Online Supplementary Information

Multimodal Imaging of Drug and Excipients in Rat Lungs following an inhaled administration of controlled-release drug laden PLGA microparticles.

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Reference MS spectra were first generated for the drug to aid the SIMS (**Figure S1**) and MALDI MS imaging (**Figure S2**) interpretation. **Figure S1** for the SIMS analysis, shows the MS spectrum for the drug molecule, GSK1, across the mass range 30 – 600 Da. The molecular ions, used for monitoring GSK1, were present as $[M+H]^+$ ions at *m/z* 482.1 and *m/z* 484.1 from the two chlorine isotopes. Major fragment ion signals were also detected at lower mass values e.g. $C_3H_8N^+$ at *m/z* 58.1 and $C_5H_{12}N^+$ at m/z 84.1.

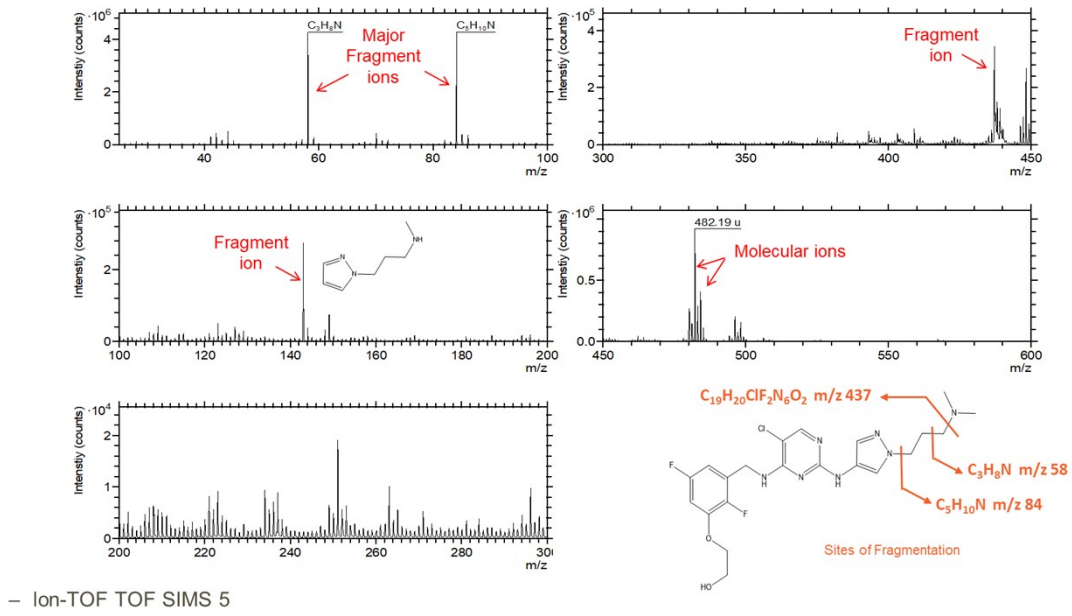

Figure S1 – Reference Mass Spectra: Positive polarity mass spectrum for GSK1 standard from TOF-SIMS.

SIMS spectrum for the drug molecule, GSK1, across the mass range 30 – 600 Da depicting the molecular ions, $[M+H]^+$, at *m/z* 482.1 and *m/z* 484.1 from the two chlorine isotopes. Fragment ions were also detected, with the most intense fragment ion signals being observed as $C_3H_8N^+$ at *m/z* 58.1 and $C_5H_{12}N^+$ at m/z 84.1. The proposed sites of fragmentation are also illustrated.

Figure S2 illustrates the MALDI MS spectrum (LIFT MSMS mode) obtained for a standard of GSK1. (A) In the mass range 20 - 580 Da, the most intense signals observed were for the fragment ions m/z 437.1 and 439.1 respectively. (B) The same spectrum zoomed in the range 420 – 480, reports the typical 3:1 signal ratio for a chlorine containing molecule or fragment. Therefore, for all MALDI MS imaging analysis (LIFT MSMS mode) all the displayed intensities/images were for the fragment ion at *m/z* 437.1.

