Analyte of Interest Calibration Procedure for a Lonestar® 3.0

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001	28/02/2017	Isabel Marquez, Céline Lainé	The Original

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Introduction

This document details the procedure to calibrate a Lonestar® system for an analyte of interest in a matrix background.

The Lonestar® calibration procedure finalises the method development. This procedure can only be based on the optimised detection method of the analyte of interest in the matrix background. The optimised detection method results of the correct sample preparation and the developed Lonestar® online configuration.

For more information on method development, please consult additional documents on the Owlstone® support website such as:

http://support.owlstonenanotech.com/entries/23604688-Lonestar-Method-Development-Procedure

The calibration of the Lonestar® system is necessary to determine unknown concentrations of the analyte of interest. Analysis of unknown samples on the Lonestar® system consists with running them against the created calibration curve to determine the analyte of interest concentration.

The Lonestar® calibration procedure can be divided in two major parts:

1. Analysis on the Lonestar® system of a set of calibration standards containing the analyte of interest in the matrix background
2. Creation of a calibration curve for the analyte of interest using the Owlstone® offline software

For further details on how to use the Lonestar® system and its software, please consult the documentation available on the Owlstone® website, in the support section.

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Set up and Components

Please ensure that you are familiar with the hardware naming before generating the system blank.

Figure 1 shows the final setup of the Lonestar® when used with an ATLAS™ sampling system.

Figure 2 details the Sampling Module Assembly part of the ATLAS™.

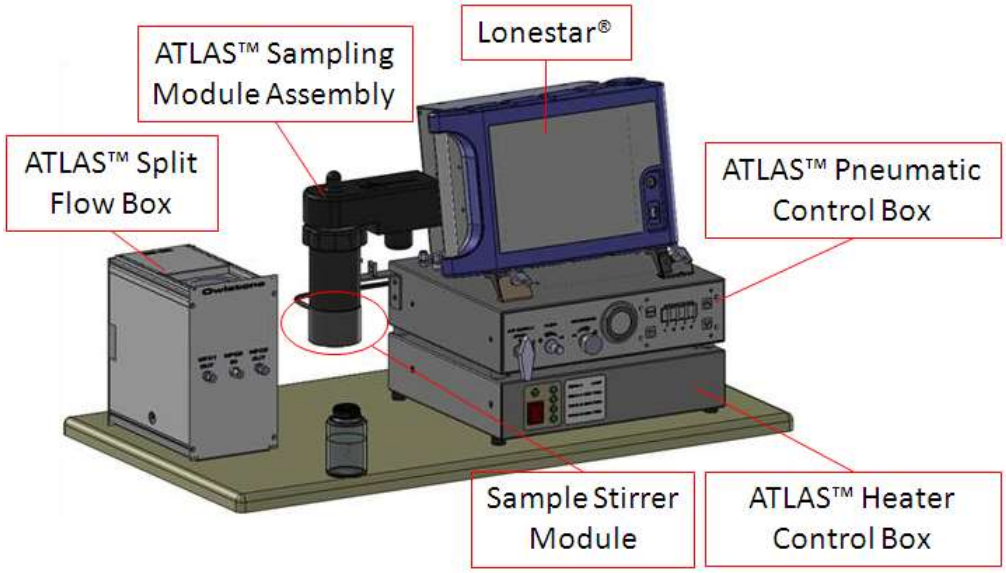

Figure 1 Lonestar® ATLAS™ Split Flow Box installation

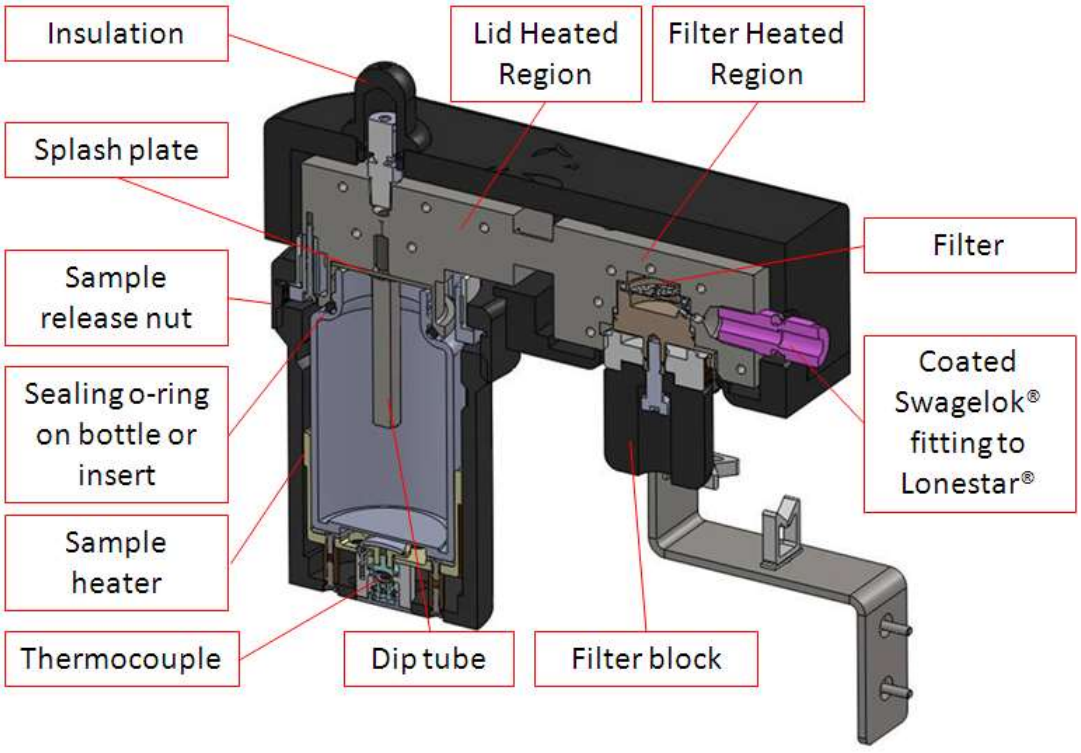

Figure 2 Diagram of components of the ATLAS™ Sampling Module Assembly

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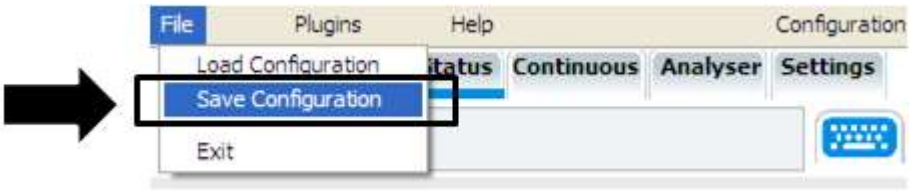
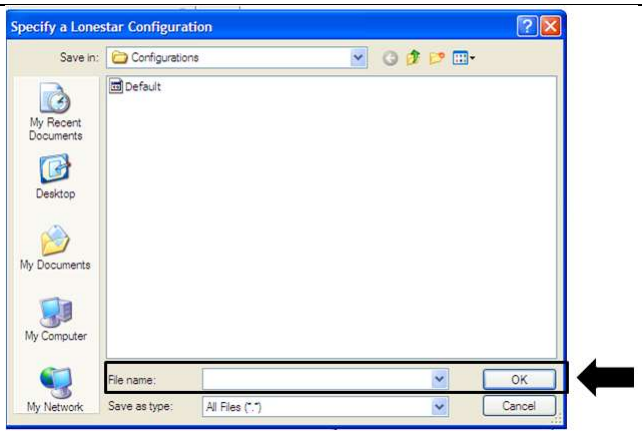
Instructions

