RESEARCH ARTICLE

A Comparison of DESI-MS and LC-MS for the Lipidomic Profiling of Human Cancer Tissue

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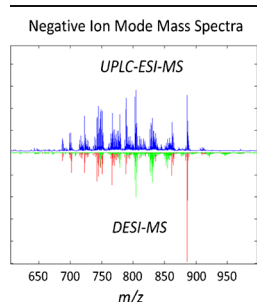
Abstract. In this study, we make a direct comparison between desorption electrospray ionization-mass spectrometry (DESI-MS) and ultraperformance liquid chromatography-electrospray ionization-mass spectrometry (UPLC-ESI-MS) platforms for the profiling of glycerophospholipid (GPL) species in esophageal cancer tissue. In particular, we studied the similarities and differences in the range of GPLs detected and the congruency of their relative abundances as detected by each analytical platform. The main differences between mass spectra of the two modalities were found to be associated with the variance in adduct formation of common GPLs, rather than the presence of different GPL species. Phosphatidylcholines as formate adducts in UPLC-ESI-MS accounted for the majority of differences in negative ion mode and alkali metal adducts of phosphatidylcholines in DESI-MS for positive ion mode. Comparison of the relative abundance of GPLs, normalized to a common peak, revealed a correlation coefficient of 0.70 ($P < 0.001$). The GPL profile detected by DESI-MS is congruent to UPLC-ESI-MS, which reaffirms the role of DESI-MS for lipidomic profiling and a potential premise for quantification.

Keywords: DESI-MS, UPLC-ESI-MS, Lipidomics, Lipids, Esophageal cancer

Introduction

Lipidomics is defined as the systems level analysis of lipids and factors that interact with lipid species [1]. The discipline has experienced significant advances in the last decade, allowing for unambiguous identification of specific lipids in complex biological matrices [2]. Liquid chromatography-mass spectrometry (LC-MS) has been the analytical technique of choice because of its capability of quantification and detection of isobaric lipid species [3, 4].

The LC-MS technique relies on prior extraction of lipids from a block of tissue with no histologic differentiation of tissue/cellular subtypes found within them. Therefore, data from a block is difficult to interpret because of its spatially averaged nature. For instance, in a cancer sample, there are carcinomatous cells and surrounding stromal cells, which would collectively contribute to the lipidomic profile of the specimen. Therefore, the routinely used homogenization without separation of cell types precludes any detailed comparison between carcinoma and its corresponding normal cell/tissue types. Tissue separation techniques such as laser capture micro-dissection have been used to mitigate this problem; however the process can be time-consuming.

Mass spectrometry imaging (MSI) provides a solution by allowing the analyst to map identified lipids to histological areas of interest [5, 6]. Two-dimensional MSI of tissue sections can be co-registered with its corresponding histologic image, and mass spectra representative of specific cell types can be extracted for the purpose of comparative analysis [7]. Desorption electrospray ionization-mass spectrometry (DESI-MS) [8, 9] is one of the common desorption ionization techniques widely used for MSI, together with matrix assisted laser

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desorption ionization (MALDI) [10, 11] and secondary ion mass spectrometry (SIMS) [12, 13]. DESI-MS requires minimal sample preparation and no matrix deposition; it can be performed under ambient conditions [14] and is nondestructive, which means that the analyzed tissue sections can directly undergo staining and subsequent histologic assessment for comparative analysis after MSI acquisition [15, 16]. In DESI-MS, a beam of high-velocity electrically charged solvent micro-droplets is directed at a surface of interest, where the impact of droplets results in the ionization of chemical species present on the surface [17–19].

Despite the obvious advantages of delivering spatially resolved information, DESI-MS, as with other MSI techniques, lacks the ability of quantification because of difficulties in introducing internal standards into the tissue matrix. Doping of solvent or covering the surface of the section with internal standard has limitations because of different ionization efficiencies of the dopant and species present in the sample. There is also a general skepticism for quantification by desorption ionization mass spectrometry, citing unknown ion suppression or matrix effects. Mass spectrometric imaging is therefore currently considered as a profiling method.

Histologic classification based on DESI-MS lipid profiling has been demonstrated in multiple studies [15, 20, 21], which suggest that corresponding lipid signal intensities have an acceptable dynamic range and are correlated with the actual tissue concentrations (i.e., the detected intensities are not random). Furthermore, in our previous study we have shown DESI-MS precision to have a co-efficient of variation (CV) of 20% [22], which is in line with FDA guidelines for chromatographic analytical techniques [23]. The low CV values indicated an acceptable level of precision and theoretical scope for relative quantification of lipids with MSI.

In the current study, a direct comparison of the analytical performance of lipid profiling is made between DESI-MS and UPLC-ESI-MS. The optimized systems utilizing different high resolution mass spectrometers, solvents, and analytical techniques were used to profile complex lipids in a set of esophageal cancer samples. Comparisons were made with regard to the raw spectral contents, ionization mechanisms, and the congruency of their relative abundances between DESI-MS and UPLC-ESI-MS.

Methods

Tissue Samples

Tumor samples from 10 patients with esophageal adenocarcinoma were retrieved for analysis. Written informed consent was obtained from the patients prior to sample retrieval, which was performed immediately after surgical removal of the tumor. The samples were snap-frozen and stored at –80°C. Several cryosections were cut at 15 µm from each of the 10 tumor samples for the purpose of DESI-MS and the remainder of each specimen was preprocessed for UPLC-ESI-MS.

DESI-MS

The cryosections of tumor samples were stored in closed containers at –80°C and were allowed to thaw at room temperature under nitrogen flow for a standardized 5 min prior to DESI-MS acquisition. DESI-MS analysis was performed using an Exactive Orbitrap MS (Thermo Fisher Scientific Inc., Bremen, Germany) controlled by XCalibur 2.1 software. The following instrumental parameters were used: nominal mass resolution of 100,000 (mass accuracy of <4 ppm), injection time was set to 1000 ms, mass to charge (m/z) range was 150–1000, capillary temperature was set to 250°C, capillary voltage was 50 V, tube lens voltage was –150 V, and skimmer voltage was –40 V. DESI-MS was performed in negative and positive ion modes on separate adjacent tissue sections.