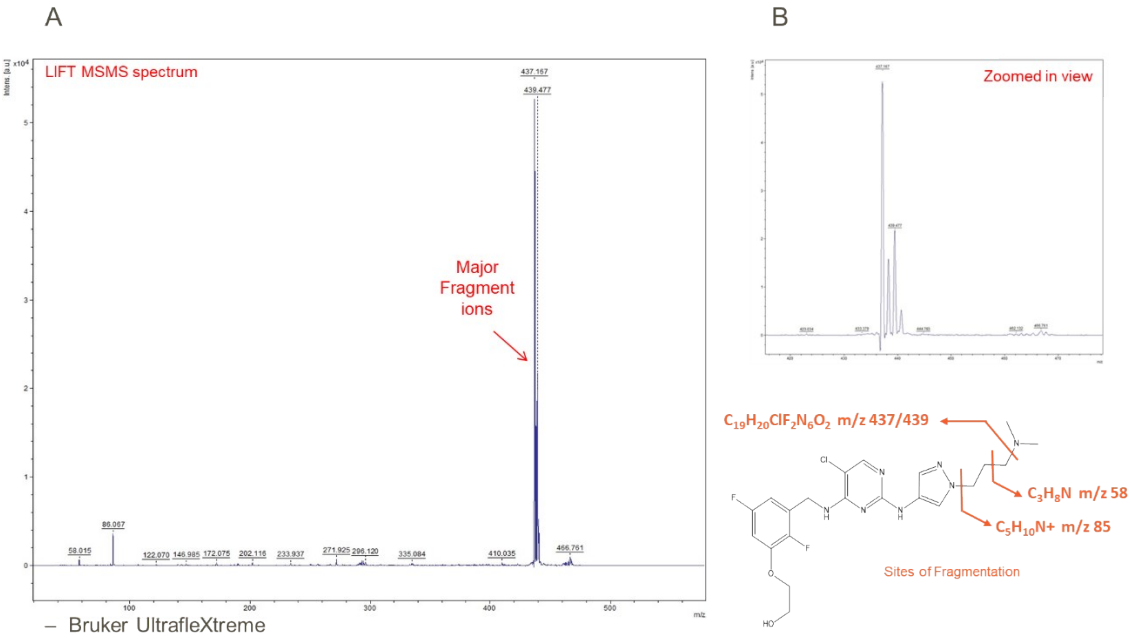

Figure S2 – Reference Mass Spectra: Positive polarity mass spectrum for GSK1 standard from MALDI MS (performed in LIFT MSMS mode).
A) In the mass range 20 - 580 Da, the most intense signals observed were for the fragment ions m/z 437.1 and 439.1 respectively. (B) The same spectrum zoomed in the range 420 – 480, shows the typical 3:1 signal ratio for the two isotopes of chlorine and a chlorine containing molecule or fragment. The proposed sites of fragmentation, including the one to generate the fragment ions at 437.1 and 439.1, are also illustrated.

TOF-SIMS analysis of the drug-laden particles was initially performed to determine if GSK1 could be detected in association with the microparticles. For this evaluation, drug-laden particles were sprinkled onto carbon tape mounted on a glass slide and analysed using the TOF.SIMS 5 instrument. **Figure S3** depicts the successful detection of the molecular ion, $[M+H]^+$ at *m/z* 482.1 and the fragment ion *m/z* 58.1, consistent with the presence of GSK1 associated with the microparticles.

