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Inter-platform assessment of performance of high-throughput desorption electrospray ionization mass spectrometry

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ABSTRACT

High throughput analysis is increasingly relevant in both industry and academia, with applications reaching from enzymology and drug discovery to organic reaction optimization and diagnostics. Currently, chromatography-free mass spectrometry (MS) techniques have acquired an important role in this field due to their inherent speed, versatility, and chemical specificity. Desorption electrospray ionization (DESI) MS is one of these approaches, which allows for direct analysis of complex samples in the ambient environment, with throughputs better than 1 sample per second and no need for sample treatment. Here we assess the evolution of the high throughput DESI-MS analytical performance from the early DESI source developed by Prosolia Inc. to the recently commercialized version of Waters Corporation. The evaluation was carried out through both quantitative and qualitative analysis of biologically relevant compounds including metabolites, peptides, lipids and pharmaceuticals. Our results indicate that both platforms are successful at the direct analysis of these species even from complex matrices such as bioassay buffers. However, the newest iteration of the DESI stage, when combined with a quadrupole time-of-flight (Q-ToF) instrument, provides higher sensitivity (1-3 orders of magnitude lower limits of detection) and reproducibility (ca. 10% average reduction in coefficients of variation for quantitation using an internal standard). The cases explored in this study also showcase the broad applicability of high throughput DESI-MS for bioanalysis and quality control.

Introduction

High throughput analysis is one basis of modern pharmaceutical discovery and development.[1] For decades the use of high throughput approaches (particularly optical and radiometric methods) to determine the activity of large sets of compounds against biological targets (e.g. inhibition of an enzyme, binding with a receptor) has been essential for the identification of lead candidates in drug discovery campaigns [1,2]. More recently, high throughput experimentation has extended to the organic synthesis realm [2,3]. The rapid exploration of reaction conditions (e.g. temperature, stoichiometry, catalysts, pH, solvents), as well as reactant combinations, allow for an efficient exploration of the chemical reactivity space, offering an alternative paradigm to classical factor-by-factor process development [2,4]. Consequently, many high throughput technologies have been developed and implemented, in most cases pushing towards sample miniaturization, broader applicability, and higher analysis speeds.[4–6]

Mass spectrometry (MS) is acquiring a role in high throughput analysis methodologies, as it allows high analysis speeds (mostly sampling-time limited) together with high versatility, sensitivity and chemical specificity.[7–9] Initially coupled to chromatography, which limits speeds even when using novel ultrafast approaches, mass spectrometry is currently a stand-alone technology for the exploration of high density arrays [7,8]. Multiple methodologies have been developed under this chromatography-free category, including approaches based on matrix assisted laser ionization (MALDI) [10,11], rapid solid phase extraction (SPE),[12] acoustic droplet ejection (ADE) [13,14], and desorption electrospray ionization (DESI) [15,16]. In all cases, MS-based high throughput analytical platforms must be able to handle complex matrices without a significant compromise in sensitivity or reproducibility, or a major trade-off between analysis speed and data quality [8, 17].

DESI-MS in particular has demonstrated promise as an imaging technique [18–20] and as an ambient ionization analysis method. [21–25] In the context of high throughput analysis, it has proven useful for both the rapid screening of organic reactions to identify reactivity profiles [26–28] and so optimize synthetic conditions for scale-up [29, 30], as well as in the development of label-free biological assays.[31]

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The inherent contactless nature of the DESI spray process conveys tolerance to high salt concentrations [32] making DESI ideal for high throughput analysis of complex samples without any sample preparation or dilution, and yielding reproducible quantitative results from nanoliter-sized samples with sub-second analysis times.[31] However, as with any methodology, technological advancements can lead to improvements in analytical performance. In this case, improvements in the DESI ion source (the least efficient of the steps in MS) might provide higher sensitivity and reproducibility for high throughput analysis.

First described in 2004,[33] DESI was initially commercialized by Prosolia Inc., until 2018 when Waters Corporation acquired exclusive rights. Each company developed its own ion source, with the newest Waters iteration being released in 2021 (**Figure 1**). In this study we assess the evolution in the analytical performance from the Prosolia DESI stage, a product no longer in the market but that has been extensively used for high throughput experimentation as part of the Purdue Make It system (an automated and integrated system for high throughput synthesis, screening and bioanalysis) [15,34], to the Waters DESI stage, equipped with a new prototype sprayer and transfer capillary. This inter-platform assessment, which was focused on the progress of the DESI technology, was carried out on different types of small molecules (metabolites, peptides, lipids, pharmaceuticals). At the same time, this study seeks to demonstrate the power of automated DESI-MS for rapid and small-scale high throughput analysis, using sub-nanogram sample amounts directly from complex matrices, illustrating as well the potential of this technique in multiple applications, including bioanalysis, biological assays, and quality control.