Optimization of the DESI sprayer for the purpose of lipid analysis in human tissue has been reported by our group in a previous publication [22]. We used the same optimized DESI sprayer settings for the purpose of this analysis: sprayer to surface distance of 2 mm, sprayer to MS inlet capillary distance of 14 mm, solvent flow rate of 1.5 µL/min, gas flow rate of 7 bar, 90:10 v/v methanol/water solvent composition, electrospray potential of 5 kV, and an incidence angle of 75°.

After DESI-MS analysis, the tissue slides were stained with hematoxylin and eosin for the purpose of histologic analysis by an expert pathologist. The tissue sections were analyzed to confirm the presence of esophageal adenocarcinoma in all tissue sections.

UPLC-ESI-MS

The 10 tissue blocks were used for UPLC-ESI-MS-based lipidomic profiling. Lipid extraction was performed by homogenizing each tissue block and using methyl-t-butyl ether (MTBE) as a lipid solvent [24] (Online Resource 1).

Chromatography was performed on an Acquity UPLC-ESI-MS system (Waters Ltd., Elstree, UK) using a charged surface hybrid (CSH) C18 (1.7 µm, 2.1 × 100 mm) column (Waters Corporation, Milford, MA, USA) kept at 55°C. The separation conditions have been previously established for lipid profiling [25]. The mobile phases consisted of 0.1% formic acid and 10 mM ammonium formate in 60:40 (v:v) of acetonitrile (ACN)/water (A), and 0.1% formic acid and 10 mM ammonium formate in 90:10 (v:v) of isopropyl alcohol (IPA)/ACN (B) at a flow rate of 0.4 mL/min. Starting conditions were 60% A and 40% B and the gradient changed as follows: increase to 43% B over the first 2.0 min; increased to 50% B from 2.0 to 2.1 min; increase to 54% from 2.1 to 12.0 min; increase to 70% from 12.0 to 12.1 min; increase to 99% from 12.1 to 18.0 min, after which the solvent composition returned to starting conditions over 0.1 min, followed by re-equilibration for 1.9 min prior to the next injection. The injection volume was 5 and 10 µL for the positive and negative ion mode analysis, respectively.

Mass spectrometry was performed on a Xevo-G2 Q-TOF mass spectrometer (Waters Ltd., Manchester, UK) in both positive and negative ion electrospray ionization (ESI + and

ESI–) modes. The MS parameters were set as follows: capillary voltage 1.5 kV, sample cone voltage 30 V, source temperature 120°C, desolvation temperature 600°C, desolvation gas flow 1000 L/h, and cone gas flow 50 L/h. For mass accuracy, a LockSpray interface was used with a 2 ng/μL leucine enkephalin (m/z 556.2771 in ESI+, m/z 554.2615 in ESI−) solution at 10 μL/min for lock mass. Data were collected in centroid mode with a scan range of 50–2000 m/z, with lockmass scans collected every 30 s and averaged over 3 scans to perform mass correction.

To ensure system suitability and stability, a quality control (QC) sample was prepared by combining equal aliquots of all the samples and injected at regular intervals throughout the analytical run. This QC sample was also used to condition the column (10 injections) prior to the analysis of both the ESI+ and ESI– mode batches. Blank samples (i.e., injection of the reconstitution solvent) were also run to check the presence of artifact or contaminant peaks.

Data-dependent acquisition (DDA) and MS^E analysis of the QC sample were performed for structural elucidation. In the DDA automated MS/MS mode of operation, the quadrupole selects the precursor ion based on a preset threshold of the total ion intensity, whereas in the MS^E mode of operation both precursor and fragment spectra are acquired simultaneously during a single chromatographic run by alternating between low and high collision energies.

Data Importing and Preprocessing

Data from the UPLC-ESI-MS and MS^E analyses were converted from the Waters .raw file format to mzXML using MSConvert (Proteowizard) [26] prior to importing them into MATLAB environment (MathWorks, ver. R2014a). The resulting data is a common mass to charge and retention time array and 10 time-bin/intensity matrices. Further processing involved binning the data into a common mass to charge vector at 0.01 Da bin size. The DESI-MS data, acquired on a Thermo Exactive instrument, were first converted to imzML file format, uploaded to MATLAB, and then binned to a common mass range with 0.01 Da bin size.

The lipid profiles from the full tissue in both techniques were compared as the UPLC-ESI-MS does not have the advantage of separating the cancer-specific areas. The UPLC-ESI-MS data was summed across all scans for each single analysis and then a median spectrum across all 10 samples was obtained. For DESI-MS, a region of interest was drawn in a MATLAB script such that all spectra located 'on tissue' were separated from 'off tissue'. The off-tissue pixels were used to create a spectrum comprising of background peaks, which was then subtracted from the 'on tissue' spectra. These processed on-tissue spectra were then averaged over all pixels and a median spectrum of all 10 samples was created.

Principal Component Analysis (PCA)

Comparison of data from both modalities was performed by compiling a single data matrix of spectra binned to 0.1 Da and by normalization of each spectrum to the total ion current (TIC). Resulting data was then subjected to principal component analysis by using the (non-linear iterative partial least squares (NIPALS) algorithm. The resulting score and loadings were plotted in MATLAB with the first two principal components explaining approximately 80% and 90% of the variance, respectively, for positive and negative ion modalities.

