**Differentiation of lactose sample batches from surface impurities by OrbiSIMS**

Bin Yan1\*, Junting Zhang1, Eleonora Paladino2, Mark Nicholas2, Paulina Rakowska1,3, Tony Bristow4, and Ian Gilmore1\*

1. National Physical Laboratory, NiCE-MSI, Teddington, Middlesex, UK.

2. Inhalation Product Development, Pharmaceutical Technology and Development, Operations, AstraZeneca, Gothenburg, Sweden.

3. National Biofilms Innovation Centre, University of Southampton, Southampton, UK.

4. Chemical Development, Pharmaceutical Technology and Development, Operations, AstraZeneca, Macclesfield, UK.

**Contact:** bin.yan@npl.co.uk; ian.gilmore@npl.co.uk

**Abstract**

Impurities present on the surface of pharmaceutical formulations for drug delivery significantly impact their stability and efficacy. Hence, the characterization of impurities on the surface is essential to ensure efficacy, quality, and safety. Secondary ion mass spectrometry (SIMS) is gaining increasing popularity for surface analysis in the pharmaceutical industry, due to its high sensitivity and spatial resolution. Time-of-Flight (ToF) SIMS instruments have been successfully used. However, their application has been limited since the mass resolving power and mass accuracy are too low to separate complex peaks and give identification with confidence. The OrbiSIMS instrument overcomes this issue owing to its Orbitrap analyzer with a mass resolving power of > 240,000 at *m*/*z* 200 and a mass accuracy of < 2 p.p.m. We use the OrbiSIMS in combination with multivariate analysis to differentiate notionally identical batches of lactose from different suppliers using the detection of small differences in surface impurities.

**1. Introduction**

The characterization of impurities on the surface of a drug formulation is a critical aspect of pharmaceutical quality control. Impurities can affect the efficacy and the stability of a drug and could lead to adverse effects.1, 2 Whilst the active ingredient is most likely manufactured in-house to a high level of purity and consistency, the excipients used in the formulation may be from external suppliers. Consequently, it is important to be able to determine the batch-to-batch variability of impurities and diagnose any variability in formulated performance that may arise as a result.

Time-of-flight secondary ion mass spectrometry (ToF-SIMS) provides chemical analysis of surfaces and interfaces, and has been widely applied in various types of pharmaceutical analysis, such as locating structurally-similar or low concentration impurities on the crystal surface,3, 4 differentiating crystalline and amorphous phases, 5, 6 detecting and monitoring surface phase separation and recrystallisation at single particle scale and at the uppermost surface in different types of pharmaceutical solid products,7-9 and studying content change and distribution of active components in complex Chinese medicines with growth cycle.10 However, the limited mass resolution and mass accuracy of a time-of-flight analyzer make it challenging to study complex sample systems, in which unresolved ion peaks and inaccurate peak assignment often occur.11

With the recent development of 3D Orbitrap secondary ion mass spectrometry (OrbiSIMS) using a dual Orbitrap and ToF analyzer approach,12 the inherent drawbacks of ToF-SIMS are alleviated. The Orbitrap anayzer of the instrument improves the mass resolving power to 240,000 at m/z 200, which dramatically reduces peak overlapping and improves the accuracy of peak assignment (mass deviation <2 ppm). The relatively gentle argon cluster ion beam combined with Orbitrap MS enables the detection of large molecules with much less fragmentation. In addition, the tandem MS capability provides an extra dimension of validation for ion identification. Less than a decade since its development, OrbiSIMS has demonstrated its powerful capabilities in numerous biological studies by detecting and imaging endogenous metabolites,13-15 fatty acids,16 lipids,17 semi-volatile organic compounds,18 amino acids,17 proteins19 as well as exogenous metal ions20 and drug molecules21 from a variety of sample systems including fresh and archived tissues,13, 15, 18, 21 single cells,14 cell membranes,22 skins,16, 19 biofilms,17 etc. Recently the ability to directly identify trace amounts of degradation molecules in blue OLED and localisation within a few nanometres of the interface has been demonstrated.23

Here, we report the application of OrbiSIMS to identify impurities on the surface of inhalation-grade lactose, which is one of the most commonly used excipients in the pharmaceutical industry. The combination of multiple analysis methods including Orbitrap MS, tandem MS, depth profiling and 3D imaging enables confident annotation of detected ions and identification of impurities. Furthermore, batch separation of lactose samples is achieved by multivariate analysis of the spectral data.