The calibration procedure detailed below is divided in two major parts:

1. Analysis on the Lonestar® system of a set of calibration standards containing the analyte of interest
2. Creation of a calibration curve for the analyte of interest using the Owlstone® offline software

Step #	Instructions	Pictures
PART 1 – Calibration standards analysis		
1	Once the method development is complete, save the Lonestar® online configuration that has been optimised. Select **File/Save configuration** in the Lonestar® online software top taskbar.	
2	A new window opens called **Specify a Lonestar® Configuration**. Type the configuration name and select **OK**.	

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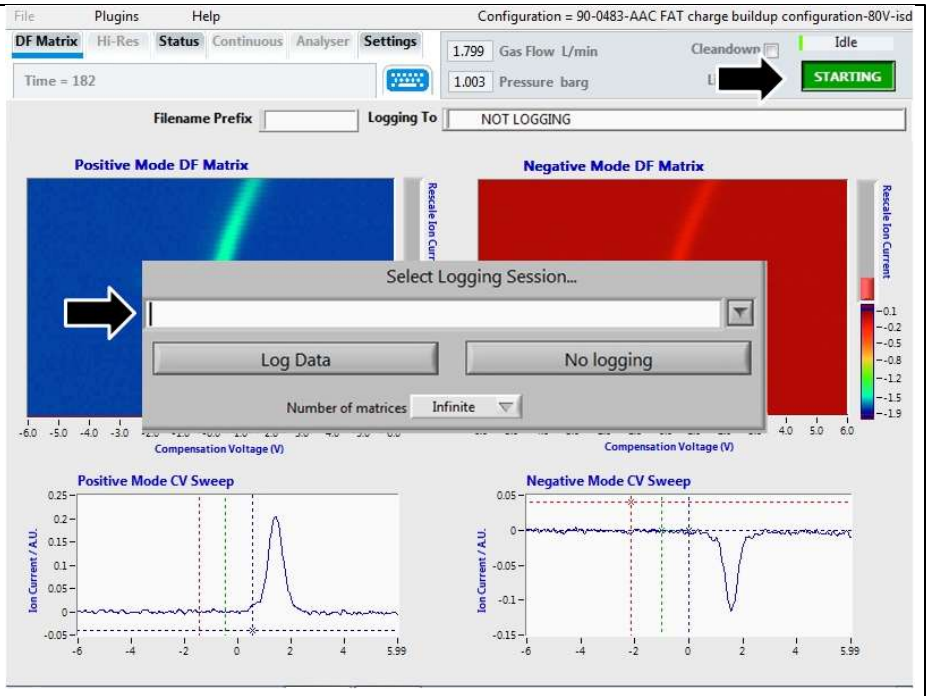
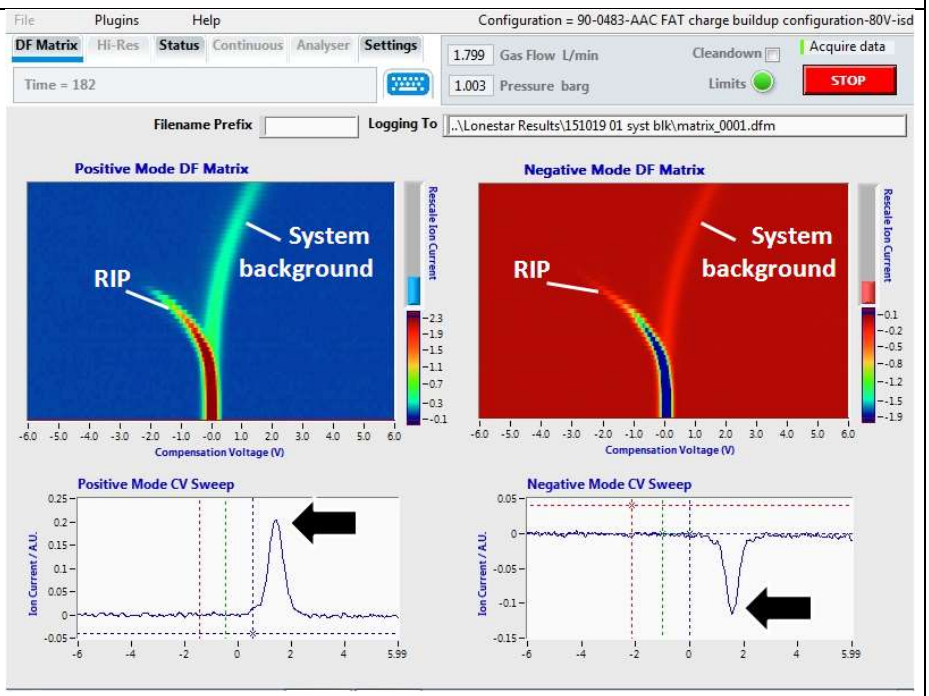
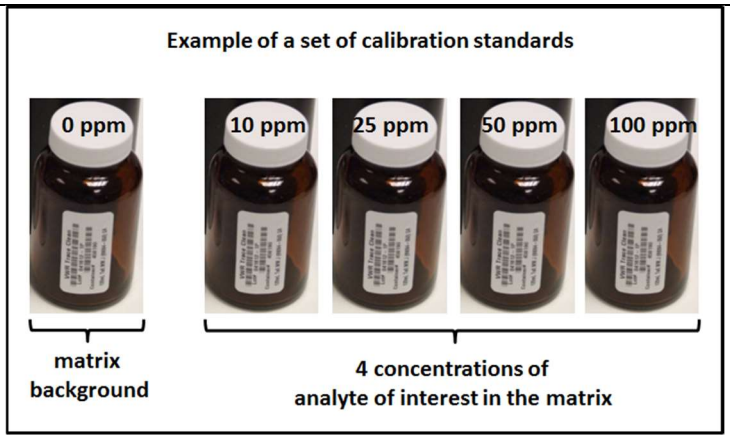
3	Before analysing any standards, perform a Lonestar® system cleanliness check by running the system with an empty sample bottle. Press **START** to open the **Select Logging Session** window. Type the system blank reference name, typically **YYMMDD 01 system blank** and select **Log Data** to record it.	
4	A typical Lonestar® cleanliness matrix shows the RIP (Reactant Ion Peak) curving to the left hand side and the system background peak curving to the right hand side. For the cleanliness check to pass the system background ion current should stay below 0.2 A.U. at DF 90 %.	
5	To create a calibration curve it is recommended to analyse at least four concentration points across the analyte in the region of interest.	