Lipid Annotation of Differentiating Spectral Features

For UPLC-ESI-MS, additional refinements of lipid assignments were made combining the retention time information with the MS data. The accurate m/z values were searched against available online databases including Metlin [27] and Lipidmaps [28]. The isotopic distribution and fragmentation patterns in both positive and negative ion modes (from the DDA and MS^E analysis) were used to identify the lipid species to the mass spectrometrically attainable level. For DESI-MS, tentative lipid identities were determined using full resolution m/z values and searching the Lipidmaps structure database (LMSD) [27] for $[M − H]^−$ ions (negative ion mode) and $[M + H^+/Na^+/K^+]^+$ (positive ion mode) with a mass tolerance of 5 ppm.

Lipid Annotation and Intensity Correlation Analysis for Common Spectral Features

Median mass spectra of each of the 10 tissue samples in negative ion mode, obtained by DESI-MS and UPLC-ESI-MS were used for the purpose of lipid profile correlation analysis. The data was then reduced to the 600–1000 m/z range for the identification glycerophospholipids (GPL). Peaks present in less than 50% of the samples for each modality were considered to be noise and were removed. Automated peak annotation was carried out based on exact mass measurement data by comparing m/z values with theoretical values of an in-house built in-silico GPL database. While the mass deviation of large intensity peaks was ≤5 ppm (63% of identified lipids), the mass deviation of smaller intensity peaks is naturally larger. Therefore, a peak was considered matched if the mass deviation between measured and theoretical m/z value was below 10 ppm. Isotope peaks identified from the GPL database were excluded from analysis. If several database entries were matched for the same measured m/z value, the species with the smallest mass deviation was taken. Peak annotations were verified by tandem mass spectrometry. This resulted in 52 individual GPL species common to both analytical platforms (Online Resource 5a). Both UPLC-ESI-MS and DESI-MS datasets were normalized to the common peak of m/z 722.5 for inter-instrument comparison of GPL intensities with Spearman correlation analysis.
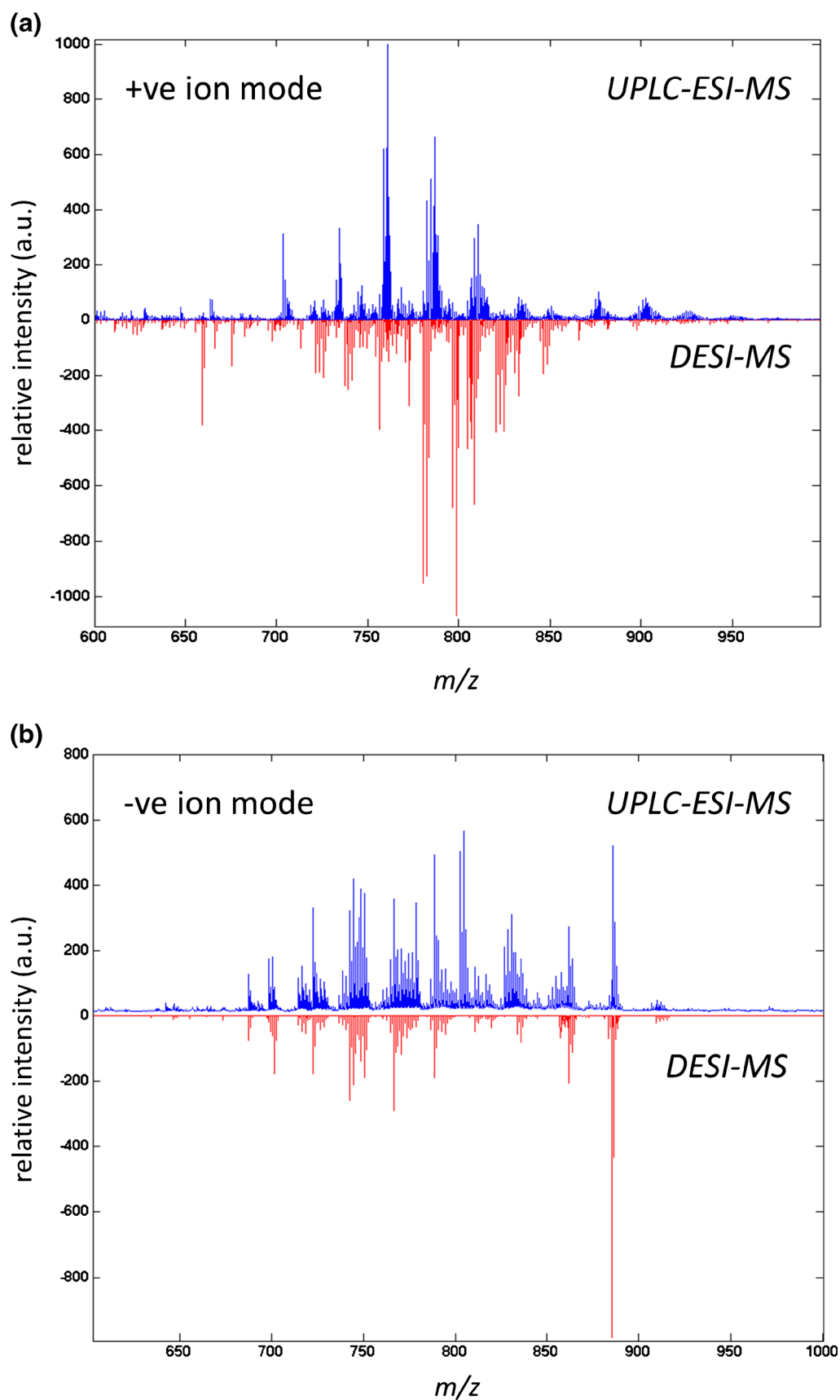
Figure 1. Median mass spectra of 10 esophageal cancer samples determined by UPLC-ESI-MS (blue) and DESI-MS (red) in positive and negative ion mode
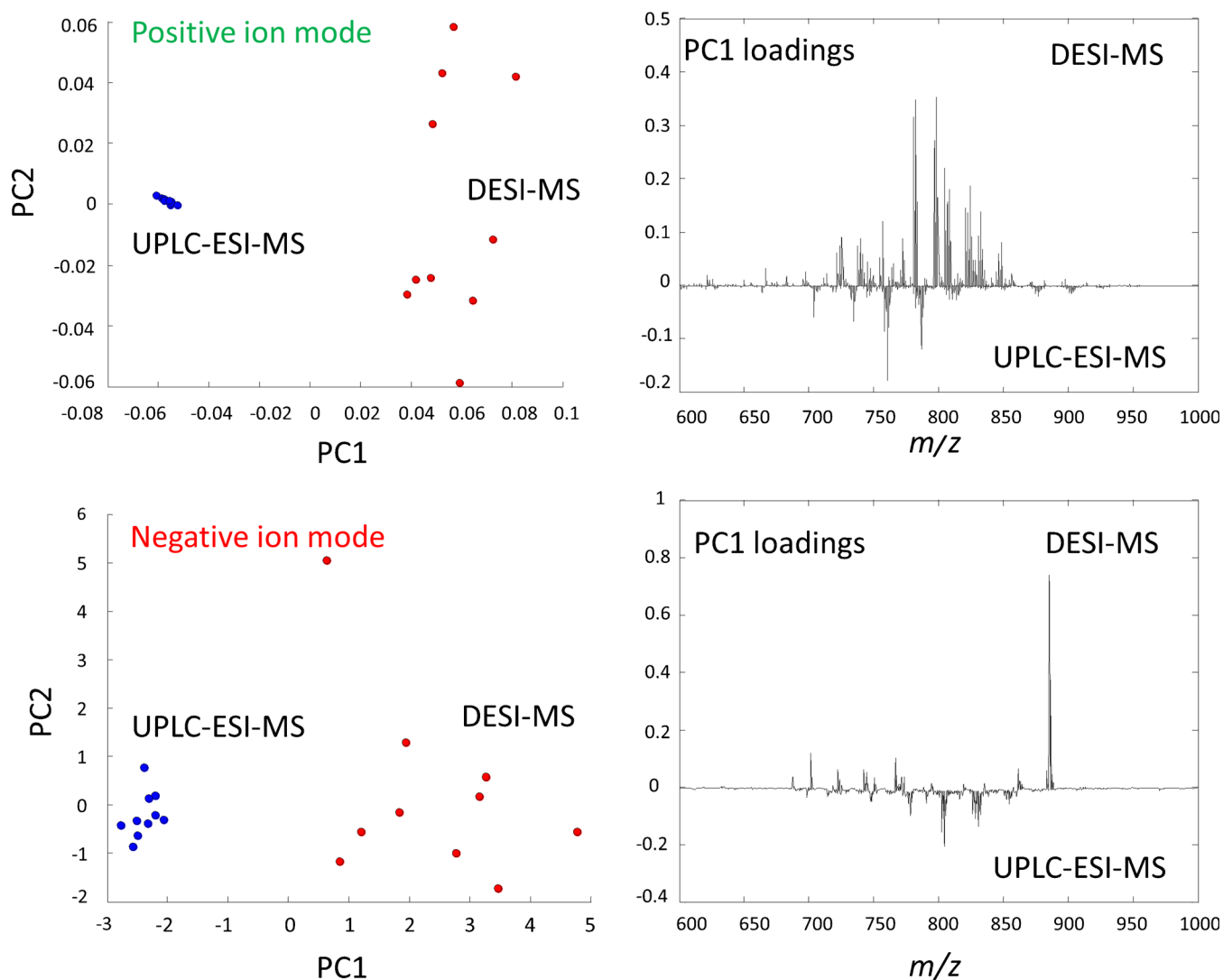
Figure 2. Loading plots to determine differences between mass spectra of 10 esophageal cancer samples analyzed by DESI-MS and UPLC-ESI-MS in positive and negative ion mode

