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A VAMAS Interlaboratory Study for Desorption Electrospray Ionisation (DESI) Intensity Repeatability and Constancy: Protocol for Analysis

E Gurdak, T L Salter, S A Smith, M P Seah, I S Gilmore and F M Green

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Analytical Science Division

ABSTRACT

This report describes the protocol for analysis in the VAMAS 2013 DESI interlaboratory study. General guidance for DESI settings are provided to help establish equivalence between different instruments. Two reference materials are supplied for this study, a thin layer of Rhodamine B on a glass slide and double-sided adhesive tape on a glass slide. No sample preparation is required by the user and specific guidance is given on sample handling. Forms for reporting the data are provided at http://www.npl.co.uk/science-technology/surface-and-nanoanalysis/research/international-standardisation-and-traceability/versailles-project-on-advanced-materials-and-standards-(vamas)

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Approved on behalf of NPLML by Dr Michael Adeogun, Head of Division

CONTENTS

1 INTRODUCTION

The innovation of desorption electrospray ionisation (DESI)[1,2] in 2004 heralded a new era of ambient surface mass spectrometries. Since then there has been significant progress in understanding the fundamentals and a rapid expansion in the applications covering a diverse range of science and technologies, from tissue imaging to characterisation of medical devices and from forensics to food analysis. To promote the wider uptake of DESI, especially by industry, it is important that measurements are repeatable and reliable. Ideally, for laboratory conformance with accreditation, standards consistent with ISO 17025[3] should be available for analysts. To achieve this, a robust measurement infrastructure needs to be established. For effective International Standards it is important that interlaboratory studies are conducted to evaluate if methods are effective and fit-for-purpose. It is especially important to test that methods are transferable between many different instrument designs and that the procedures are clear. The Versailles Project on Advanced Materials and Standards (VAMAS)[4] provides an excellent mechanism for such studies. Within this platform, NPL has conducted five interlaboratory studies in secondary ion mass spectrometry. This has resulted in an ISO standard covering repeatability and constancy,[5,6] mass scale calibration[7,8] and linearity of the intensity scale [9].

This VAMAS interlaboratory study for DESI has two principal objectives: (i) to determine the current achievable repeatability and constancy of DESI instruments and (ii) to evaluate the equivalence of results between different laboratories and instruments. Since, this is the first such study it is hoped to also provide a survey of measurement issues and also help establish a community of users interested in metrology issues for DESI.

2 TIMETABLE

You should complete the analysis of the samples provided for this work by the 31st March 2013. If you cannot do so and need extra time please inform Elzbieta Gurdak via elzbieta.gurdak@npl.co.uk.

3 THIS PACKAGE

This package contains: (i) this protocol for analysis, (ii) one container with one glass slide sample in a sealed light-tight black plastic bag and (iii) one container with four glass slide samples. Inspect the packaging to check if it has been opened by customs and if the integrity of the samples have been compromised. If you are in doubt please contact NPL. Please inform us that you have received the package and that everything received is in order. It is important to read this protocol completely before opening the samples.

☐ I have emailed NPL that all is OK with the samples on …./…. /2013

4 OUTLINE OF PROPOSED METHOD

In this work, we propose to measure DESI mass spectra using two types of reference materials, prepared on standard glass slides (approximately 76 mm x 26 mm x 1 mm) with one end frosted. The reference material is on one side only with the reverse side clearly marked. The two reference samples are a thin layer (approximately 800 nm thick) of Rhodamine B coating one half only of the glass surface, and a self-adhesive tape (“Mammoth tape”, Everbuild) prepared as three strips arranged in rows along the long side. The adhesive tape has a protective non-stick paper layer, which should not be peeled off until the sample is to be analysed. This is described later. Each sample has a unique reference code.

The Rhodamine sample (A) is used in 7.1 to measure the absolute intensity repeatability. The adhesive tape samples (B1, B2, B3, B4) are used in 7.2 to measure the relative intensity repeatability and in 7.3 to measure the constancy of the relative intensities over a defined time period.

The VAMAS Reporting Form is provided as Excel spread-sheets for entering data and recording experimental parameters; sheets "A", "B1" and B2,3,4" and "Experimental Parameters", respectively. The VAMAS Reporting Form can be found and downloaded from http://www.npl.co.uk/science-technology/surface-and-nanoanalysis/research/international-standardisation-and-traceability/versailles-project-on-advanced-materials-and-standards-(vamas)

Table 1. Summary of the reference samples.

	Reference sample	
	A	**B1, B2, B3, B4**
Sample Type	Rhodamine B film of ~800 nm thickness on a glass slide	Adhesive tape on glass slides: B1, B2, B3 - three strips, B4 - one strip
No of provided samples	1	4
Purpose	Absolute intensity repeatability	Relative intensity repeatability and constancy

5 INSTRUMENTAL OPERATING CONDITIONS

5.1 MASS SPECTROMETER

A great benefit of DESI is that it is compatible with a wide variety of mass spectrometers including ion trap, time-of-flight and Fourier Transform spectrometers. It is therefore not possible to provide specific guidance for the mass spectrometer settings and operation. Analysts are requested to optimise the mass spectrometer settings in the positive ion mode according to their standard procedures. Ensure that the mass scale of the spectrometer has been calibrated in the mass range from *m/z* 400 to 1400.