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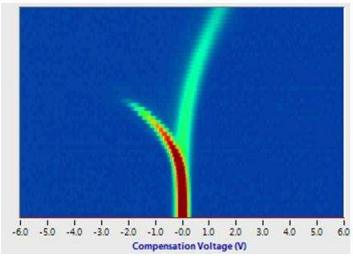
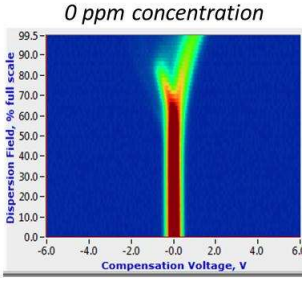
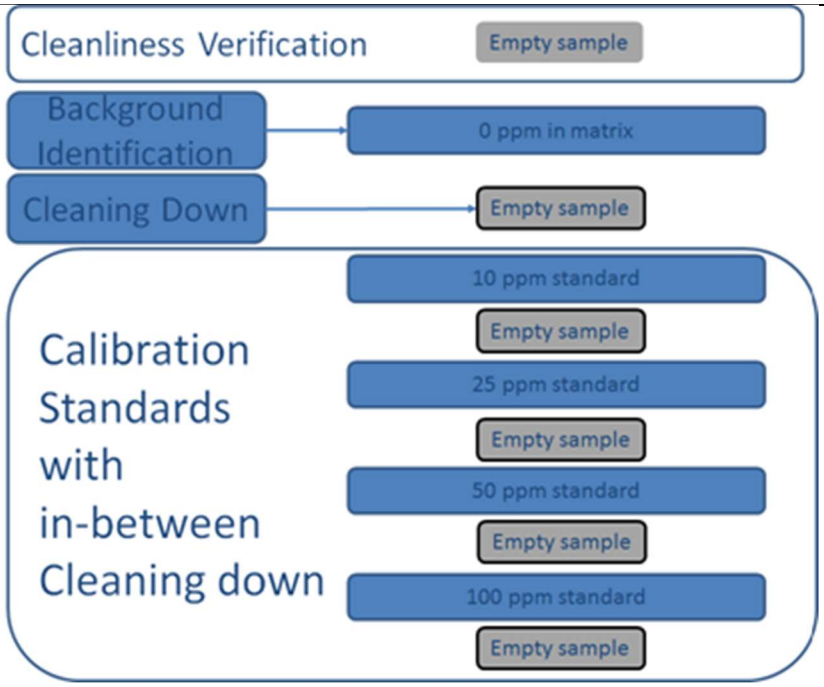
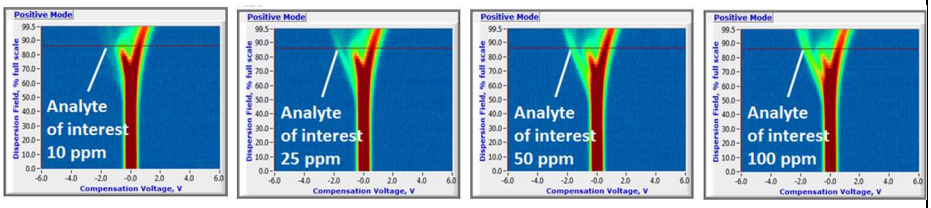
6	Start the calibration standard analysis with the 0 ppm concentration of analyte of interest. Running this matrix blank first allows recording the matrix background.	**Lonestar® system blank** *Empty sample bottle*  **Analyte of interest matrix background** *0 ppm concentration* 
7	After each sample, run a Lonestar® system blank by inserting an empty sample bottle to avoid any carry over from one sample to another. To limit the clean down time in-between samples, run calibration standards from lower to higher concentration of analyte of interest.	
8	Observe the peak height increasing with the analyte of interest concentration and select one DF showing a peak distinct from the matrix background.	

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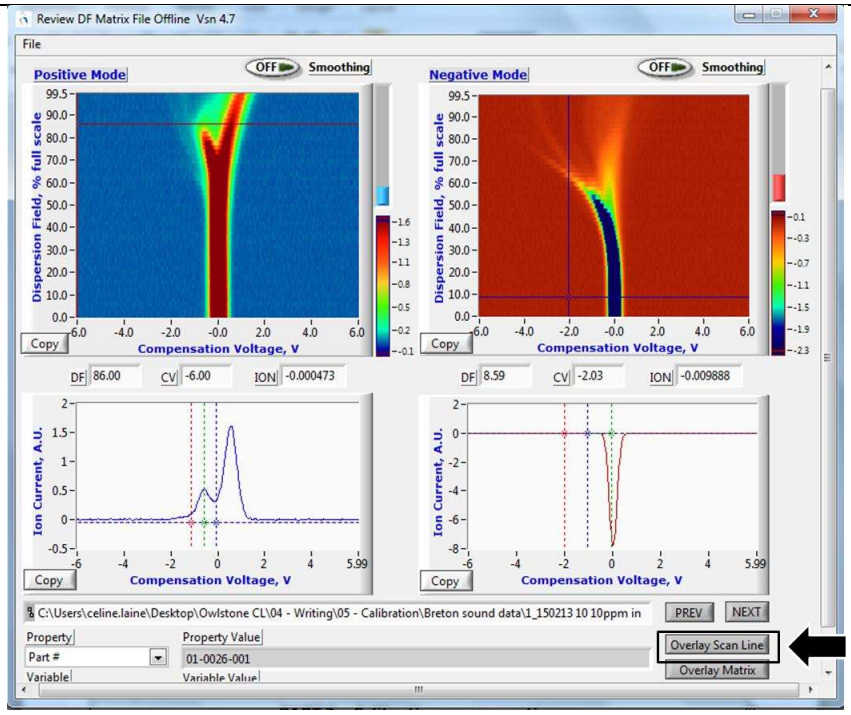
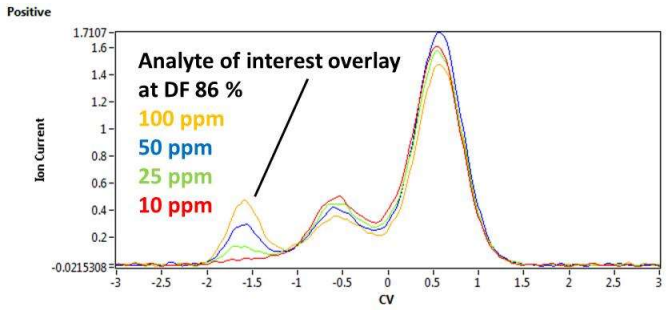
9	Verify that the analyte of interest peak height increases with the concentration. Use the **Overlay Scan Line** button located at the bottom right corner of **the Review DF Matrix File** offline software. Apply this overlay to all analyte of interest concentrations making sure to select the same matrix number (example matrix_003.dfm) to create the overlay chart.	 