Results and Discussion

Mass Spectra Comparison

Individual average mass spectra/chromatograms/MSI of each cancer sample, as determined by DESI-MS or UPLC-ESI-MS, is provided in Online Resource 2. The positive and negative ion median mass spectra of the 10 cancer samples were compared between DESI-MS and UPLC-ESI-MS (Figure 1). Mass spectral comparison was performed in the 600 to 1000 m/z range, to limit spectral features to those of complex lipid species. Visual comparison of the positive ion mode median spectra shows no obvious similarities. Median spectra in negative ion mode show similarities in terms of the distribution and relative abundance of spectral features, with some missing features in the 780–850 m/z range for DESI-MS.

In order to objectively compare the median mass spectra between the two analytical techniques, we performed a PCA to determine spectral features that were different between DESI-

MS and UPLC-ESI-MS (Figure 2). The data points of DESI-MS and UPLC-ESI-MS are separated across principal component 1, in both ion polarities. Separation is due to consistent variation in mass spectra between the two different analytical techniques. In addition, the UPLC-ESI-MS data was more reproducible than DESI-MS in both ion polarities. The better reproducibility of data in UPLC-ESI-MS can be related to superior analytical precision of the instrument or due to the averaging of data from a larger volume sample. Analysis of a larger volume of homogenized tissue may dilute more pronounced changes as a consequence of tumor heterogeneity, which can better revealed with DESI-MS tissue section analysis.