**2. Experimental**

2.1. Sample preparations

In this study, five different lactose batches were utilized: LH100 and SV010 batches were purchased from DFE Pharma and three technical batches were sourced from a contract manufacturing organization associated with AstraZeneca. Lactose particles were mounted on poly(methyl acrylate) (PMA) adhesive-coated Si wafers.

* 1. OrbiSIMS experimental methods

3D OrbiSIMS (Hybrid SIMS, IONTOF GmbH, Germany) was used for data acquisition. Mass calibration of the Q Exactive Orbitrap mass analyzer was performed daily using silver cluster ions Ag3+. Electrons with an energy of 21 eV and a current of 10 µA, and argon gas flooding were used for charge compensation (pressure in the main chamber was maintained at 1.0 × 10−6 mbar). The target potential was optimized for each sample. For all Orbitrap data, mass spectral information was collected from a mass range of 80-1200 Da. The Orbitrap analyser was operated with the mass-resolution of 240,000 set at m/z 200 (512 ms transient time).

For spectra and depth profiling, 6 to 8 different areas of the sample with the field of view 400 × 400 or 450 × 450 µm were sputtered using defocused 20 keV Ar3000+ in random raster mode for 240 μs per cycle. The total ion dose was 3.88-7.75 × 1015 ions/cm2 for depth profiling, while for full MS and tandem MS experiments, a significantly lower ion dose (approximately 3 orders of magnitude less) was used to minimize sample damage.. The scan number was 500-1000 for depth profiling while 20 for full MS. For the tandem MS, the primary ion beam parameters were the same as above. The normalized collision energy was set to 30 – 50 eV and the isolation width was 2 u. For 3D imaging experiments, the dual beam-dual analyser mode was used. 450×450 µm and 300×300 µm images containing 256 × 256 pixels in positive and negative ion mode respectively were acquired by a pulsed 30 keV Bi3+ ion beam with a beam current of 0.2 pA and ToF mass analyser. These images were interleaved with Orbitrap MS acquired by 20 keV Ar3000+ ion beam sputtered on the same field of view. 30 and 50 depth scans were accumulated in the ToF images, which correspond to a total primary ion dose of 1.82 × 1012 and 6.82 × 1012 ions/cm2 in positive and negative ion mode, respectively. Reference mass spectra of the poly(methyl acrylate) (PMA) substrate were also acquired under identical experimental conditions and used to identify and exclude substrate-derived peaks during data preprocessing.

* 1. Data analysis

The 3D OrbiSIMS was controlled by SurfaceLab Version 7.0 (IONTOF GmbH, Germany) software, which used the application programming interface (API) provided by Thermo Fisher for both control of the Orbitrap mass analyzer portion of the instrument as well as online retrieval of the data. Both ToF and Orbitrap mass analyzer image analyses were performed using SurfaceLab Version 7.0 (IONTOF GmbH, Germany). Peak assignment was performed with a mass accuracy tolerance of 3 ppm, taking into account potential impurities and fragments that may arise during lactose production and analysis. A custom code was written in MATLAB for data pre-processing for principal component analysis (PCA) and PLS-DA, which includes sum of peak intensities of first 2 scans, normalization to total ion counts (TIC), removing PMA substrate peaks and scaling by the inverse square root of the mean value for each peak across all samples. PCA analysis and visualization was performed on processed data by OriginPro 2020. PLS-DA analysis was conducted by using the web-based platform MetaboAnalyst 6.0.24 To evaluate spatial correlation between ions in ToF-SIMS images, the correlation coefficient tool in OriginPro 2020 was used to compare pixel-resolved ion intensity maps. This tool automatically calculates the Pearson correlation coefficient based on pixel-wise intensity values for the selected ion images.