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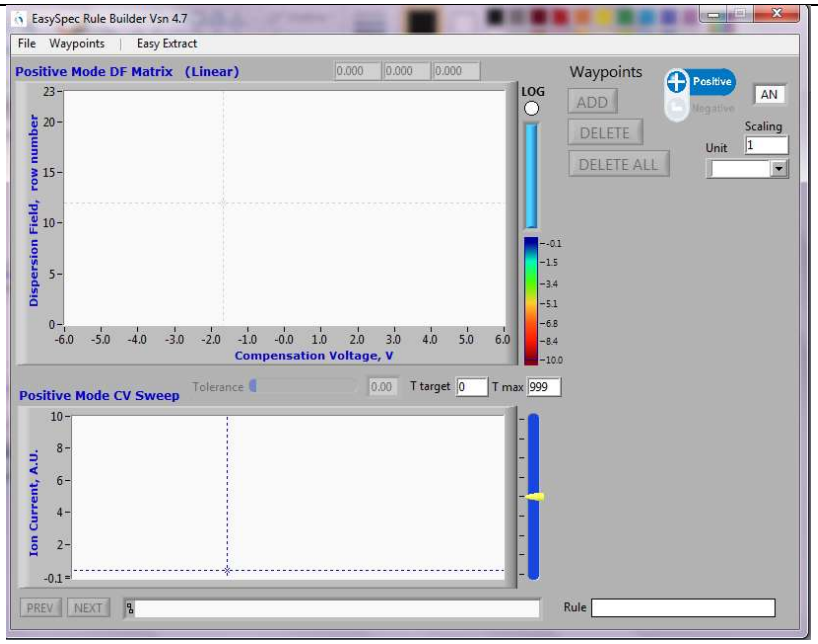
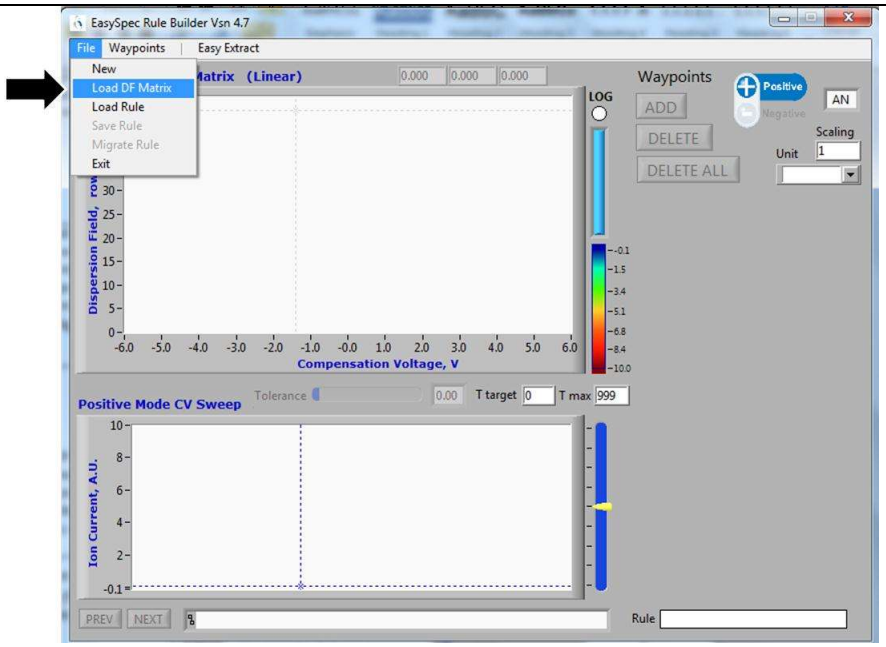
PART 2 – Calibration curve creation

Note: All data analysis is carried out offline, using a separate computer that has Owlstone® offline tools Vsn 4.7 software installed on. Data are copied from the Lonestar® analyser using a USB drive and pasted to the separate computer for analysis.

10	The Owlstone® **EasySpec Rule Builder** offline software is used for data extraction to create the calibration curve.	
11	Load a DF matrix showing the analyte of interest fingerprint distinct from the matrix background. Select **File/Load DF Matrix** in the offline software top taskbar.	

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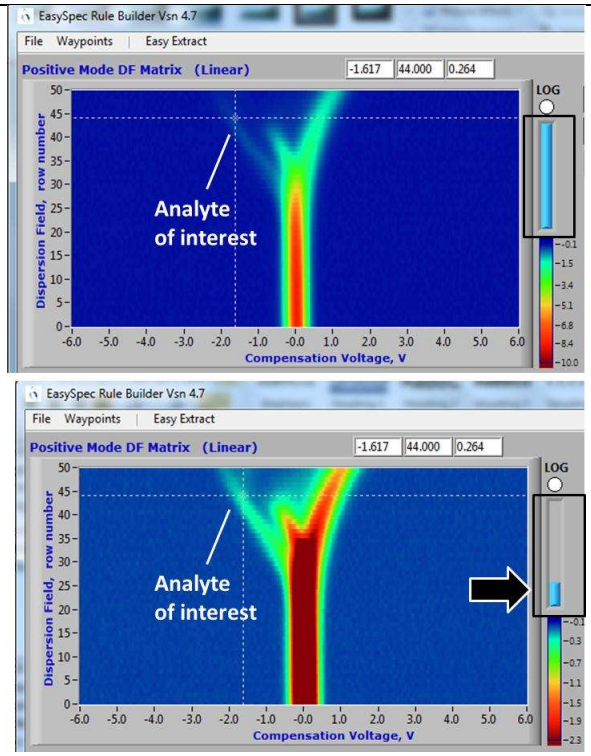
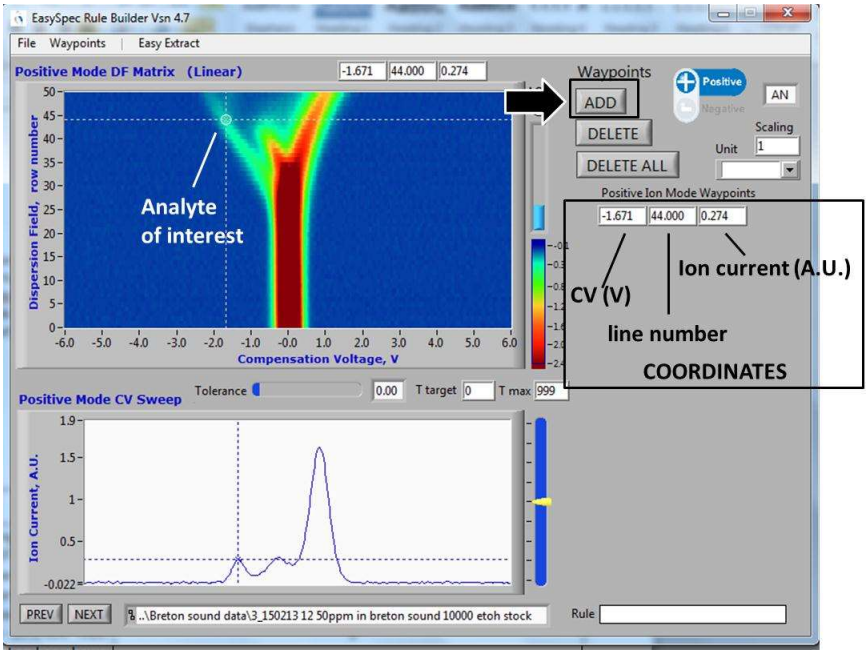
12	Adjust the contrast bar to make the analyte of interest peak appearing clearly.	
13	Select a waypoint in the analyte of interest fingerprint by moving the cross and using the **ADD** button. Waypoint coordinates (-1.671 V, line 44, 0.274 A.U.) appear on the right of the DF matrix. The **DELETE** and **DELETE ALL** buttons can be used to modify the waypoint(s) selection.	