In positive ion mode DESI-MS, the data are clustered in two separate areas as a function of principal component 2. This inconsistency is explored further as part of the description of varying metal adducts formation in Figure 3. The data are more tightly clustered in negative ion mode DESI-MS with one sample outlier (TO30, refer to Supplementary Figure 1).
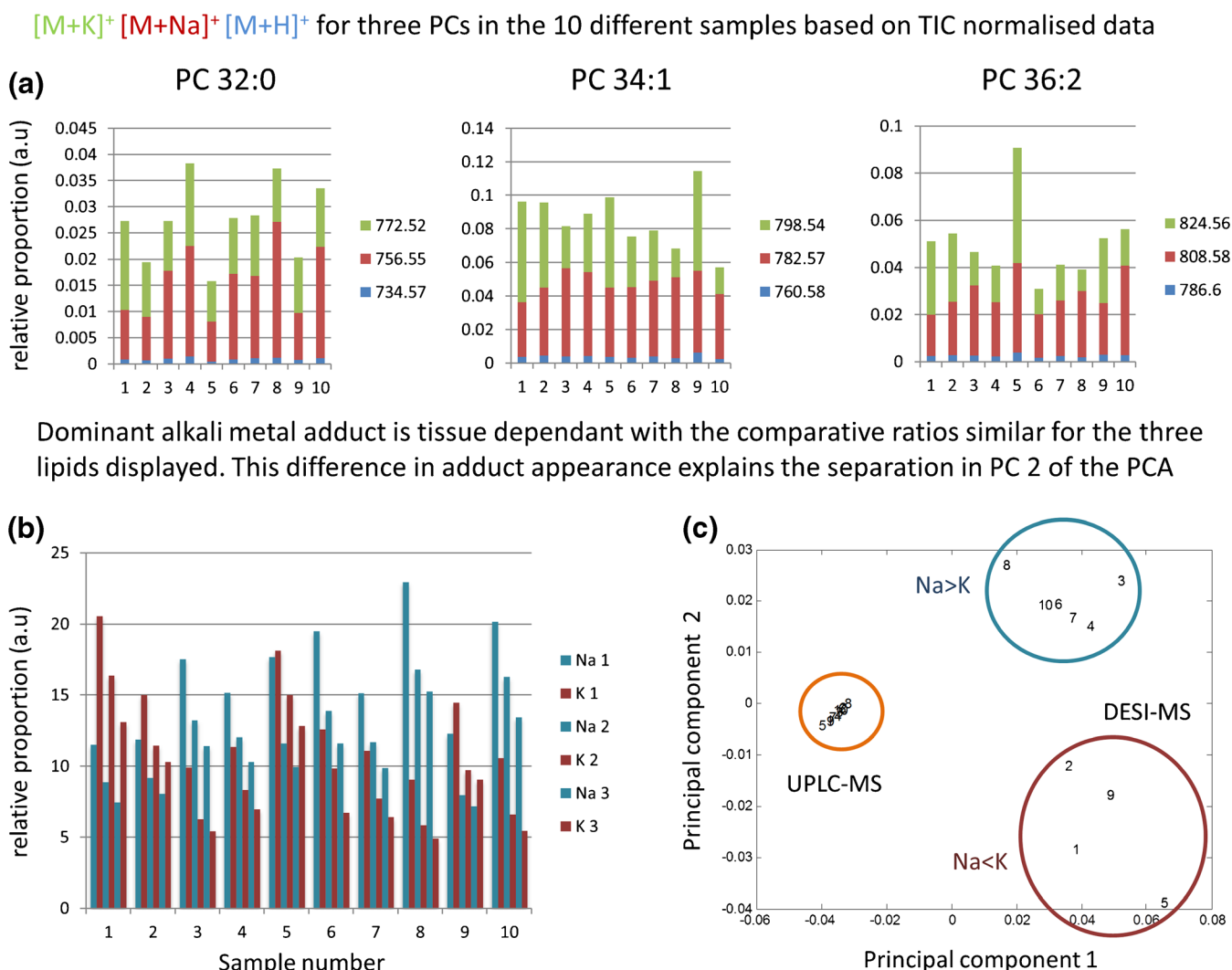
[M+K]⁺ [M+Na]⁺ [M+H]⁺ for three PCs in the 10 different samples based on TIC normalised data

Dominant alkali metal adduct is tissue dependant with the comparative ratios similar for the three lipids displayed. This difference in adduct appearance explains the separation in PC 2 of the PCA

Figure 3. Protonated versus sodium and potassium adduct patterns for positive ion DESI-MS. (**a**) [M + K]+, [M + Na]+, and [M + H]+ adduct ratios of three phosphatidylcholines (PC) in 10 different tumor samples based on Total Ion Current normalized data; (**b**) relative proportion of sodium (Na1-3) and potassium adducts(K1-3) of the 3 PCs in the 10 different samples; (**c**) principal component analysis of the positive ion mode data for UPLC-ESI-MS and DESI-MS, the latter showing separation of data points (sample number) in principal component 2 based on majority sodium adducts (red circle) and majority potassium adducts (circled blue)

Further interrogation of this outlying sample shows a unique base peak of *m/z* 861.550 between the mass range 600, and 1000, in comparison to the base peak of *m/z* 885.549 found in the other nine samples. The same peak is also prominent in the UPLC-ESI-MS analysis of this sample but does not dominate the spectra and, therefore, has less influence on the PCA.

From the loading plots of Figure 2, peaks with significant weightings either positively or negatively were tentatively identified based on accurate mass and MS/MS (Online Resource 3). The data in Online Resource 3 shows that the differentiating spectral features found between median mass spectra from the two techniques can be accounted for by formation of varying ion adducts. The majority of lipids give Na⁺/K⁺ adducts in positive ion mode DESI-MS. This is associated with the high physiological concentration of these salts in unprepared human tissue. Identical phosphatidylcholine species were found exclusively as protonated molecular ions in

UPLC-ESI-MS because of the chromatographic separation performed in an alkali-free acidic environment. In addition, the presence of ammonium in the carrying solvent allows for formation of ammonium adducts of triglycerides, which are absent in DESI-MS.