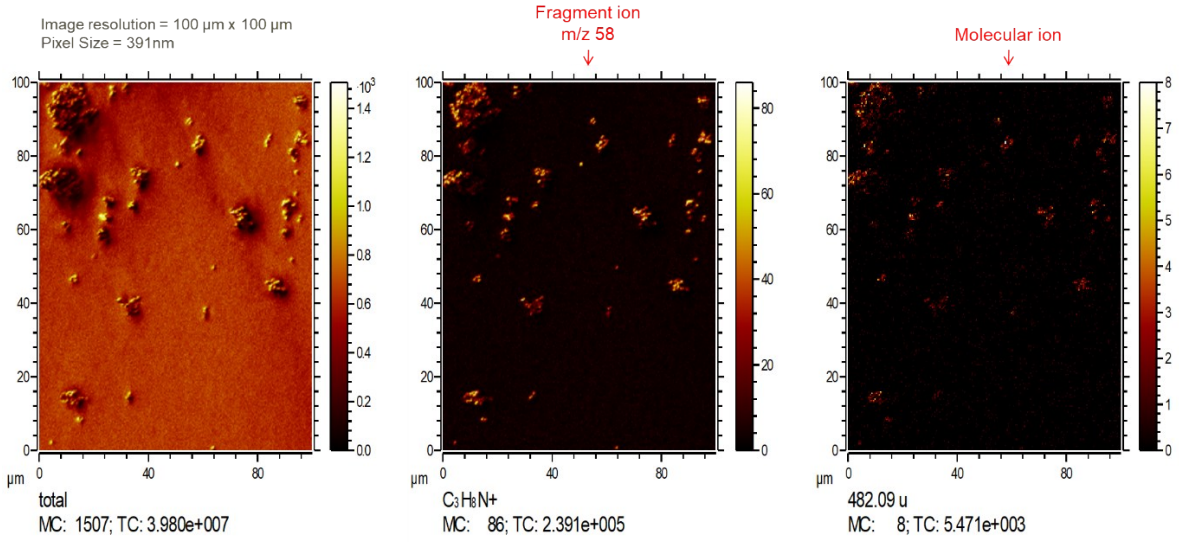

Figure S3 – TOF-SIMS images of drug-laden PRINT particles on carbon tape.
Positive secondary ion micrographs of drug-laden particles sprinkled on carbon tape analysed with an Ion-Tof, ToF.SIMS 5 instrument, equipped with a Bi_3^{2+} liquid metal ion gun and a 5 keV Ar_{1700}^{+} ion gun, showing the distribution of specific ion images obtained from the drug molecule, GSK1. Image size = 100 × 100 µm in 256 × 256 pixels = 391 nm/pixel in the original images. Maximum counts (mc) and total count (tc) is presented below each specific ion image. Images are represented in a colour scale ranging from black, to red to bright yellow as shown in the image colour scale bar.
(Left) The total ion image, indicating the presence of the drug-laden microparticles on the carbon tape. (Middle) The ion image generated for the fragment ion of GSK1, i.e. $C_3H_8N^+$ at *m/z* 58.1. (Right) The specific ion image for the $[M+H]^+$ ion at *m/z* 482.09 from GSK1. In the (Middle) and (Right) ion images the intense yellow spots indicate the presence of GSK1 associated with the microparticles.

TOF-SIMS depth profiling was used to analyse 11 microparticles (**Figure S4**) to elucidate the vertical distribution of GSK1 within the microparticles. In this experiment, the particles were gradually sputtered using the Ar_{1700}^{+} ion beam and analyzed using the Bi_3^{2+} beam. The average depth distribution of GSK1 within the 11 microparticles was determined to be reasonably homogenous throughout the microparticles, see **Figure S4**, i.e. GSK1 is not just located on the surface/periphery of the microparticles, as this would have given a U-shaped distribution profile.