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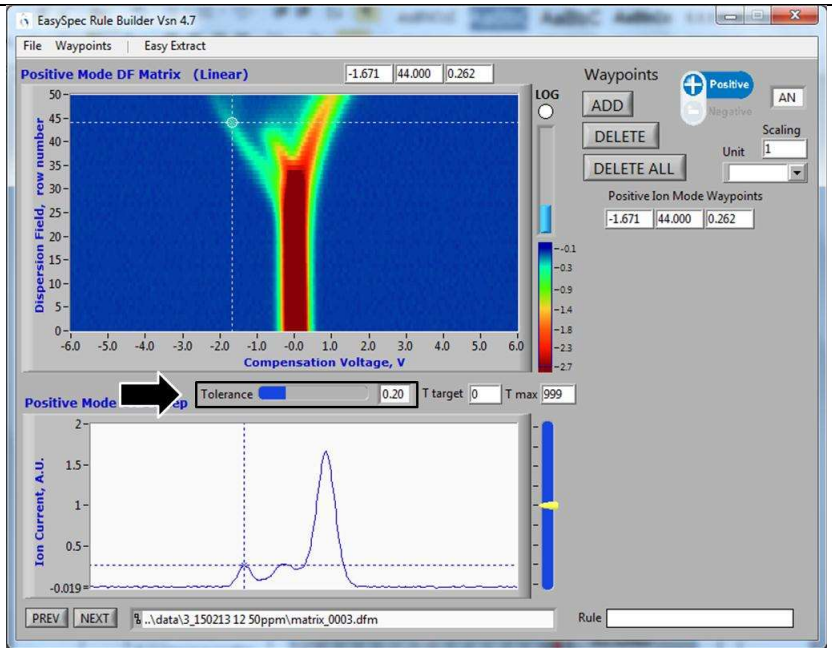
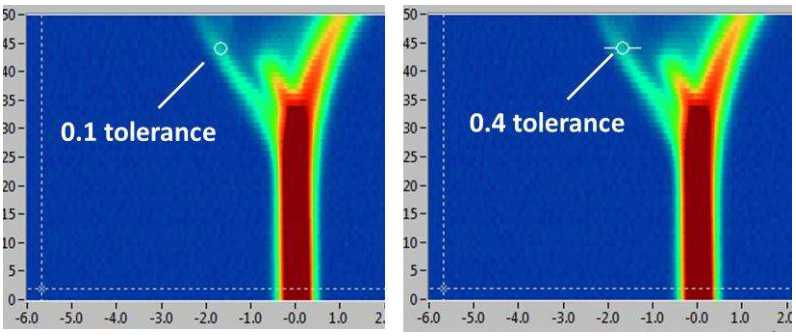
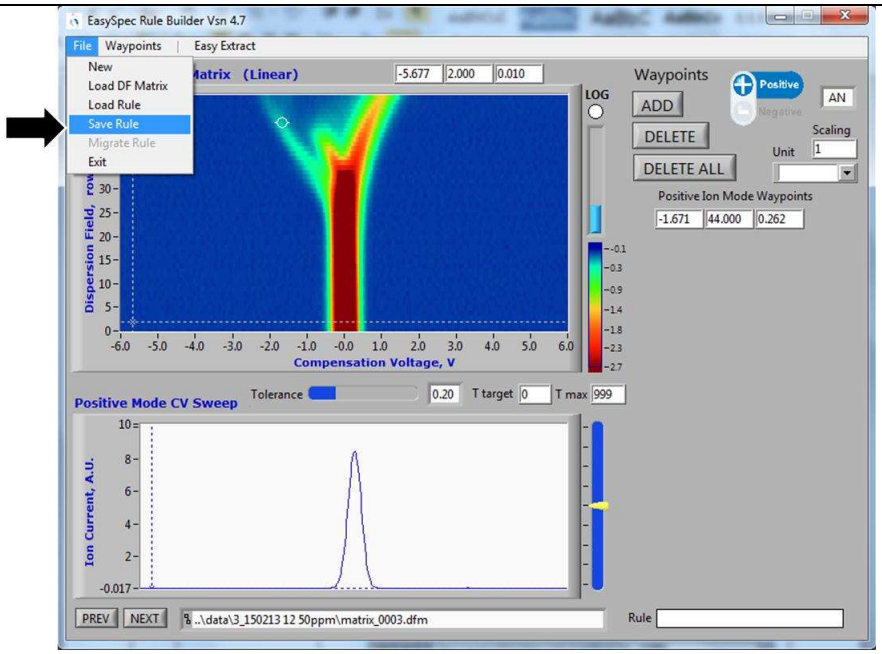
14	Set the tolerance by typing an arbitrary number between 0 (lowest) and 1 (highest). Typically 0.1 or 0.2 tolerance is chosen. Tolerance allows peak selection within waypoint bars in case the analyte of interest fingerprint compensation voltage is slightly affected by external parameters.	 
15	Save the rule by selecting **File/Save Rule** in the offline software top taskbar.	