Figure 3a shows the relative abundance of [M + H]⁺, K⁺, and Na⁺ adducts of three different lipids across all 10 cancer samples in positive ion mode DESI-MS. There was a negligible contribution of [M + H]⁺ ions and varying abundance of Na⁺ and K⁺ adducts observed. However, the relative quantities of the alkali metal adducts are not entirely random. As it is shown in Figure 3b, tumor samples 1, 2, 5, and 9 have more abundant Na⁺ adducts across all three lipids. A similar pattern is discovered for K⁺ adducts across the remaining cancer samples. This phenomenon of varying adduct formation in samples of the same tissue type from different humans is difficult to explain as physiological concentrations of sodium and potassium are

theoretically constant in human tissue. Furthermore, this phenomenon is the main determinant of the separation in principal component 2 of the PCA (Figure 3c). These findings suggest that multivariate analysis of raw positive ion data in DESI-MS, for multiple samples in biomarker discovery experiments are systematically flawed unless the spectral features are aligned into a common mass channel (i.e., the spectra are de-isotoped and de-adducted prior to multivariate statistical analysis). Therefore difference will not exist due to varying adduct formation but rather the spectral features that define that sample. Similar patterns of metal adduct formation have previously been identified in MALDI lipidomic experiments, highlighting the challenges of comparative analysis and absolute lipid quantification [29, 30].

In negative ion mode, the majority of spectral features present in DESI-MS are also present in the UPLC-ESI-MS datasets. The additional peaks found in the negative ion mode UPLC-ESI-MS can be accounted for by the presence of formate (FA) adducts of phosphatidylcholines [31, 32], not normally seen in negative ion spectra for DESI-MS using conventional methanol:water solvent. Online Resource 4 shows and example of this, demonstrating the MS/MS data and chromatograms for PC (36:3), which is detected as $[M + H]^+$ in positive ion mode and $[M + FA - H]^-$ in negative ion mode.

The main differences seen in the spectral profiles obtained by DESI-MS and UPLC-ESI-MS are due to differences in the types of ionic species rather than the presence or absence of lipid species. Although mass analyzers using different physical principles were used resulting in completely different dynamic ranges, the resulting spectra were surprisingly similar. We therefore performed in-silico conversion of the DESI-MS median spectra to follow the same ionization/adduct formation rules as the UPLC-ESI-MS median spectrum for the purpose of direct mass spectral comparison. In positive ion mode, this was performed by using an algorithm that searches for peaks that are 21.9818 Da, 38.0904 Da, or 16.1086 Da greater than another in the DESI-MS spectrum (H→Na, H→K, Na→K) and aligning the intensity values as a total sum such that the resulting peaks are an estimate of $[M + H]^+$ ions only.

For the negative ion mode, the *m/z* values of the corresponding UPLC-ESI-MS positive ion spectral peaks, specific to phosphatidylcholines, were increased by 43.9861 Da (mimicking a $[M + FA - H]^-$ ion) and superimposed over the DESI negative ion spectra. Figure 4 shows distinct similarities in the distribution and relative abundance of spectral features in the negative and positive ion mode median spectra of both modalities.

The majority of the observed differences in adduct formation were presumed to be due to different composition of solvent used in case of the two analytical techniques. To investigate this further, we added 10 mM ammonium formate to the solvent (90:10 v/v methanol:water) of the DESI-MS to see if we could replicate similar mass spectra as found in UPLC-ESI-MS. As shown in Figure 5, the addition of ammonium formate to the DESI solvent resulted in the appearance of phosphatidylcholine formate adducts in negative ion mode as seen in the UPLC-ESI-MS spectrum. In positive ion mode, the
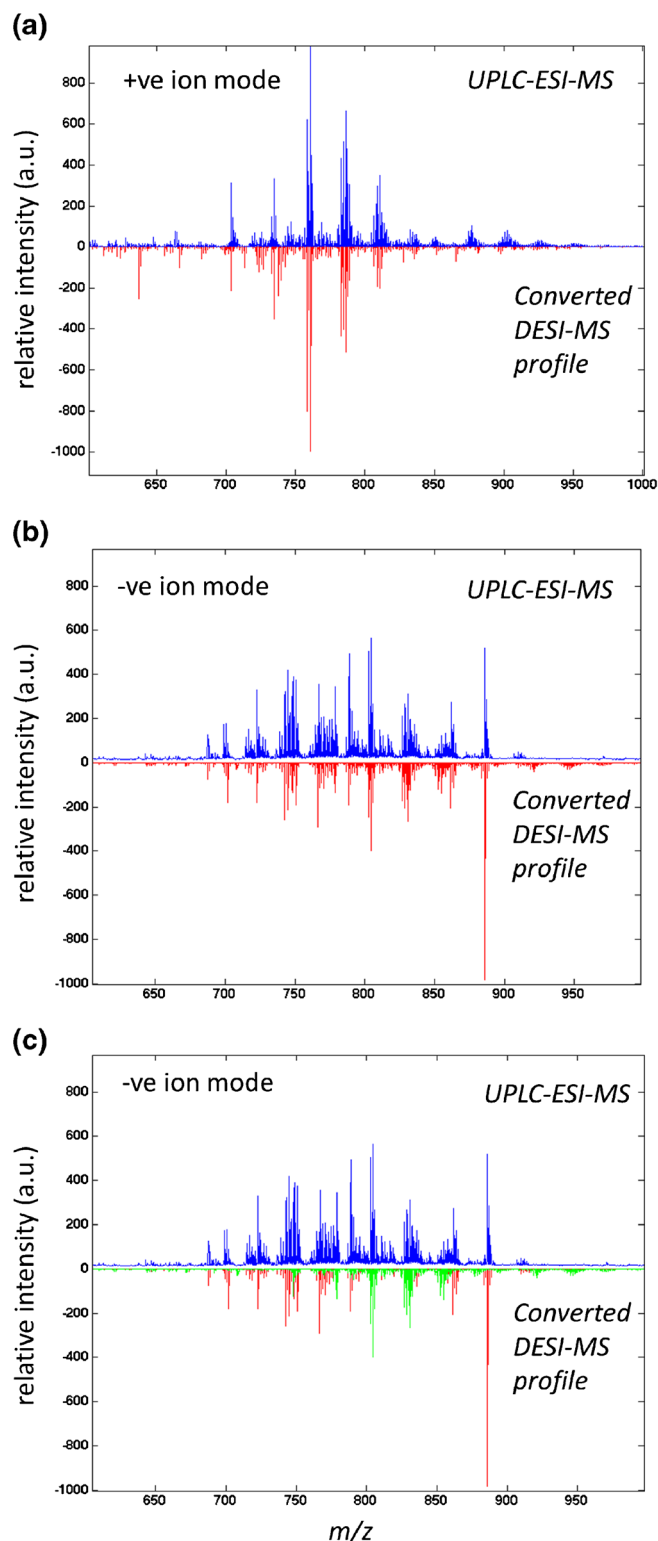