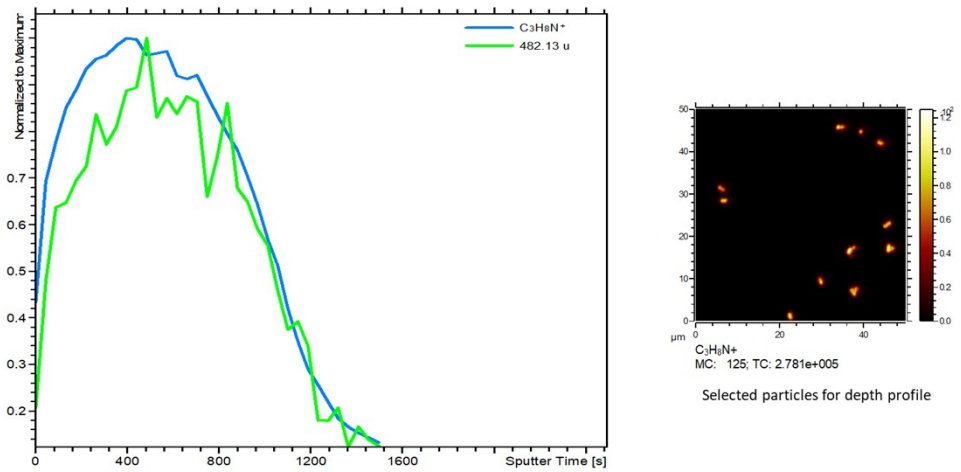

Figure S4 – TOF-SIMS depth profiles of drug-laden microparticles on tape. (Note: the depth profile corresponds to the ion count for all the eleven selected particles).
The eleven particles illustrated in the specific ion image (Right) obtained for the fragment ion of GSK1, i.e. $C_3H_8N^+$ at *m/z* 58.1, were gradually sputtered using the Ar_{1700}^+ ion beam and analyzed using the Bi_3^{2+} beam on the ToF.SIMS 5 instrument. From the profile (Left) the average depth distribution of GSK1 within the 11 microparticles was determined to be reasonably homogenous throughout the microparticles, i.e. GSK1 is not just located on the surface/periphery of the microparticles, as this would have given a U-shaped distribution profile.

Determination of specific ion species representative of the PLGA particles required identification of a characteristic secondary ion(s) of the microparticles ions against any background ions arising from the rat lung tissue. This was achieved by sprinkling the drug-laden particles onto an untreated rat lung section mounted on a glass slide and performing chemical and spatial analysis of the microparticles by TOF-SIMS. Prior to the TOF-SIMS measurements, secondary electron images (**Figure S5**) of the particles sprinkled on the tissue were produced to aid the identification and localisation of the particles on the tissue section.

Particle size – 900 nm x 1020 nm

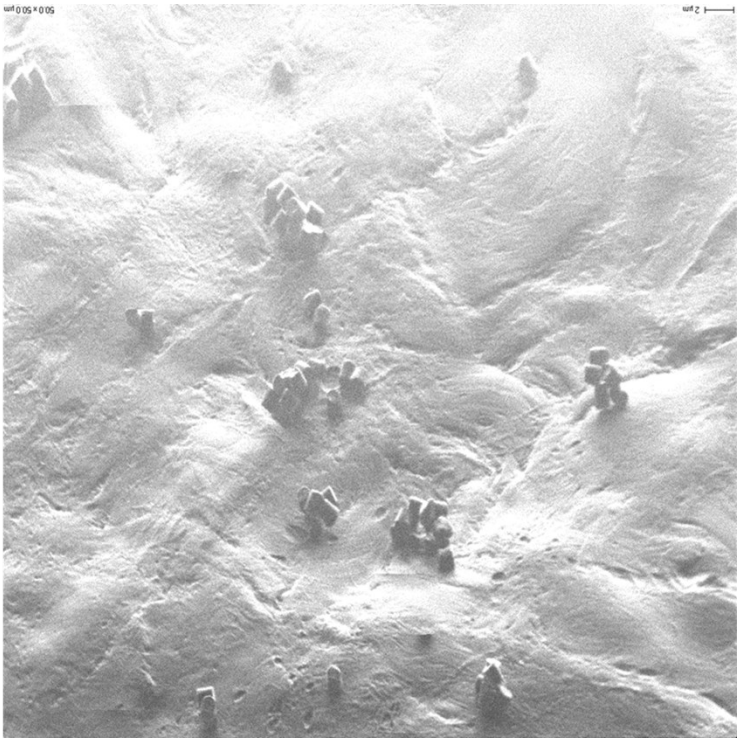

Figure S5 – Secondary electron images of drug-laden microparticles sprinkled onto a section of rat lung tissue.

The TOF-SIMS 5 instrument was utilized to obtain ion-induced secondary electron images of the microparticles using a 30 keV Ga^+ focused ion beam. The dimensions of the particle were typically 900 nm x 1020nm.