5.2 DESI SOURCE

There are no single, optimum operating conditions for DESI. For example, Takats et al. have shown that different substances (proteins, steroids, etc.) have different optimum electrospray to surface distances as well as different optimum spray impact angles [10]. The DESI source and mass spectrometer should be operated under conditions that give the most stable performance. For the optimum intercomparison, a common set of operating conditions are needed, however due to the variety of instruments available, no single set of conditions can do this. In DESI, there are many parameters that can affect signal intensity and repeatability. These are illustrated below in Figure 1. Analysts may use their standard operating conditions or alternatively, guidance is provided below on the effect of some of these parameters. Further details are given in references 11 and 12.

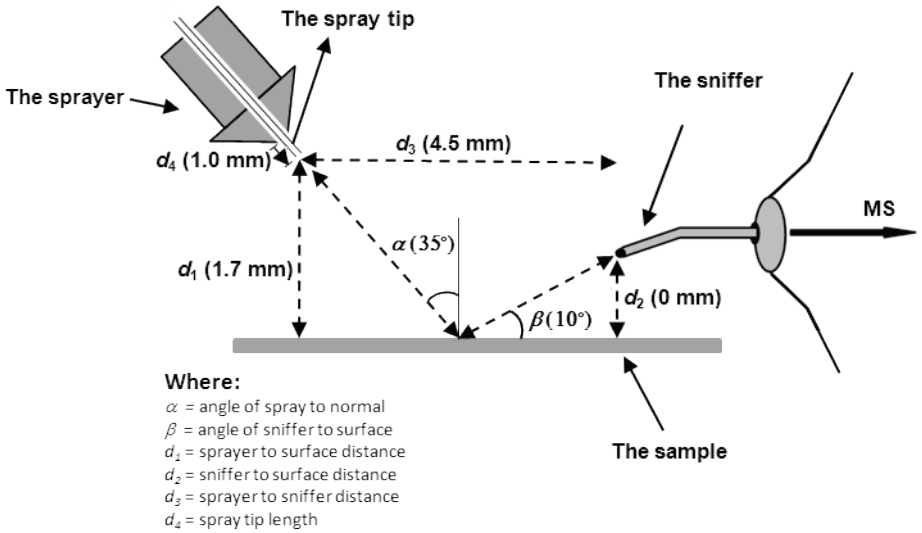

Figure 1. Parameters affecting DESI signal intensity and repeatability, adapted from reference 11. The values of these parameters, used in the study, should be recorded in the Electronic Reporting Form.

For reference, the settings for the NPL instrument using a 2D automated Omni Spray Ion Source (Prosalia, Inc, Indianapolis) with an LTQ Orbitrap Velos mass spectrometer (Thermo, Bremen) are given in Table 1. For analysts who wish to optimise or check the parameters of their DESI configuration, some general guidance is provided below.

Table 2. Important DESI source and mass spectrometer parameters for intensity repeatability and constancy and the values used in the NPL instrument consisting of an Omni Spray Ion Source with an LTQ Orbitrap Velos mass spectrometer

DESI source parameter (unit)	**Value**
Sprayer to surface distance, d_1 (mm)	1.7
Sniffer to surface distance, d_2 (mm)	0
Sprayer to sniffer distance, d_3 (mm)	4.5
Spray tip length d_4 (mm)	1
Angle of spray to normal, α (degrees)	35
Angle of sniffer to surface, β (degrees)	10
Solvent volume fraction, X	Acetonitrile : water / 90:10
Additives to solvent	0.1% formic acid
Electrospray solvent flow rate, S (µl/min)	2
Electrospray voltage, Vs (V)	+5000
Spray tip (material)	Fused silica
Mass spectrometer parameter (unit)	**Value**
Capillary temperature (°C)	275
Ion polarity	Positive
Resolution	100 000
N_2 gas pressure (psi)	100
Acquisition time (s)	30

5.2.1 Geometrical parameters (d_1, d_2, d_3, d_4, α, β)

A key issue for repeatability is that any slight change in geometry, particularly spray tip length d_4 and tip to surface distance d_1, can have a large effect on sensitivity. The effect of the spray tip length, d_4, on the signal intensity and the spot shape is shown in Figure 2. It is also important to ensure that the spray tip is positioned centrally within the gas flow. If not, the DESI erosion area will be off-axis which results in a lower detection efficiency. Similar symptoms may be observed if deposits form on the tip and so regular cleaning is recommended.

