions to detect, remaining highlighted peak on left is due to $O_2^-$ and right is $H_3O^+$, (faint peak is contamination in air line and is present in blanks as well)

As no ions were detected from raw drug using this sampling method a detection algorithm could be very simple to implement; any detectable changes in the fingerprint obtained will signify the presence of an additional volatile compound.

**Individual solvent responses**

Tests were carried out on a range of individual solvents to obtain reference fingerprints and enable initial peak identification and show approximate dynamic range for quantification of concentration.

**Chloroform**

The first solvent on the USP list tested was chloroform (CHCl₃, CAS number 67-66-3) as an example of a halogenated solvent on the list. It produces stable negative and positive ions against which identification and quantification algorithms can be generated.

![Image of chloroform molecule](image)

*Trichloromethane – boiling point 61.2°C, 119.38amu; chlorine can pick up a negative charge while the hydrogen can pick up a positive hydronium ion allowing detection via the presence of both positive and negative ions by the Lonestar*
Positive(left) and negative (right) ion fingerprints of chloroform

The rule builder software provided with the Lonestar can be used to pick out a series of waypoints which can be then stored on a library to allow peak identification.

Identification waypoints selected using rule builder

Peak height can then be linked to concentration using various quantitative methods, in this case an exponential dilution was carried out. A flask held above the solvent boiling point is injected with the solvent and clean air is flushed through at a known rate so the concentration is dropping at a known rate. If the injection volume is known then an approximate concentration versus peak height relationship can be developed and used for real-time concentration calculations using the following equation.

\[
[i]_T = [i]_{EDF}e^{-\frac{TF}{V}}
\]

\([i]_T = \text{concentration at time } T,\ [i]_{EDF} = \text{Initial concentration, } T = \text{time (s)},\ F = \text{flow (litres/sec)},\ V = \text{Volume (litres)}\)
Peak height versus concentration for chloroform generated from an exponential dilution

In practice the intrinsic errors in an exponential dilution means calibration by prepared standards should be used for accurate quantification, however this method is an effective way of quickly producing basic calibration curves.

Diethyl ether
The response to a trace amount of diethyl ether (C₄H₁₀O, CAS number 60-29-7) was also tested; the fingerprint obtained is shown below.

Diethyl ether – Boiling point 34.6, 74.12 amu; 828 kJ/mol proton affinity allows significant formation of positive ions

Diethyl ether fingerprint
The double peak structure represents a monomer and dimer of the diethyl ether, this does need to be taken into account when quantifying the Lonestar response, at high concentrations the dimer peak (furthest right) dominates while at low concentrations the monomer peak (middle, left is air peak) is more significant. It is therefore necessary for some chemicals to produce two calibrations for different concentration ranges. Below is a plot of falling diethyl ether concentration, as the concentration drops the monomer (middle peak) becomes larger than the dimer peak.

Monomer/dimer ratio changes as concentration decreases
Other solvents – Acetone, Ethanol, Hexane and Toluene

Below are example fingerprints for Acetone (C₃H₆O, CAS number 67-64-1), ethanol (C₂H₅OH, CAS number 64-17-5), hexane (C₆H₁₄, CAS number 110-54-3) and toluene (C₇H₈, CAS number 108-88-3) at low ppm levels. This illustrates how the ion FAIMS fingerprint changes for different analytes.

Approximate limits of detection were found by a modified exponential dilution method. These detection limits could be exceeded with sampling optimisation but such a method may sacrifice the flexibility to detect all the solvents simultaneously.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Approximate Limit of detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>methanol</td>
<td>100ppb</td>
</tr>
<tr>
<td>diethyl ether</td>
<td>&lt;10ppb</td>
</tr>
<tr>
<td>chloroform</td>
<td>&lt;10ppb</td>
</tr>
<tr>
<td>hexane</td>
<td>50ppb</td>
</tr>
</tbody>
</table>
Ethanol 30ppb

**Methanol**

Methanol (CH$_3$OH, CAS number 67-56-1) is the smallest solvent on the list it and consequently is one of the most difficult to detect. Methanol’s mobility is similar to that of the air molecules which make up the reactive ion peak making resolving the ion peak challenging.

\[
\text{H-C-O-H} \\
\text{H} \\
\text{H}
\]

*Boiling point 64.7, 32.05 amu, proton affinity 754 kJ/mol gives positive ion formation*

The figure below shows the results of testing at a concentration of 4ppm methanol, the second plot shows a single slice of the fingerprint at 48% dispersion field with the methanol peak (middle peak) resolved from the reactive ion peak (left peak).