* 1. ToF-SIMS experimental methods and data analysis

Comparison data were acquired using a TOF.SIMS5 (IONTOF GmbH, Germany) on the same batches. 200 × 200 µm images containing 1024 ×1024 pixels were acquired in positive and negative ion mode over 5 to 7 different areas of each sample using a pulsed 30 keV Bi3+ ion beam with a beam current of 0.05 pA (primary ion dose of 4.50 × 1011 ions/cm2) and a ToF mass analyser, with an extraction delay of 0.310 µs. Mass spectral information was collected over a mass range of 0-1100 Da. Electrons with an energy of 21 eV and a filament current of 2.30 A were used for charge compensation. Data acquisition and spectral calibration were performed using SurfaceLab Version 7.2 software (IONTOF GmbH, Germany). Using the home-written MATLAB code mentioned above, similar data pre-processing including TIC normalization, PMA substrate peaks removal, and inverse mean square root scaling was applied to each collected spectrum. The processed data were imported to OriginPro 2020 for PCA analysis and visualization.

1. **Results and discussion**
   1. Surface impurity analysis by high mass resolution, mass accuracy, and tandem mass spectrometry

The positive ion OrbiSIMS spectrum of all studied lactose (C12H22O11) (**Fig. 1a**) is dominated by its salt adduct ions, i.e., [M+Na+ *m/z* 365.1055 and [M+K]+ *m/z* 381.0798 with only a weak signal for [M+H]+ *m/z* 343.1237. Fragments including [M-H2O+Na]+ *m/z* 347.0952 and [M-C2H4O2+Na]+ *m/z* 305.0846 are observed with low intensity. Below *m/z* 300, many peaks associated with the PMA substrate (highlighted in the inset) are detected. Some impurities are observed in the highmass region, including sodiated and potassiated C20H35NO16 near *m/z* 600. Furthermore, several salt adducts of C18H32O16 and C24H42O21 are observed, which may be due to either intermolecular interaction between lactose molecules (potentially through non-covalent association or dehydration-driven combination with its monomeric fragments or another lactose unit during secondary ion formation) or impurities of trisaccharide and tetrasaccharide.



**Fig. 1.** Orbitrap positive (a) and negative (b) ion mass spectrum of lactose (C12H22O11)using 20 keV Ar3000+. Putatively assigned peaks with a mass accuracy of 2 ppm deviation are annotated. Comparison of spectra between sample and substrate in the *m/z* region below 300 is displayed in the inset.

The tandem MS (**Fig. 2a**) shows that activation of C18H32O16Na+ or C24H42O21Na+ leads to the loss of C6H10O5 (breakage of the glycosidic bond), as the main cleavage pathway, in addition to loss of water and C2H4O2. This fragmentation pattern was previously observed in tandem MS studies of both oligosaccharide (e.g., panose, maltotriose, and cellotetraose)25, 26 and a dimer of disaccharide (e.g., isomaltose).27 Therefore, there are several possible routes to the formation of C18H32O16 and C24H42O21 salt adduct ions.