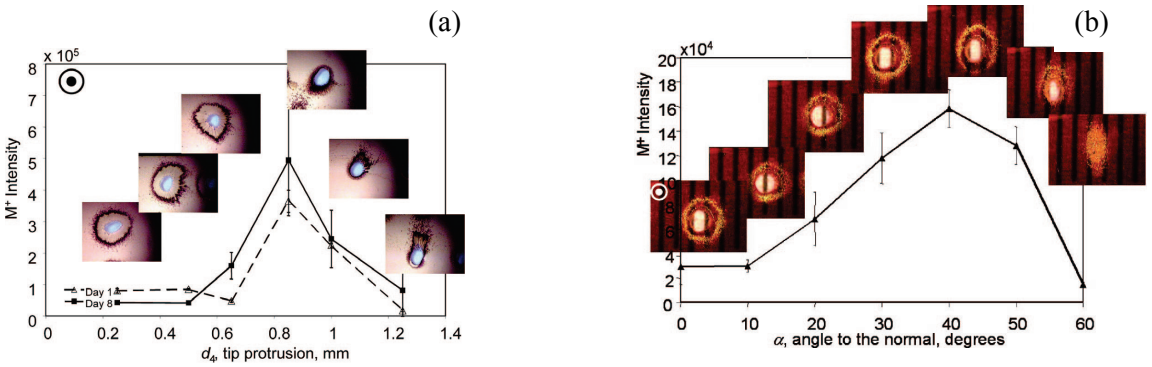

Figure 2. The effect of two geometrical parameters on the DESI signal intensity and spot shape (a) tip protrusion, d_4 and (b) electrospray angle of incidence, α. For more details see reference 11.

Similar analyses found that the tip position relative to the surface is optimized when d_1 is set between 1.5 and 2 mm, and the tip position relative to the sniffer is optimized when d_3 is set to ~5 mm. The inlet to the mass spectrometer ("sniffer") needs to be close to the sample surface as demonstrated in simulations by Costa et al.[13] It has been found that setting d_2 to be as small as possible, whilst allowing the sample stage to move freely gives the best sensitivity.

Ensure that the DESI spray tip, analysis spot and the inlet to the mass spectrometer "sniffer" are on axis. This can normally be achieved by adjustment of the DESI spray tip mounting device and ensuring the spray tip capillary is positioned centrally within the gas flow.

5.2.2 Chemical parameters

The electrospray uses a solvent-water mixture. Figure 3 shows that the spot shape improves with a higher solvent fraction with the diameter reducing approximately linearly and the finger-like structure on the trailing edge disappearing.[14,15] Additionally, it was found that the DESI intensity increases with solvent fraction. Comparison with direct electrospray ionisation data shows this is not simply an ionisation effect and is thought to be due to smaller droplets having higher efficiency for sample collection into secondary droplets. Further details are given in reference 12. We recommend using an electrospray solvent composition of acetonitrile:water with a 90:10 volume fraction with 0.1% formic acid. All solvents should be LC-MS grade or better.

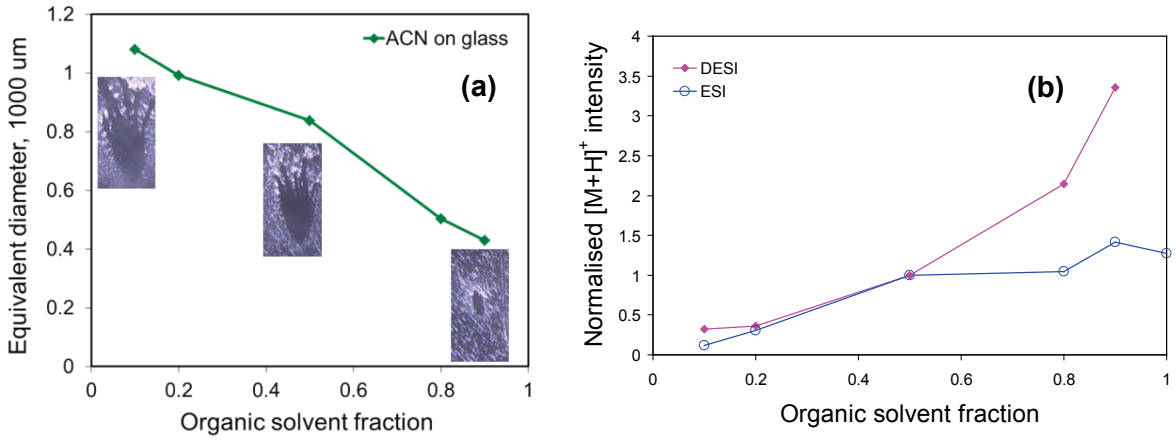

Figure 3. The effect of organic solvent fraction on (a) the DESI spot shape and size and (b) the signal intensity. The effect for the ESI with analyte is shown for comparison. For more details see reference 12.

The solvent flow rate is also found to affect the spot size. This is optimized at 2 µl/min, further details are in reference 12.

5.2.3 Electrical parameters

It is found that the signal intensity generally rises with electrospray voltage, since more charge is available to create ions.[16] However, we find that, at voltages greater than 5000 V, the signal intensity is unstable, perhaps due to the build-up of charge at the surface of insulating samples. Therefore, we recommend 5000 V as the optimum electrospray voltage.