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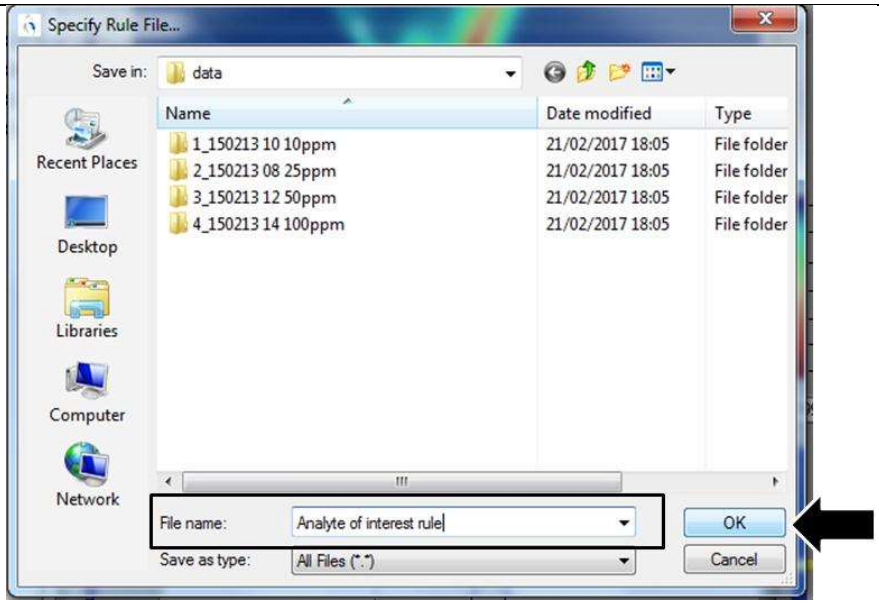
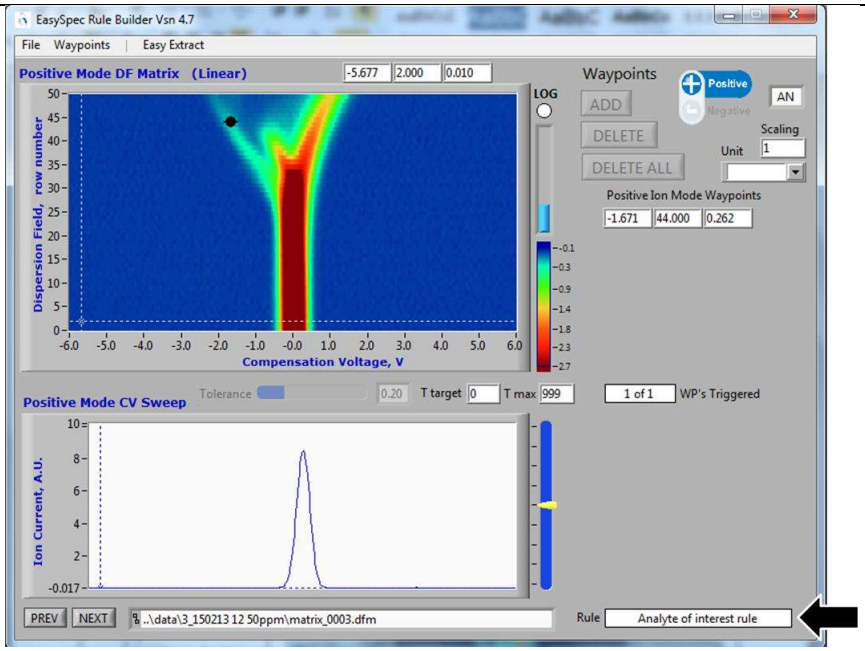
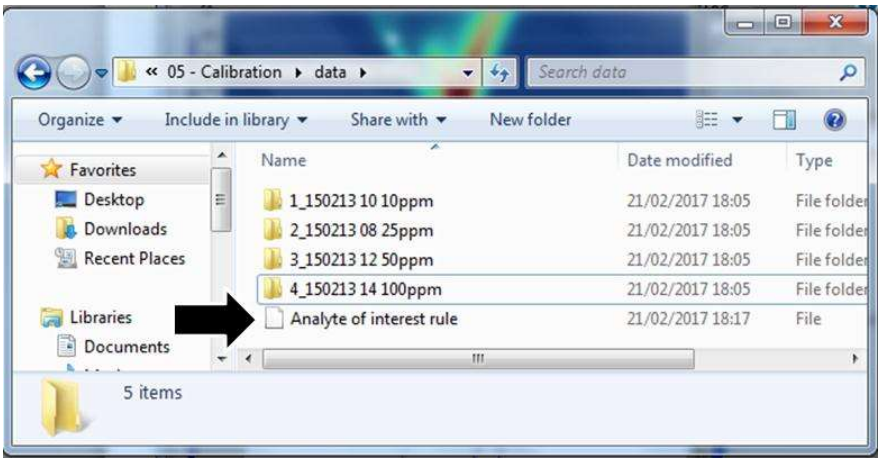
16	A separated window opens named **Specify Rule file**… NB: The created rule must be saved at the same level as the data folder for correct data extraction. Type a rule name and press **OK**	
17	Once the created rule has been saved, its name appears in the right corner of the **EasySpec Rule builder** software. The rule name appears also below the data to be extracted by the rule. This is the correct location for the rule to be saved for further data extraction.	 