**Fig. 2.** 20 keV Ar3000+ GCIB Orbitrap positive (a) and negative (b) MS/MS spectra of sodiated lactose C12H22O11Na+, C18H32O16Na+, C24H42O21Na+, and C12H21O14S-. The normalized collision energy was set to 30-50 eV and the isolation window was 1 u. In positive MS/MS, same fragmentation pattern was observed in previous tandem MS studies of both oligosaccharide (e.g., panose, maltotriose, and cellotetraose)25, 26 and dimer of disaccharide (e.g., isomaltose).27

The negative ion OrbiSIMS spectrum of lactose is more complex (**Fig. 1b**). In addition to the [M-H]- at *m/z* 341.1092 and [M+Cl]- at *m/z* 377.0861 and *m/z* 379.0831, there is an intense peak corresponding to sulphate-related adduct of lactose [M-H+SO3]- at *m/z* 421.0663 (tandem MS shown in **Fig. 2b**). The high mass resolution of the Orbitrap mass analyser enabled identification of a separate peak at *m/z* 421.0750, which could be tentatively assigned to phosphate-related adduct of lactose [M+PO3]-. As with the positive ion mode spectrum, fragmentation of lactose by losing C2H4O2 or H2O was also noted in the negative mode. In the region below *m/z* 300, some peaks are likely related to lactose fragments, such as C6H9O5- and C6H11O6-. In the high mass region, peaks at *m/z* 683.2259, 665.2154 corresponding to dimerization of lactose were detected, though in very low abundance. Some ions that may originate from impurities were also detected, including C11H16O8N- (*m/z* 290.0883), C23H38NO19- (*m/z* 632.2049) and some unannotated peaks.

* 1. Improving confidence in distinguishing impurities from lactose through depth profiling



**Fig. 3.** Orbitrap MS positive ion intensity depth profile with 20 keV Ar3000+ sputtering of a lactose particle for selected lactose and impurity ions. All the signals are summed over a 400 µm × 400 µm area.

To study the correlation between the depth distribution of lactose and impurity ions, depth profiling was performed using Orbitrap MS with 20 keV Ar3000+ sputtering. In positive ion mode (**Fig. 3**), it is found that the intensities of lactose [M+H]+ and [M-OH]+ are relatively constant throughout the total ion dose of 3.88 × 1015 ions/cm2 (corresponding to a total depth of ~8 µm, considering a typical sputtering rate for organic materials of 200 nm3 per 20 keV Ar3000+ ion, i.e., 20 nm per 10¹³ ions/cm² fluence)28, whilst the intensity of sodiated lactose falls rapidly in the near surface region, followed by a gentle decline before stabilising by an ion dose of approximately 1 × 1015 ions/cm2. This is consistent with the sodium salt being enriched on the surface of the sample, and similar behaviour is seen for the contaminant annotated as C20H35NO16Na+ until falling below the detection at an ion dose of 1 x 1015 ions/cm2. It is noteworthy that C24H42O21Na+ (labelled as [2M-H2O+Na]+ in **Fig. 3**) exhibits a similar profile to sodiated lactose and hence is more likely due to lactose intermolecular interactions rather than originating from other oligosaccharides. In contrast, the depth profiles of the impurity ions C10H12N2O5K+ and C7H6N2O2K+ are distinct with an initial sharp rise in intensity in the near surface followed by a gradual decline. This suggests the presence of an overlayer (the sampling depth of SIMS is a few nanometres) with the contaminants within the lactose material. The source of the contaminants is unknown and further study is required to identify the source from a range of possibilities including from the feed, packaging materials or processing. As expected, the PMA substrate ions C10H8+ and C13H9+ have an initial sharp rise in intensity as surface contamination is sputtered away. This is followed by a gradual increase, which we attribute to the progressive exposure of the underlying PMA substrate as the overlying lactose layer is sputtered away rather than diffusion of the substrate into the lactose., A rapid decline is then observed at around 2.6 x 1015 ions/cm2,indicating the depletion of the PMA layer itself. However, the PMA intensity does not drop to zero, since areas previously covered by lactose were protected from sputtering. The ratio of PMA intensities at the beginning and end of the profile allows an estimate of the surface coverage by lactose of approximately 75%. The depth information provides an additional dimension, allowing separation of the main analyte from impurities. Similar benefits are found from the negative ion depth profile data (Supplementary **Fig. 1**). Except for a very mild drop in the near surface region, relatively constant signal of lactose [M-H]- and its dimer [2M-H]- was measured throughout the total ion dose of 7.75 × 1015 ions/cm2, while a slow rise was observed for sulphate-related and phosphate-related adduct ion intensities. For impurity ions C32H35N8O6S2- and C27H22N2O9Na-, a distinct sharp drop of intensities was noted in the near surface.