6 SAMPLE HANDLING

6.1 We now give specific guidance for sample handling and data acquisition. The glass slides are stored in a container to reduce contamination. The Rhodamine B sample A is supplied in a black plastic bag to prevent exposure to light. Please keep the sample inside the black bag until you are ready to analyse it. All the samples should be stored out of direct heat and light in a normal laboratory environment. The sample A is to be analysed in the "as received" condition with no further sample preparation. For the samples B1, B2, B3 and B4 a protective non-stick paper layer strip should be peeled off just before analysis. The Material Safety Data Sheet (MSDS) for Rhodamine B is supplied separately.

6.2 DESI is sensitive to trace contaminations and it is important, therefore, that the samples are kept clean and are analysed as soon as possible after the containers are opened and are then returned to the container immediately after analysis. Samples should only be handled at their edge and held using powderless polyethylene gloves (or gloves with low levels of release agent) and clean tweezers. Ensure the correct side of the sample is analysed as marked. It is necessary to keep the blank half of the glass slide clean too as this is used to generate a spectrum of the local ambient background and contaminants in the electrospray solution.

7 DATA ACQUISITION

A flow chart for the study is given in Figure 4 and a schedule for the analysis is provided in Table 3. Note that the exact timings of analysis are not important and should be integrated with the laboratory workflow at your convenience.

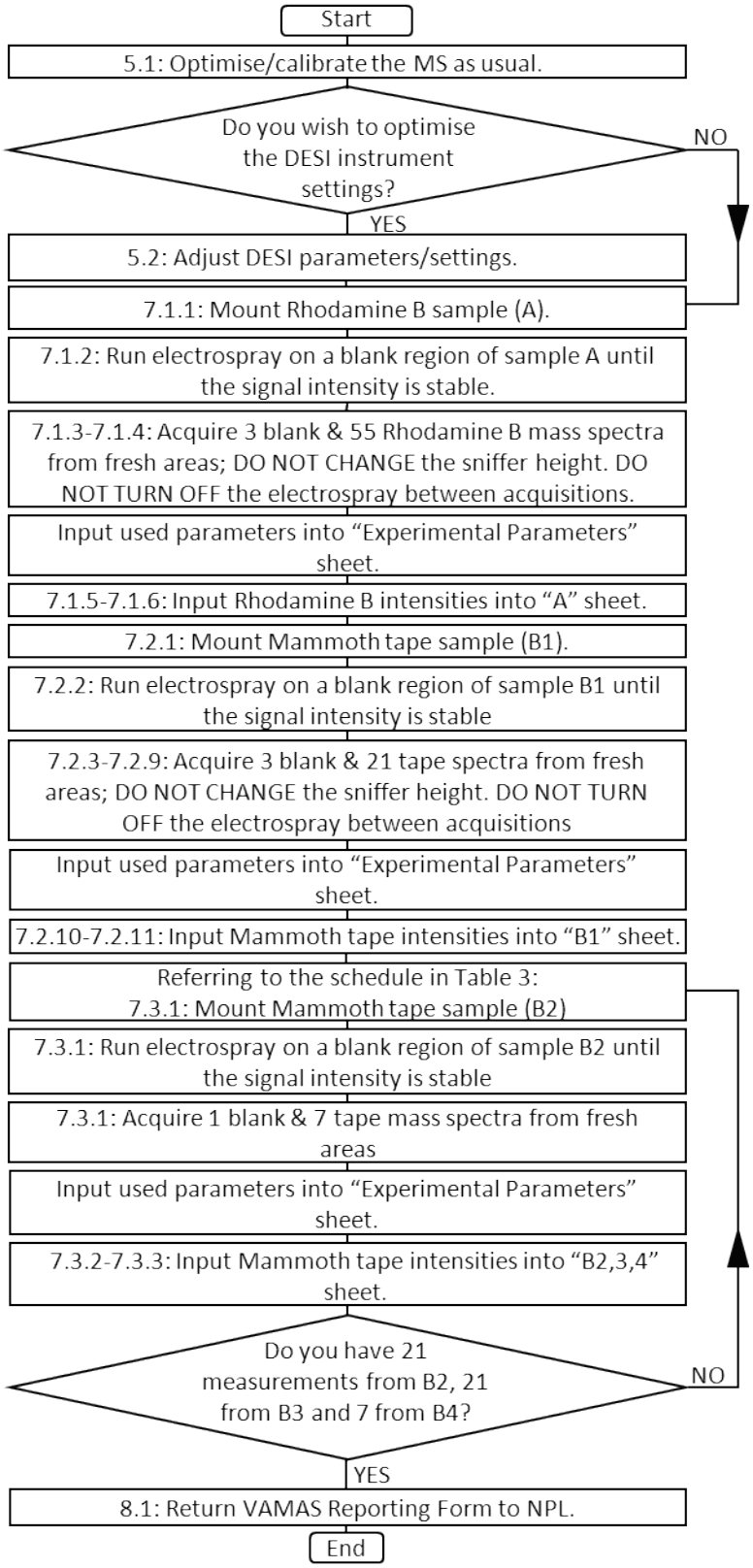

Figure 4. Flow chart of the work.

Table 3. Repeatability and constancy time table for reference samples A, B1, B2, B3 and B4.