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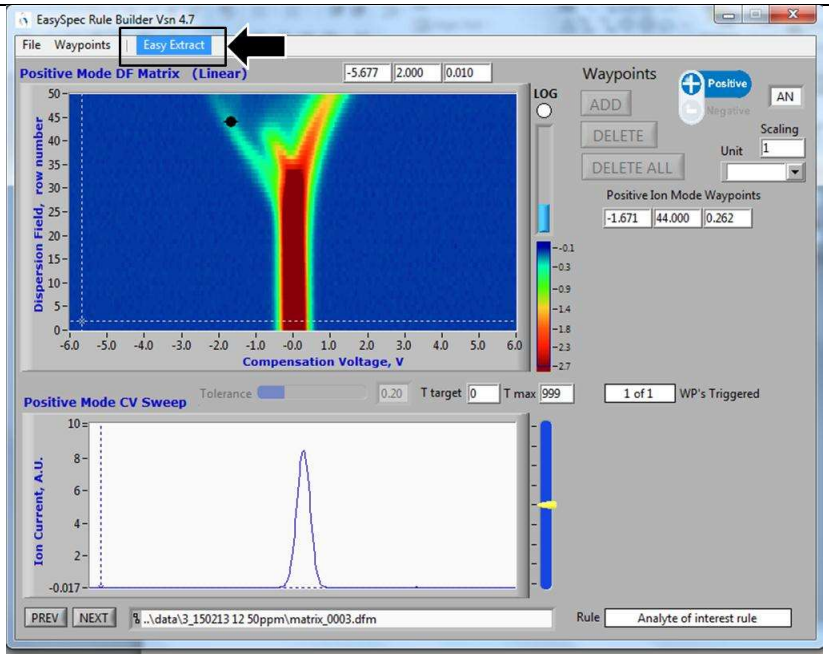
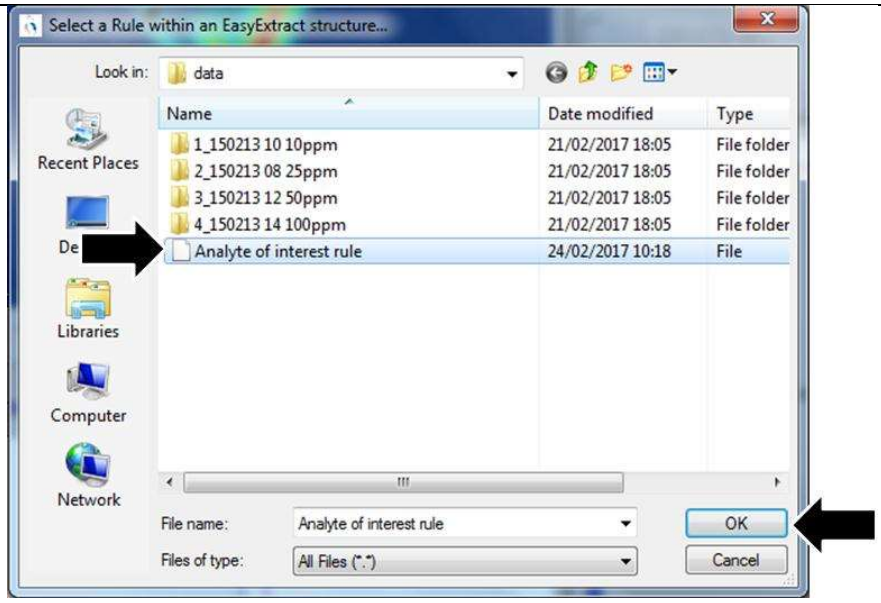
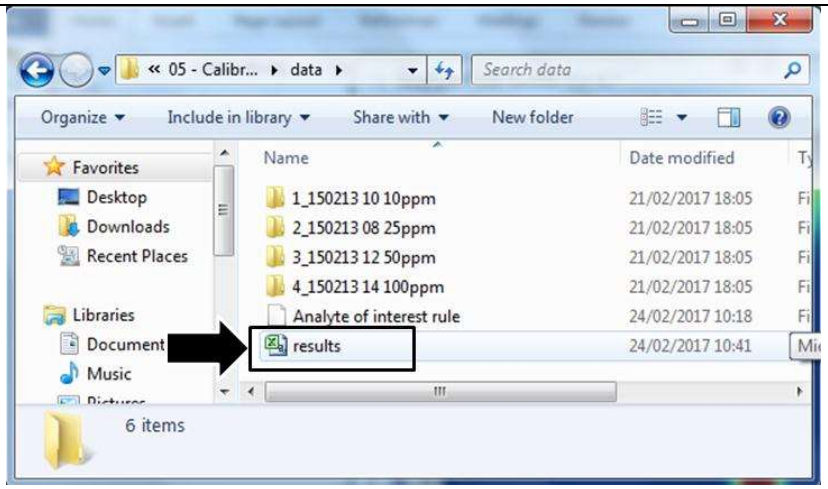
18	To start data extraction, select **Easy Extract** in the offline software top taskbar.	
19	A separated window called **Select a Rule within an EasyExtract structure…** opens. Select the wanted rule and press **OK**.	
20	Data extraction creates an Excel comma separated values (csv) file named **results** located in the data folder.	

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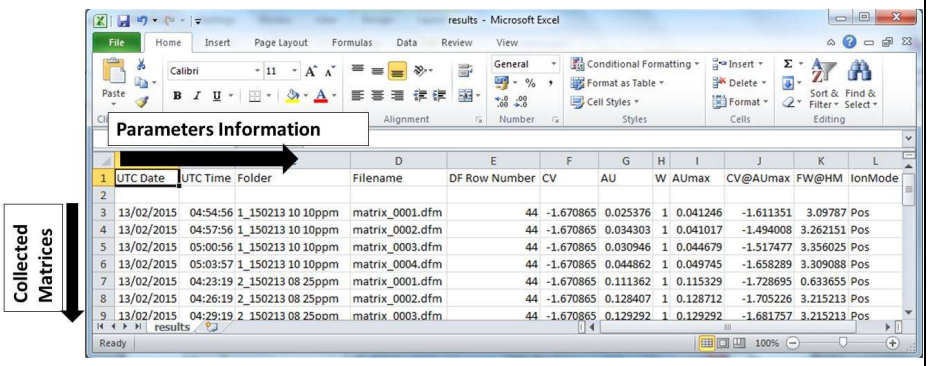
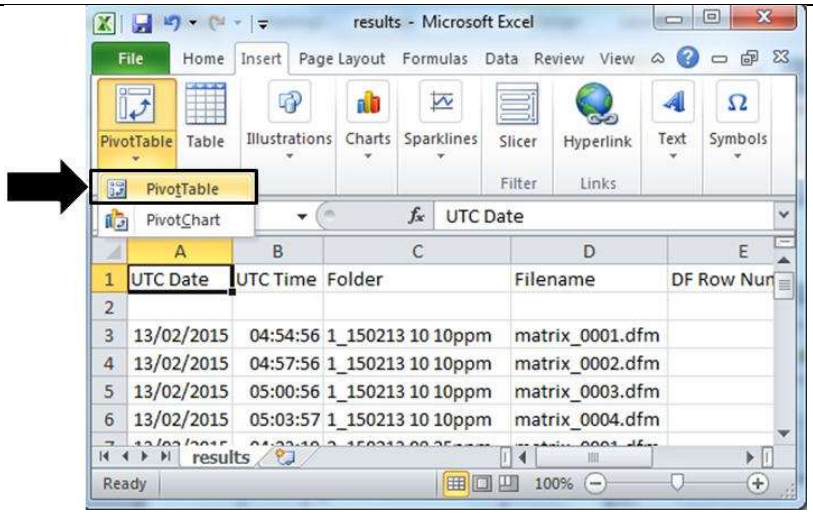
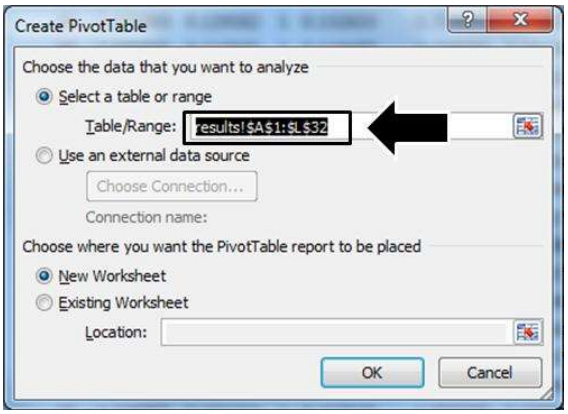
21	The Excel csv spreadsheet displays all data ordered in rows. For each collected matrix, the Lonestar® parameters are ordered in column: date, time, folder name, filename, DF Row number, CV, AU, weight, AUmax, CV@AUmax, fullwidth@halfmaximum, ion mode.	
22	Extracted data can be analysed manually or using the Excel tool called PivotTable. Select **Insert/Pivot table** in the Excel top taskbar.	
23	A separated window opens called **Create PivotTable**. Choose the data to be analysed by selecting the table/range manually and select **OK**. Make sure to include the first row with all column titles in the data selection.	