* 1. Spatial distribution correlation and differences between lactose and impurities



**Fig. 4.** 2D 30 keV Bi3+ ToF MS positive ion images of lactose acquired by dual beam-dual analyzer operation mode. The field of view is 450 × 450 µm which contains 256 × 256 pixels and in total 30 scans are accumulated along the depth. Ions are annotated based on their m/z values detected by orbitrap MS interleaved with ToF-SIMS images. The distribution differences between lactose and its salt adducts or impurities are marked with green and blue circles, respectively.

The OrbiSIMS can be operated in a dual beam dual analyser mode, where high-spatial resolution images are acquired with a 30 keV Bi3+ primary ion beam in combination with ToF MS, interleaved with high-mass resolution spectra measured by a 20 keV Ar3000+ sputtering beam coupled to the Orbitrap mass analyser. The sequence is repeated creating a 3D ToF MS data set with a synchronised Orbitrap MS depth profile. The high-mass resolution Orbitrap data aids the interpretation of the lower mass resolution ToF-SIMS data. Furthermore, the Orbitrap depth profile has a much higher duty cycle than ToF-SIMS, resulting in over two orders of magnitude increased sensitivity.29 Rendered 3D ToF MS images (**Supplementary Fig. 2**) of the lactose ions [M+Na]+, [M-OH]+, and the contaminants on the surface, along withthe depth scan-resolved 2D images, show no significant change in the 2D distribution of the ions with increasing depth. Each scan has a 20 keV Ar3000+ dose of 7.75 × 1012 ions / cm2. Annotation of ToF peaks is supported by the high mass resolution Orbitrap MS data (**Supplementary Fig. 3**). It is found that the peak at *m/z* 153.05 detected by ToF MS can be attributed to multiple ions. The 2D images summed over all scans of selected ions are shown in **Fig. 4**. Similar pattern is observed between the distribution of protonated lactose (**Fig. 4e**) and its fragments (**Fig. 4a, f**). In contrast, the sodium adducted ions of lactose and its dimer (**Fig. 4b, g and h**) display slightly different distribution (marked by green circle), while the difference is more significant (marked by blue circle) in the distribution of impurity ions (**Fig. 4c, i, j and k**). The overlay ion image (Fig. 4d) of selected peaks clearly shows the differences. Scatter plots (**Supplementary Fig. 4**) of pixel intensities for pairs of secondary ions reveal that the signal of lactose fragment [M-OH]+ and C6H11O5+ are highly correlated with each other (Pearson correlation coefficient (PCC) = 0.913). The distribution of sodiated lactose is similar to that of lactose fragments [M-OH]+ and C6H11O5+ (PCC = 0.712 and 0.790, respectively) but is moderately correlated with the impurity C7H6N2O2K+ (PCC = 0.504). In addition, ToF-SIMS negative ion images were acquired (**Supplementary Fig. 5**), which also demonstrated the spatial distribution correlation and differences between different lactose adducts and impurities. Not surprisingly, the distribution patterns of deprotonated lactose, its dimer, fragments, and chloride adduct ions are very similar, whereas those of the impurities are significantly different.