Reference Sample	Day	Comment
A	1†	Analyse the sample on 58 fresh areas (including 3 blank areas). DO NOT CHANGE the sniffer height and DO NOT TURN OFF the electrospray between acquisitions.
B1	1†	Analyse the sample on 24 fresh areas (including 3 blank areas). DO NOT CHANGE the sniffer height and DO NOT TURN OFF the electrospray between acquisitions.
B2	1†	Analyse the sample on 8 fresh areas (including 1 blank) (first strip)
B2	2	Analyse the sample on 8 fresh areas (including 1 blank) (middle strip)
B2	3	Analyse the sample on 8 fresh areas (including 1 blank) (third strip)
B3	4 *	Analyse the sample on 8 fresh areas (including 1 blank) (first strip)
B3	5 *	Analyse the sample on 8 fresh areas (including 1 blank) (middle strip)
B3	6 *	Analyse the sample on 8 fresh areas (including 1 blank) (third strip)
B4	7 *	Analyse the sample on 8 fresh areas (including 1 blank) (first strip)
† Note: Samples A and B1 should be analysed on the same day. Sample B2 first strip may be analysed on the same day or another day. * Note: It is not critical that this is the 4th, 5th, 6th or 7th day. Please schedule it at a time that is convenient for you, but on different days.		

7.1 INTENSITY REPEATABILITY MEASUREMENT USING RHODAMINE B (SAMPLE A)

7.1.1 Set up the mass spectrometer and DESI source according to your standard operating conditions or refer to sections 5.1 and 5.2. Record relevant parameters in the "Experimental Parameters" sheet. Mount sample A into the position for analysis with the writing on the underside of the sample as shown in Figure 5. Please ensure that the syringe, or other solvent supply, has enough solvent for the whole analysis; 300 µl should be sufficient.

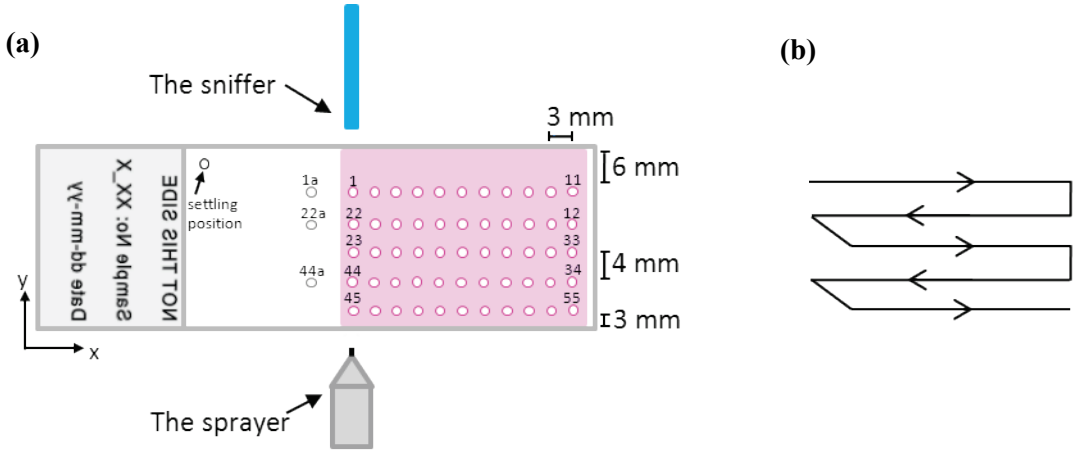

Figure 5. (a) Top view of the analysis side of reference sample A, including the directions of the sniffer and the sprayer, the analysis direction indicated by spots and (b) illustrated as a directional sequence. The axes, *x* and *y*, are also defined.

7.1.2 First, some time is required for the DESI source to settle. In our instrument we find 30 minutes is required. Place the sample so that the sniffer and the electrospray are on a blank area of the

glass slide well away from the analysis area depicted in Figure 5. Turn on the DESI source and leave it running until the signal intensities are stable; we recommend doing this for at least 30 minutes, at a position shown as settling position in Figure 5(a), before acquiring data.

7.1.3 Move to position 1a on a fresh blank area and record one mass spectrum for 30 s covering the mass range *m/z* 50 to 600.

7.1.4 Before moving onto the Rhodamine coated side ensure there is about 6 mm space in the *y* direction from the glass slide edge, into the Rhodamine film. Before moving to position 1 on the Rhodamine B film, change the mass range to *m/z* 400 to 500; use the same settings for everything else. Move to position 1 on the Rhodamine B film and acquire a mass spectrum for 30 s. For reference, an example spectrum is provided in Figure 6(a). Move to the next position defined in the pattern in Figure 5 and acquire a mass spectrum. Repeat this until 58 mass spectra have been acquired (including 3 blanks). Note the directional sequence of the 58 spectra; please use this sequence (Figure 5(b)). Ensure that the spots are separated by 3 mm in the *x* direction and 4 mm in the *y* direction as shown in Figure 5(a). The DESI source should be left on (with the pump running) between analysis positions. Move quickly between analysis spots to avoid consuming too much material. After analysing the last spot, position 55, immediately switch off the electrospray and the gas supply. Lift the sniffer from the surface, move the sample stage so that the blank side of the sample is in the analytical position and remove the sample from the holder. Return the sample to the sample container. Put it back into the black plastic bag for return to NPL.