Figure 4. In-silico conversion of DESI-MS data. (**a**) In-silico correction of positive ion mode DESI-MS spectrum by identifying and shifting both [M + Na] + and [M + K] + ions into [M + H] + mass channels. (**b**) In-silico correction of negative ion mode DESI-MS by superimposing the new positive ion mode spectrum shifted by 43.99 Da to replicate formate adducts [shown in green, (**c**)]
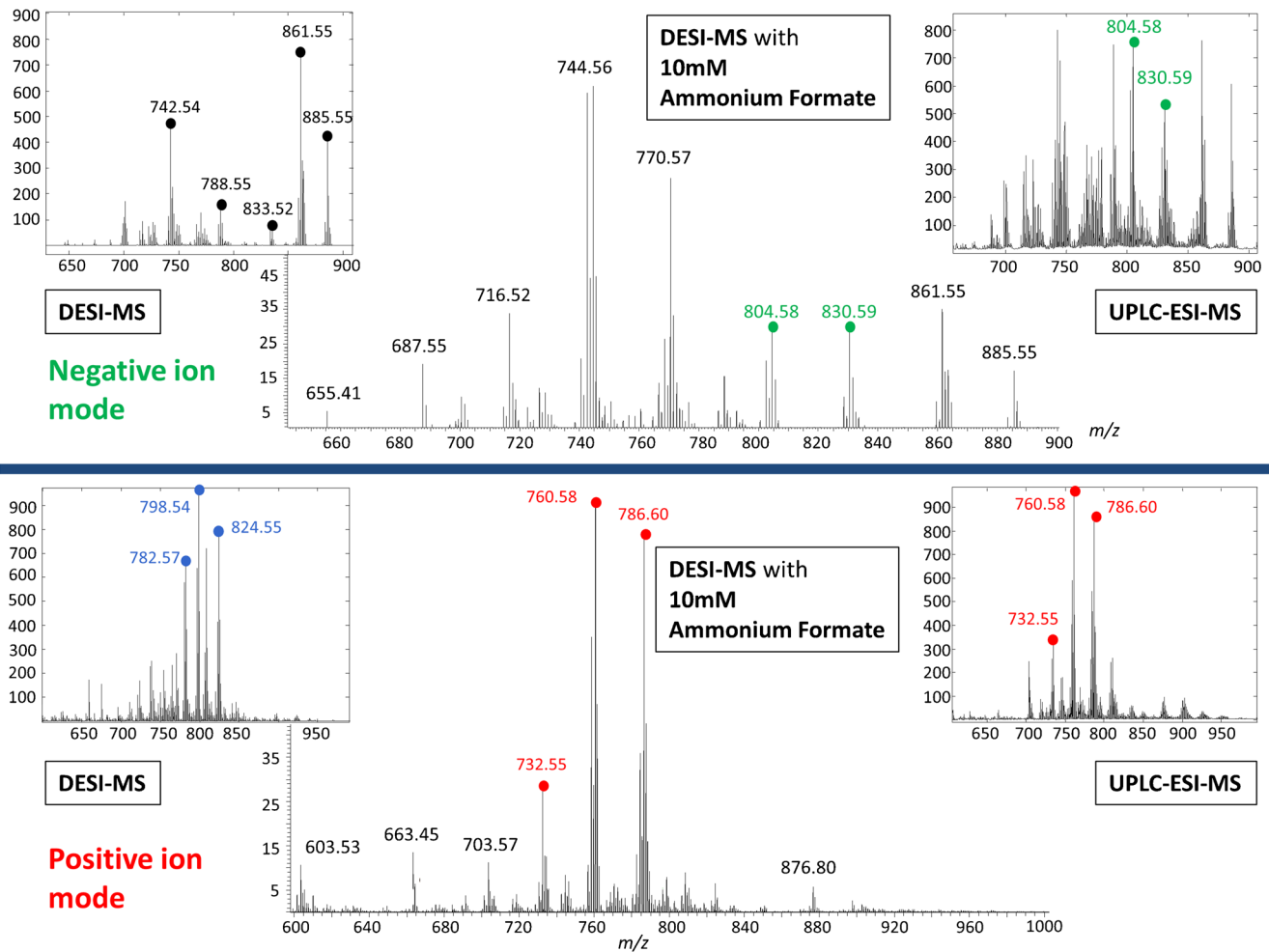
Figure 5. Alteration of DESI-MS spectrum with the addition of ammonium-formate to the solvent. In negative mode, there is the appearance of PCs with formate adducts (green). In positive mode, the spectral features have changed from alkali metal adducts (blue) to [M + H]+ (red)

dominant ionic species type becomes $[M + H]^+$, similar to the UPLC-ESI-MS data. The initial experiments were not conducted with ammonium formate as the standard DESI-MS setup utilizes a methanol:water solvent, which has been shown to produce more stable and higher signal intensity [32]. In addition, ionic dopants are not used in the majority of studies because of the much lower onset voltage of corona discharge. Nevertheless, if the signal from a lipid is split over three mass channels as is the case with methanol:water solvents in positive ion mode, the signal-to-noise for that lipid could be worse even if the overall signal is better in the absence of a dopant. The use of a dopant to mitigate the problem of metal adducts formation in positive ion mode DESI-MS has been previously described in a study using sodium acetate [30, 33]. In addition, washing the tissue sample with ammonium acetate prior to analysis has been shown to desalt the sample, remove contaminants, and improve spectral quality in MALDI-MS experiments [34].