* 1. Lactose batch separation through multivariate analysis of OrbiSIMS spectra

We used multivariate analysis to differentiate sample sources or batches. Principal component analysis (PCA) of the negative ion OrbiSIMS spectra (**Fig. 5a**) differentiates batches of lactose. Most data points from the same batch are grouped together on the score plot of the first two principal components. Although batch 2 & 3 overlap, better separation can be achieved if only these two batches are studied (**Supplementary Fig. 6**). The PC1 and PC2 loadings plotted against m/z (**Fig. 5b** and **Supplementary Fig. 7a**) shows that the separation is primarily driven by lactose sulphate (C12H21O14S-) and phosphate (C12H22O14P-) anions, which are likely introduced as trace contaminants from cleaning agents, raw materials, or process water used during lactose production. . In addition, their C2H4O2 loss fragments (e.g., C12H21O14S-, C12H22O14P-, etc) and certain nitrogen-containing impurities also contribute to the differentiation. Partial Least Square-Discriminant Analysis (PLS-DA) also does not separate batch 2 from 3 (**Fig. 5c**). As in PCA, the key ions for differentiation are related to lactose sulphate, phosphate anions, and their fragments (**Fig. 5d and Supplementary Fig. 7b**).

PCA of the positive ion spectra (**Supplementary Fig. 8a**) gives less effective separation of the five batches, with substantial overlap of the 95% confidence regions—particularly between batches 2 and 3, and batches 4 and 5. PLS-DA (**Supplementary Fig. 8c**) yields slightly improved separation, though batches 2 and 3 remain indistinguishable. As with the negative ion mode, performing separate pairwise analyses (**Supplementary Fig. 9**) enhances batch separation where overall PCA shows overlap. While negative ion mode separation is primarily driven by lactose sulphate and phosphate adducts, in the positive ion mode, batch differentiation is mainly influenced by lactose salt adducts, fragments, and impurities (**Supplementary Fig. 8b, d; Fig. 9b, d**).



**Fig. 5.** PCA and PLS-DA of inhalation-grade lactose from five different batches using first two scans (uppermost surface) of 20 keV Ar3000+ Orbitrap negative ion spectra. (a) Scores plot for PC 1 and PC 2. (b) PC1 Loadings vector plotted as loadings coefficient versus peak m/z values for the data set. (c) PLS-DA Scores plot for the first two components. (d) Key ions driving the separation of different batches identified from component 1 scores of variable importance in projection (VIP). The coloured boxes on the right indicate the relative intensities of the corresponding ion in each batch under study. The spectra data was processed by normalization to total ion counts, removing PMA substrate peaks and scaled30 by the inverse square root of the mean value for each peak across all samples.

SIMS enables chemical imaging of the surface and, in combination with gas cluster ion beam sputtering, the bulk material is accessed as a 3D image or a chemical depth profile. Traditionally, a time-of-flight mass spectrometer is used but that has limitations in terms of the mass resolution and mass accuracy necessary for confident identification of molecules, e.g., only 3 ions were detected by ToF-SIMS out of 9 ions (labelled in Fig. 5b), identified by OrbiSIMS, that drive the separation of different batches. For comparison, we also analysed the 5 batches of lactose with a TOF.SIMS 5 using a 30 keV Bi3+ primary ion beam. Principal component analysis (**Supplementary Fig. 10**) was largely ineffective in clearly distinguishing between different batches, except for batch 1 in negative ion mode, which could be separated from the others. Similar results were observed in the PLS-DA analysis. Moreover, even when analyzing a small subset of mixed batches (e.g., batches 2 and 3) separately using PCA, although some separation was observed, it was insufficient for clear differentiation.

**4. Conclusions**

We show that OrbiSIMS can distinguish between different batches of lactose using its high mass resolution, mass accuracy and tandem MS to putatively annotate trace amounts of surface contamination. ToF-SIMS enables fast 3D imaging with sub-micron spatial resolution but the limited mass resolution prevents the resolving overlapping peaks and identification of surface impurities is ambiguous resulting in less effective batch separation. However, different strengths of Orbitrap and ToF mass spectrometers compensate for their respective weaknesses so that complementary analysis using the hybrid analyser approach of the OrbiSIMS is effective.