7.1.5 Export the data for the extracted absolute intensity ion chromatograms for the Rhodamine $[M]^+$ mass peak at *m/z* 443.233 for all the acquired Rhodamine mass spectra. A mass range of *m/z* 443 to 443.5 is recommended but this will depend on the mass spectrometer used. An example is shown in Figure 6(b).

7.1.6 A typical extracted ion chromatogram is shown in Figure 7 exhibiting an initial drop in intensity followed by a plateau or gradual decline in intensity. The intensity profile will depend on the DESI spot size and shape as well as the solvent flow rate. This is discussed in detail in reference 10.

For best results, sum the counts in the plateau region between two fixed times, in this example between 5 s and 25 s (solid line in Figure 7). Record these times in the "Experimental Parameters" sheet. Keep the time intervals fixed for all intensity measurements. Alternatively, for some instrument software this is tedious and the intensity over the full 30 seconds should be summed.

For each of the 55 extracted ion chromatograms for Rhodamine, enter the summed intensity into the, sheet "A" in column D, rows 11 to 65. If for any reason you do not have 55 ion chromatograms enter the values of the summed intensities that you have. In the absence of the data, leave cells blank. Do not enter "zero" or "0". The form will calculate the intensity repeatability from the relative standard deviation of the intensities. Only cells highlighted in grey will be accessible to you. The rest of the cells are password protected in order to minimise accidental changes to the calculating formulas. Record the DESI and mass spectrometer settings in the "Experimental Parameters" sheet.

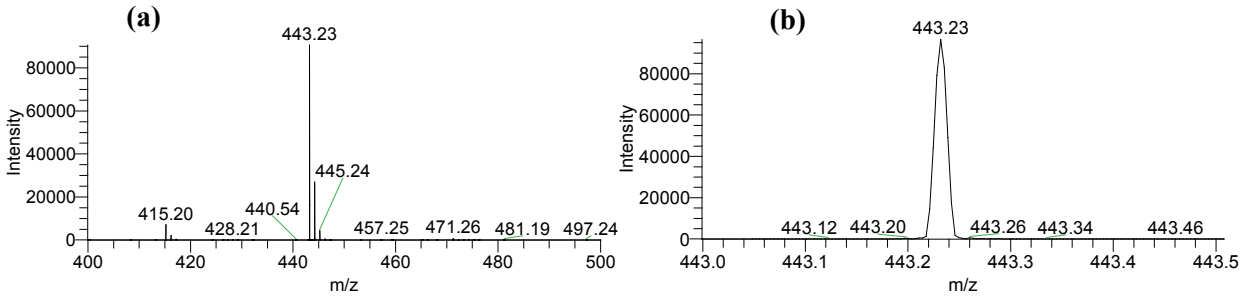

Figure 6. Positive ion DESI mass spectrum of Rhodamine B (sample A) (a) wide scan and (b) detail showing mass range selected for chromatogram.

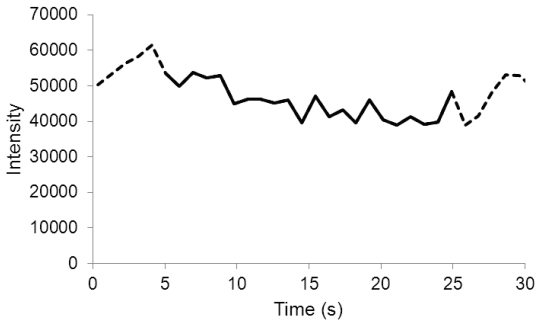

Figure 7. An example positive ion chromatogram for the Rhodamine $[M]^+$ peak (*m/z* 443.23) showing the region of more stable intensities that can be selected for improved results (solid line).

7.2 RELATIVE INTENSITY MEASUREMENT USING ADHESIVE TAPE (SAMPLE B1)

The analyses for this sample should be conducted on the same day as for sample A.

7.2.1 Set up the mass spectrometer and DESI source according to your standard operating conditions or refer to sections 5.1 and 5.2. If these parameters differ from 7.1.1 record them in the "Experimental Parameters" sheet. Mount sample B1 into the position for analysis with the writing on the underside of the sample as shown in Figure 8(a).

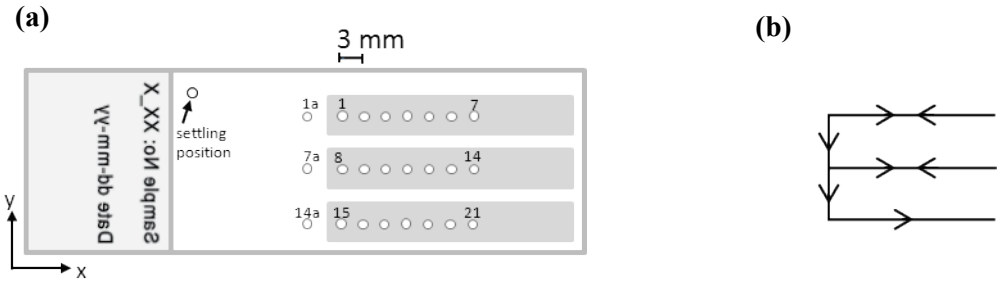

Figure 8. (a) Top view of the analysis side of reference sample B with the analysis direction indicated by numbered spots and (b) illustrated as a directional sequence. The axes, *x* and *y*, are also defined.