Correlation Analysis

A correlation analysis of lipid intensities detected by DESI-MS and UPLC-ESI-MS was performed after normalization of each
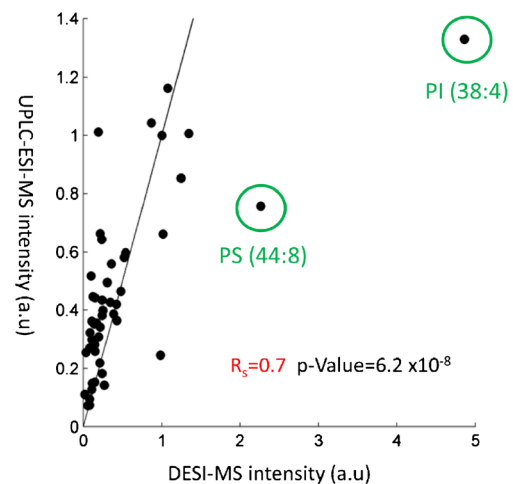

Figure 6. Correlation analysis of DESI-MS versus UPLC-ESI-MS glycerophospholipid intensities. Black line represents perfect linear correlation. R = Spearman's correlation co-efficient; PE = phophatidylethanolamines; PA = phosphatidic acid; PG = phosphatidylglycerols; PS = phosphatidylserines; PI = phosphatidylinositols

dataset to the common peak of m/z 722.5. Figure 6 shows the results of the correlation analysis between the profiles of commonly identified complex lipid ions between both analytical platforms in negative ion mode. The Spearman's correlation of 0.7 ($P = 6.2 \times 10^{-8}$) means that the relative abundance of the lipids as determined by both analytical platforms is similar. Therefore, the lipid profiles carry comparable information despite the analysis being performed with different analytical platforms, solvent systems, and mass spectrometers.

Several outliers were observed in the results of correlation analysis, which we investigated further by re-analyzing the identified lipids group patterns separately. Online Resource 5 shows the correlation analysis and resulting coefficients for phophatidylethanolamines (PE), phosphatidylserines (PS), and phosphatidylinositols (PI), which were above >0.8 and statistically significant. Analysis of phosphatidylglycerols (PG) and phosphatidic acids (PA) did not reach significant levels because of the relatively small number of data points. This analysis showed that outliers were not class-dependent and that the most significant outlier is PI (38:4). This lipid is invariably the most abundant ion in all human tissue analysis using DESI-MS and it likely has unique ionization properties, which cause it to be a significant outlier from the rest of our analysis.

This comparison could not be achieved in positive ion mode as the UPLC-ESI-MS data was composed of $[M + H]^+$ adducts, whilst the DESI-MS data was composed of varying proportions of $[M + H]^+$, $[M + K]^+$, and $[M + Na]^+$ adducts of the same lipids. Despite realigning the multiple adducts into their respective $[M + H]^+$ mass channels, in DESI-MS there were still significant problems with assigning appropriate intensity values for the peaks. Owing to the possibility of varying ionization/utilization efficiencies of different metal adducts, simple summation of their individual intensities did not produce reliable cumulative intensity values for the aligned $[M + H]+$ peaks. Furthermore, this method of adduct realignment would not account for the possibility of isobars.

Despite similarities in lipid information recovery, the analytical techniques of DESI-MS and UPLC-ESI-MS have specific qualities that differentiate their use in lipidomic applications. Targeted lipidomic quantification with the use of internal standards can only be achieved with the latter technique because of the difficulties in introducing internal standards into organic tissue matrices and at uniform concentrations for DESI-MS. Therefore, quantitative analysis of specific lipids is best performed with UPLC-ESI-MS. In addition, chromatographic separation of lipids with UPLC-ESI-MS allows for the identification of isobars, which are molecules of the same nominal mass but different exact masses [35]. With accurate mass measurement, shotgun lipidomic techniques such as DESI-MS can also identify isobars but cannot determine isomeric lipid, which have the same elemental composition but different atomic configuration. LC-MS helps with some forms of isomerism but not all. In this study, out of the common spectral features identified between UPLC-ESI-MS and DESI-MS in the $600 - 1000$ m/z range, 36% had one or more isobars

as evidenced by multiple elution peaks for the same m/z value as per the UPLC-ESI-MS data (Online Resource 6). In addition, the process of UPLC-ESI-MS can be automated for the analysis of multiple samples with better reproducibility of data in comparison to DESI-MS.

Notwithstanding the obvious advantages of UPLC-ESI-MS, we must consider the limitations of lipid extraction from blocks of tissue. For instance, tissue blocks of cancer are composed of the carcinoma cells and stromal cells. Without separation of these components with techniques, such as laser capture microdissection [36], the lipid extract will be derived from multiple tissue types and will not be an accurate representation of cancer cells. An advantage of DESI-MS is the capability of tissue-specific data extraction after the analysis has been performed. Online Resource 7 demonstrates the adenocarcinoma and stromal component of two tumor samples that have distinct mass spectral profiles. Tissue-specific data extraction with MSI creates new possibilities for accurate lipid ratio comparisons and also for the analysis of the tumor micro-environment.

Conclusion

The main differences between mass spectra of the two modalities were found to be associated with the variation in adducts of complex lipids rather than the presence of different complex lipids. Comparison of the relative abundance of complex lipids found in negative ion mode, normalized to a common peak, determined a correlation coefficient of 0.70 ($P < 0.001$). The data collected by DESI-MS is comparable to that of UPLC-ESI-MS in terms of the range of detected lipid species and their associated relative intensities. Future studies may utilize the advantage of cell/tissue-specific data extraction of DESI-MS in combination with the quantification capabilities of UPLC-ESI-MS for improved lipidomic information recovery. The compatibility of the data would be reliant on congruent mass spectra, which has been shown in the analysis of 10 cancer samples in this study.

Acknowledgments

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