Overall, multivariate analysis of the OrbiSIMS spectra acquired allows for product batch separation using differences in impurities and provides a method for quality control procedures in pharmaceutical manufacturing. Notably, impurities such as sulphate and phosphate anions, along with other trace contaminants potentially originating from chemical processing equipment, residual solvents, or packaging materials, were detected. These components may influence the stability, safety, and therapeutic performance of lactose—particularly in inhalation-grade formulations, where excipient purity and batch consistency are critical. Further investigation is warranted to trace the origins of these impurities and assess their potential impact on pharmaceutical applications.

**Funding**

This work was funded by UK Research and Innovation (UKRI) through Analysis for Innovators (A4I) program.

**CRediT authorship contribution statement**

BY - project administration, conceptualization, methodology, investigation, data curation, formal analysis, visualization, writing – original draft, writing – review and editing; JZ - investigation, methodology, data curation, formal analysis, writing – review and editing; EP - investigation, formal analysis, writing – review and editing; MN – conceptualization, methodology, resources, formal analysis; PR - conceptualization, methodology; TB – funding acquisition, conceptualization, project administration, writing – review and editing; IG - funding acquisition, supervision, project administration, conceptualization, methodology, writing – review and editing.

**Acknowledgments**

The authors would like to acknowledge Jean-Luc Vorng for preliminary optimization of instrument parameters.

**References**

1. S. Görög, *J. Pharm. Biomed. Anal.*, 2008, **48**, 247-253.

2. K. Zhang, J. D. Pellett, A. S. Narang, Y. J. Wang and Y. T. Zhang, *TrAC, Trends Anal. Chem.*, 2018, **101**, 34-42.

3. K. V. Kumar, C. Heffernan, K. A. Ramisetty, C. A. Howard and S. Beloshapkin, *CrystEngComm*, 2022, **24**, 2485-2504.

4. S. Ottoboni, M. Chrubasik, L. Mir Bruce, T. T. H. Nguyen, M. Robertson, B. Johnston, I. D. Oswald, A. Florence and C. Price, *Cryst. Growth Des.*, 2018, **18**, 2750-2758.

5. A. Iuras, D. J. Scurr, C. Boissier, M. L. Nicholas, C. J. Roberts and M. R. Alexander, *Anal. Chem.*, 2016, **88**, 3481-3487.

6. T. P. Forbes, J. G. Gillen, A. J. Souna and J. Lawrence, *Anal. Chem.*, 2022, **94**, 16443-16450.

7. E. Paladino, F. J. Doerr, E. Bordos, I. I. Onyemelukwe, D. A. Lamprou, A. J. Florence, I. S. Gilmore and G. W. Halbert, *Int. J. Pharm.*, 2022, **628**, 122191.

8. I. J. H. Barrientos, E. Paladino, S. Brozio, M. K. Passarelli, S. Moug, R. A. Black, C. G. Wilson and D. A. Lamprou, *Int. J. Pharm.*, 2017, **517**, 329-337.

9. I. J. H. Barrientos, E. Paladino, P. Szabó, S. Brozio, P. J. Hall, C. I. Oseghale, M. K. Passarelli, S. J. Moug, R. A. Black and C. G. Wilson, *Int. J. Pharm.*, 2017, **531**, 67-79.

10. M.-C. Xia, Q. Zhan, L. Cai, J. Wu, L. Yang, S. Sun, H. Liang and Z. Li, *Microchem. J.*, 2021, **164**, 106026.

11. F. M. Green, I. S. Gilmore and M. P. Seah, *J. Am. Soc. Mass Spectrom.*, 2006, **17**, 514-523.

12. M. K. Passarelli, A. Pirkl, R. Moellers, D. Grinfeld, F. Kollmer, R. Havelund, C. F. Newman, P. S. Marshall, H. Arlinghaus and M. R. Alexander, *Nat. Methods*, 2017, **14**, 1175-1183.

13. W. He, M. K. Edney, S. M. Paine, R. L. Griffiths, D. J. Scurr, R. Rahman and D.-H. Kim, *Anal. Chem.*, 2023, **95**, 5994-6001.