7.2.2 First, some time is required for the DESI source to settle. In our instrument we find 30 minutes is required. Place the sample so that the sniffer and the electrospray are on a blank area of the glass slide well away from the analysis area depicted in Figure 8(a). Turn on the DESI source and leave it running until the signal intensities are stable; we recommend doing this for at least 30 minutes, at a position shown as "settling position" in Figure 8(a), before acquiring data.

7.2.2 Move to position 1a on the fresh blank area. Record one mass spectrum for 30 s using the mass range *m/z* 50 to 1400.

7.2.3 Peel off the protective paper cover from the first strip of adhesive tape (analysis positions 1 to 7).

7.2.4 The mass spectrometer inlet "sniffer" may stick to the adhesive tape if they are in contact. Adjust the analysis position so that the sniffer runs along the glass surface very close to the edge of the tape but not in contact with the tape as shown in Figure 9.

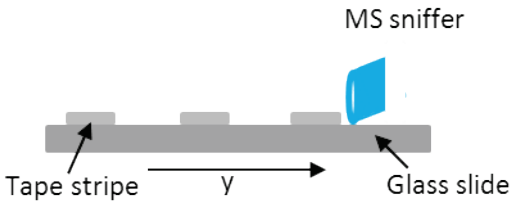

Figure 9. Schematic of the position of the mass spectrometer inlet ("sniffer") for analysis of the first adhesive tape strip. The sniffer runs along the glass surface close to the edge of the tape but not in contact with the tape.

7.2.5 Move to position 1 on the adhesive tape and acquire a mass spectrum from *m/z* 600 to 1400 for 30 s. For reference, an example spectrum is provided in Figure 10(a) along with details of specific mass ranges used later in the analysis. Move to the next position defined in the pattern in Figure 8 and acquire a mass spectrum. Repeat this until 7 spectra have been acquired on that strip of tape. Ensure that the spots are separated by 3 mm in the *x* direction as shown in Figure 8(a). The DESI source should be left on (with the pump running) between analysis positions. Be careful not to change the mass spectrometer inlet "sniffer" height, d_2, between acquisitions. After finishing the analysis on the first strip (positions 1-7) move back to position 1a and then go to position 7a.