*Methanol FAIMS fingerprint*
Methanol peak separated from reactive ion peak (single slice of FAIMS spectra at 48% dispersion field)

**Solvent drying**

An effective way of illustrating the change in the Lonestar response to solvent content is to add a mixture of solvents to a flask and monitor the change in the headspace as clean dry air is flushed through.

**Mix 1**

Methanol, diethyl ether and chloroform were added to a flask and clean dry air is flushed through at a rate of 5 flasks refreshes per minute.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>methanol</td>
<td>10μl</td>
</tr>
<tr>
<td>diethyl ether</td>
<td>10μl</td>
</tr>
<tr>
<td>chloroform</td>
<td>10μl</td>
</tr>
</tbody>
</table>

*Initial plot after 5 minute showing the additive fingerprints of each solvent, peak magnitude indicates concentration and position on plot allows identification*
Over time the peaks decay as the solvent dries/is diluted by the dry flush air. The figure below shows this evolution over time.

A mixture of three solvents drying over time as flushed with clean air till only the clean air peak is left.

The Lonestar software can be used to run simultaneous detection rules.
**Integrated application builder used to identify and set threshold levels for multiple analytes**

**Mix 2**
Adding toluene to the mix shows how dynamic range of the Lonestar can be limited, toluene high volatility puts lots in the headspace in the initial scans and masks other chemical responses, however once it drops to the low ppm concentrations the other peaks being masked become visible.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>methanol</td>
<td>10µl</td>
</tr>
<tr>
<td>diethyl ether</td>
<td>10µl</td>
</tr>
<tr>
<td>chloroform</td>
<td>10µl</td>
</tr>
<tr>
<td>toluene</td>
<td>10µl</td>
</tr>
</tbody>
</table>

**Solvent drying of four solvents, masking of some analytes by strong toluene response**

Masking by one solvent by another should not be a significant issue for pharmaceutical testing as the pass/green light requirement for the solvents will be no solvents above a certain concentration present. Also in practice the solvent levels should be low ppm or below at which point saturation masking is unlikely. Additional sample dilution with clean dry air can also be used to mitigate these issues.
Future work

Other Solvents and selectivity
Depending on the application requirements, other solvent responses will need to be obtained and cross-sensitivity issues resolved. The thresholds for each solvent would need to be calibrated accurately (accuracy required will depend on the customer specification) and classification algorithms obtained. Cross sensitivity of some solvents (benzene/toluene/pentane/heptane) would need to be investigated. In practice cross sensitivity issues should not be a significant problem as the Lonestar function may be to highlight high solvent responses and a GC/LC-MS could be used to provide more specific information on suspect samples.

An alternative testing configuration – dissolving samples in water
An alternative sampling approach would be to dissolve the drug samples in water (if the particular drug is water soluble) and sample the headspace above the water as in the figure below.

![Sampling a liquid headspace](image)

Dissolving the sample in water has the advantage of removing the variability in the physical form of the sample, surface area is simplified to the circular water surface area, rather than depending on the powdered drug’s packing density. The constant humidity generated can, in some cases, help with the detection of particular analytes. This sampling method would
change the required identification and quantification algorithms. Consequently further investigation would be needed to evaluate its potential benefits and tradeoffs.

### Summary

The testing showed that methods can be developed for making quick measurements of residual solvent content in pharmaceuticals

- The low volatility drugs tested give no measureable FAIMS response using a simple headspace measurement at room temperature. Consequently the detection of solvents is simplified as any ion response can be attributed to undesirable volatiles.

- Initial tests indicate that the solvents identified in the USP list should all be detectable down to sub 1ppm levels. Further testing would be required to confirm this but methanol which is one of the harder solvents to detect with FAIMS is detectable significantly below this concentration.

- Individual solvents can be identified and/or quantified to enable red light/green light checks on drugs for threshold solvents level. Alternatively calibrations of each solvent can be produced to enable absolute concentrations to be outputted.

- Typical fingerprints were obtained in approximately 2 minutes, however this has not been optimised, there is still redundant information which could be dropped by scanning less dispersion field values. Scan times of under a minute should be possible.

- Future work has been identified such as testing a wider range of solvents and quantifying response and cross sensitivity issues.

- Another future investigation would involve dissolving samples in water as a way of simplifying sample preparation and improving sample variability (variability from sampling a solid/powder). This method would need to be verified but has potential if solvent may be physically trapped in the drug or the sample form is variable.