14. W. Suvannapruk, M. K. Edney, D.-H. Kim, D. J. Scurr, A. M. Ghaemmaghami and M. R. Alexander, *Anal. Chem.*, 2022, **94**, 9389-9398.

15. F. Linke, J. E. Johnson, S. Kern, C. D. Bennett, A. Lourdusamy, D. Lea, S. C. Clifford, C. L. Merry, S. Stolnik and M. R. Alexander, *Acta Neuropathol. Commun.*, 2023, **11**, 1-18.

16. N. J. Starr, M. H. Khan, M. K. Edney, G. F. Trindade, S. Kern, A. Pirkl, M. Kleine-Boymann, C. Elms, M. M. O'Mahony and M. Bell, *Proc. Natl. Acad. Sci. U.S.A.*, 2022, **119**, e2114380119.

17. J. Zhang, J. Brown, D. J. Scurr, A. Bullen, K. MacLellan-Gibson, P. Williams, M. R. Alexander, K. R. Hardie, I. S. Gilmore and P. D. Rakowska, *Anal. Chem.*, 2020, **92**, 9008-9015.

18. C. L. Newell, J. L. Vorng, J. I. MacRae, I. S. Gilmore and A. P. Gould, *Angew. Chem., Int. Ed.*, 2020, **59**, 18194-18200.

19. A. M. Kotowska, G. F. Trindade, P. M. Mendes, P. M. Williams, J. W. Aylott, A. G. Shard, M. R. Alexander and D. J. Scurr, *Nat. Commun.*, 2020, **11**, 5832.

20. C. Kern, R. Jamous, T. El Khassawna and M. Rohnke, *Analyst*, 2022, **147**, 4141-4157.

21. P. McCrorie, J. Rowlinson, D. J. Scurr, M. Marlow and R. Rahman, *Pharmaceutics*, 2022, **14**, 571.

22. S. Aoyagi, Y. Kodama, M. K. Passarelli, J.-L. Vorng, T. Kawashima, K. Yoshikiyo, T. Yamamoto and I. S. Gilmore, *Anal. Chem.*, 2019, **91**, 14545-14551.

23. G. F. Trindade, S. Sul, J. Kim, R. Havelund, A. Eyres, S. Park, Y. Shin, H. J. Bae, Y. M. Sung, L. Matjacic, Y. Jung, J. Won, W. S. Jeon, H. Choi, H. S. Lee, J.-C. Lee, J.-H. Kim and I. S. Gilmore, *Nat. Commun.*, 2023, **14**, 8066.

24. J. Xia, N. Psychogios, N. Young and D. S. Wishart, *Nucleic Acids Res.*, 2009, **37**, W652-W660.

25. H. C. Hsu, C. Y. Liew, S.-P. Huang, S.-T. Tsai and C.-K. Ni, *Sci. Rep.*, 2018, **8**, 1-12.

26. H. Li, B. Bendiak, W. F. Siems, D. R. Gang and H. H. Hill, *Anal. Chem.*, 2013, **85**, 2760-2769.

27. D. Wan, H. Yang, C. Yan, F. Song, Z. Liu and S. Liu, *Talanta*, 2013, **115**, 870-875.

28. M. P. Seah, *J. Phys. Chem. C*, 2013, **117**, 12622-12632.

29. Y. Zhou, A. Franquet, V. Spampinato, A. Merkulov, M. R. Keenan, P. A. van der Heide, G. F. Trindade, W. Vandervorst and I. S. Gilmore, *J. Vac. Sci. Tech.*, 2024, **42**.

30. I. Gilmore, M. Keenan, G. Trindade, A. Pirkl, C. Newell, Y. Jin, K. Aizikov, J. Zhang, L. Matjacic, H. Arlinghaus, A. Eyres, R. Havelund, J. Bunch, A. P. Gould and A. Makarov, 2024, <https://doi.org/10.21203/rs.3.rs-3911895/v1>.