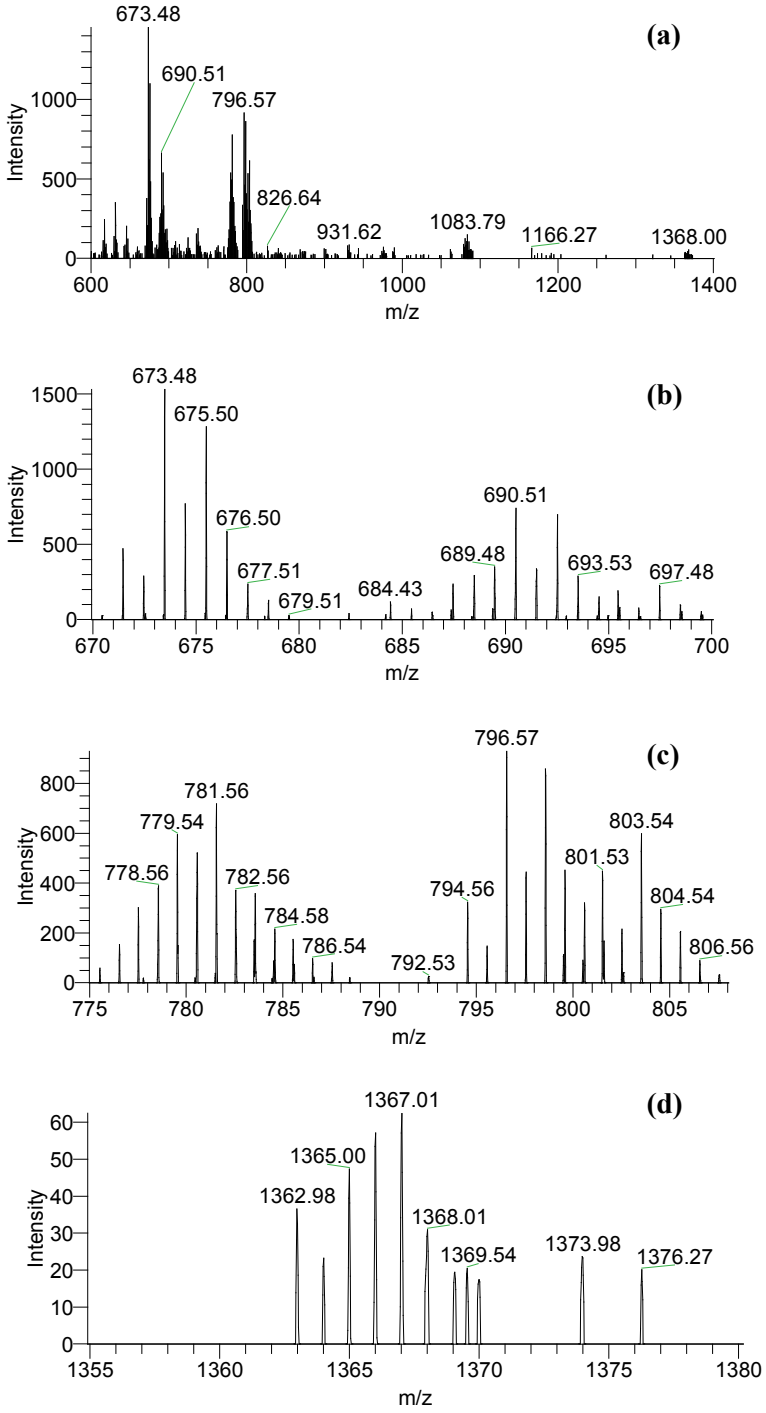

Figure 10. Positive ion DESI mass spectra of adhesive tape (sample B) (a) wide scan, (b), (c) and (d) detail showing mass ranges selected for extracted ion chromatograms.

7.2.6 At position 7a on a fresh blank area, record one mass spectrum for 30 s covering the mass range *m/z* 600 to 1400. If convenient, a wider mass range such as *m/z* 50 to 1400 is helpful to identify any contaminants. Peel off the protective paper cover from the middle strip of adhesive (analysis positions 8 to 14).

7.2.7 Repeat steps 7.2.4 and 7.2.5 for the middle strip of tape (analysis positions 8 to 14).

7.2.8 Repeat step 7.2.6 for position 14a for the last strip of tape.

7.2.9 Repeat steps 7.2.4 and 7.2.5 for the third strip of tape (analysis positions 15 to 21). After analysing the last spot on position 21, switch off the electrospray and the gas supply immediately. Lift the sniffer from the surface, move the sample stage so that the blank side of the sample is in the analytical position and remove the sample from the holder.

7.2.10 Export the extracted absolute intensity ion chromatograms from the tape mass spectra for the three mass ranges: *m/z* 670 - 700, *m/z* 774 - 808 and *m/z* 1355 – 1380, shown in Figure 10 (b), (c) and (d), respectively.

7.2.11 A typical extracted ion chromatogram is shown in Figure 7 showing an initial drop in intensity followed by a plateau or gradual decline in intensity. The intensity profile will depend on the spot size and shape of the DESI source and the solvent flow rate. This is discussed in detail in reference 10.

For best results, sum the counts in the plateau region between two fixed times, in this example between 5 s and 25 s (solid line in Figure 7). Record these times in the "Experimental Parameters" sheet. Keep the time intervals fixed for all intensity measurements. Alternatively, for some instrument software this is tedious and the intensity over the full 30 seconds should be summed.

For each of the 21 x 3 extracted ion chromatograms of the adhesive tape (sample B1), enter the summed intensity into the sheet "B1" in columns D, E and F, rows, 11 to 31. The form will calculate the relative repeatability from the intensities using the N_{ij} method; details are given in reference 17. Only cells highlighted in grey will be accessible to you. The rest of the cells are password protected in order to minimise the accidental changes to the calculating formulas. Record the DESI and mass spectrometer settings in the "Experimental Parameters" sheet.

7.3 RELATIVE INTENSITY AND CONSTANCY MEASUREMENT USING ADHESIVE TAPE (SAMPLES B2, B3, B4)

7.3.1 According to the schedule defined in Table 3, repeat steps 7.2.1 to 7.2.5 substituting the relevant strip of tape for the sample as defined in Table 3.

7.3.2 Repeat step 7.2.10.

7.3.3 Repeat step 7.2.11, inputting the data into the sheet "B2,3,4" in columns D, E and F, rows 11 to 17.

7.3.4 Repeat steps 7.3.1 to 7.3.3, inputting the data into the sheet "B2,3,4" in columns H, I and J, rows 11 to 17 etc., until all analyses in Table 3 are complete.

NOTE The schedule in Table 3 is illustrative and may be changed as explained in the table footnotes. Select the timings to suit your workflow.

8 AFTER ANALYSIS

8.1 When all the analyses are complete, check that VAMAS Reporting Form is complete and email it to NPL elzbieta.gurdak@npl.co.uk. Also, any spread-sheets with chromatogram data including the blanks from sample A, B1, B2, B3 and B4 may be emailed to help with analysis.

8.2 Return the Rhodamine sample A to NPL. This allows measurements of the spot size to be conducted.

9 ANY QUESTIONS

9.1 If you are unsure of anything please contact Elzbieta Gurdak, email: elzbieta.gurdak@npl.co.uk

ACKNOWLEDGMENTS

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