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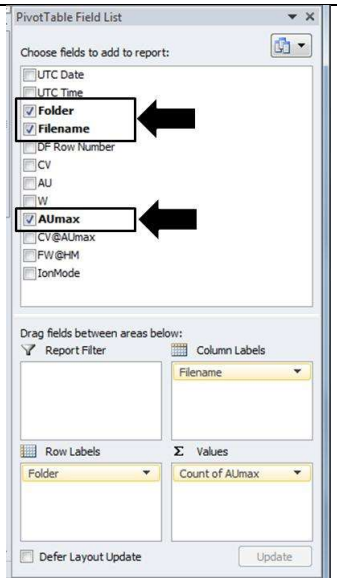
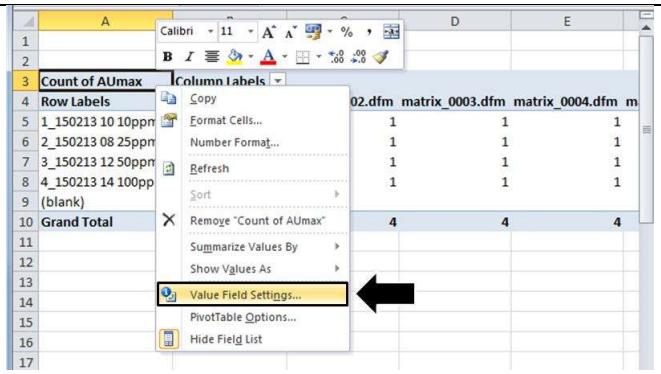
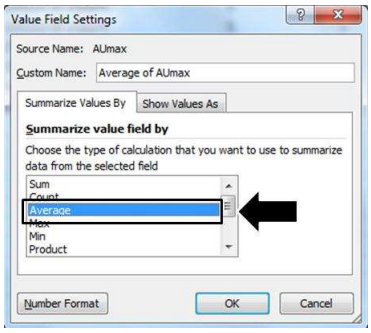
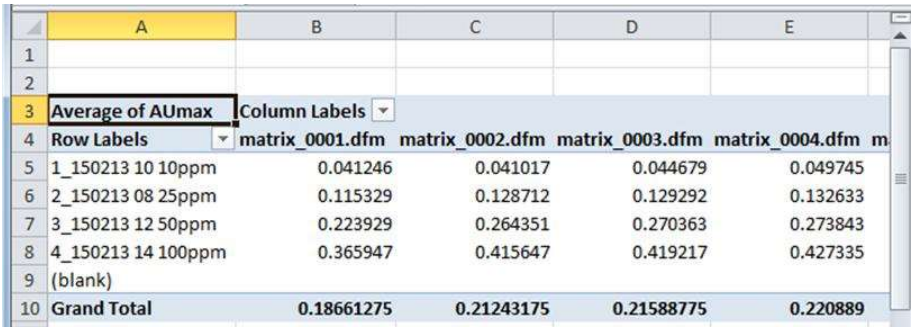
24	In the **PivotTable Field List** that opens, tick **Folder**, **Filename** and **AUmax** boxes. Organise the selected fields as: Folder – Row Labels Filename – Column Labels AUmax - Values	
25	In the Excel spreadsheet, change **Count of AUmax** for **Average of AUmax**. To do so, right click on **Count of AUmax**, select **Value Field Settings…** In the new window called **Value Field Settings**, select **Summarize Values By Average** and press **OK**.	 
26	From the PivotTable, extract data to plot them in a scatter chart.	

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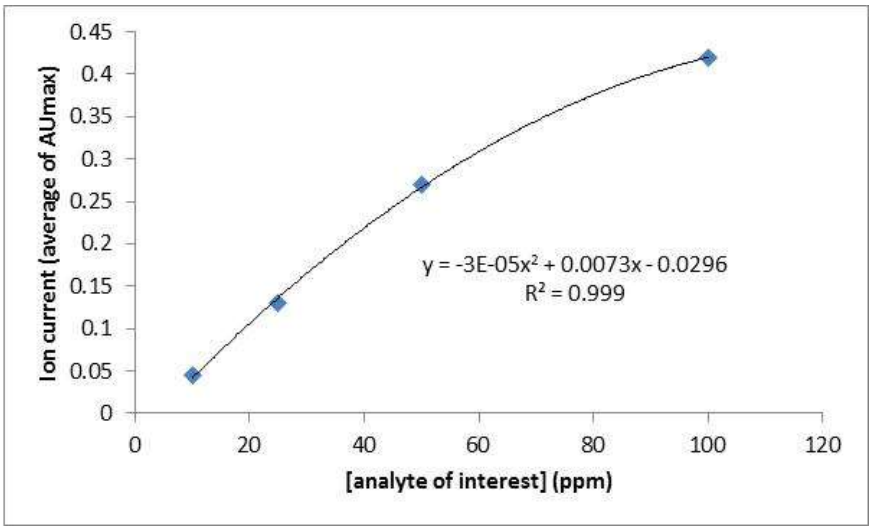
	In the example presented, matrix_003 has been chosen.	

	matrix_0003.dfm
[analyte of interest] (ppm)	Ion current (Average of Aumax)
10	0.044679
25	0.129292
50	0.270363
100	0.419217

27	Create the calibration curve by inserting a scatter chart, plotting analyte of interest concentration (ppm) in Series X Values versus Ion current (average of AUmax) in Series Y Values. The created calibration equation will used to calculate unknown sample concentrations.	

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Owlstone® develops and commercializes innovative new technologies to address the critical need for compact, dependable and cost-effective chemical and biological detection solutions for a wide range of markets.

Owlstone® was formed through the recognition of the opportunities created by the application of micro- and nano- technology to develop improved sensing solutions.

Owlstone® is focused on the innovation of detection technologies to address unmet needs, developing solutions that are flexible enough to target a range of markets with the potential for growth by enabling new application opportunities.

From homeland security to home safety, Owlstone® is working with leading manufacturers and integrators across a range of markets to develop products incorporating our microchip chemical sensing solution.

Owlstone® is headquartered in the United States and has laboratory facilities in the United Kingdom. Owlstone® Ltd was founded in 2003 with a seed investment of two million dollars from Advance Nanotech, Inc., a New York based company specializing in the investment in and commercialization of nanotechnologies.

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