Materials and methods

Chemicals

All buffer reagents as well as pregnenolone sulfate were purchased from Sigma Aldrich (St. Louis, MO, USA). Dehydroepiandrosterone (DHEA) sulfate was acquired from Cayman Chemical (Ann Arbor, MI, USA). Leucine enkephalin (Leu-enkephalin) was obtained from Bachem (Torrance, CA, USA). The small molecule library (HTS Library for Drug Discovery L5000-307) was purchased from Selleck Chemicals (Houston, TX, USA). The EquiSPLASH™ LIPIDOMIX® Quantitative Mass Spectrometry Internal Standard from Avanti Polar Lipids (Birmingham, AL, USA) was used for the lipid analysis. Methanol Optima® LC-MS grade from Fisher Scientific (Fair Lawn, NJ, USA) and water LC-MS Chromasolv™ grade from Honeywell Riedel-de Haën (Seelze, Germany) were used in all the experiments.

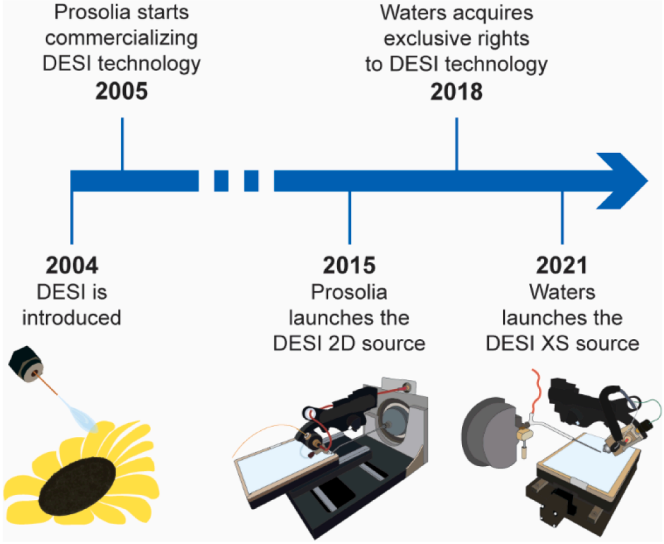

Figure 1. Brief timeline of the DESI technology since its introduction

High Throughput DESI-MS analysis

The operation of the Purdue Make It high throughput DESI-MS platform has been previously described in depth.[16] Briefly, a Biomek i7 fluidic handling robot (Beckman Coulter, Brea, CA, USA) is used to transfer 50-nL samples from a 384 well plate onto a DESI plate. This transfer is carried out using a slotted floating pin-tool (V&P Scientific, San Diego, CA). The DESI plate consists of a glass slide with the same footprint as a well plate (Abrisa Technologies, Santa Paula, CA, USA), coated with a Zytex® G115 porous PTFE membrane (Saint-Gobain, Wayne, NJ, USA). The membrane is attached to the glass slide by a thin layer of Scotch® Spray Mount™ Repositionable Adhesive (3M, St. Paul, MN). Up to 6,144 samples can be spotted on a single DESI plate by offsetting the pinning position in sixteen different ways, keeping a center-to-center distance of 1.125 mm between each pair of neighboring spots at the highest density. In this study, only four positions were pinned for the analyses, in order to obtain four instrumental replicates for each sample.

After pinning, the slide is transferred, either manually or automatically (using a selective compliance articulated robotic arm, PF3400, Precise Automation, San Diego, CA, USA) to a DESI stage for MS analysis. Custom written software is used to perform the analysis of the deposited samples in a *spot-to-spot* manner after triangulation of all sample positions using calibration marks pinned at three corners of the plate. The software synchronizes the mass spectral acquisition with the stage movement, which it controls during the entire period of analysis. The effective analysis time of the system (i.e. the time the stage is on each spot) is ca. 300 ms. The overall throughput, which depends on the density of spots in the plate, is <1 s/sample even at the lowest density (384 samples/plate). Mass spectral data is automatically processed using the same custom software together with a plate layout file input by the user. This file contains the layout of the samples in the initial 384 well plate, the pinning positions utilized, and the *m/z* values of interest. A list of the intensities for these *m/z* values in each spot is recorded and then processed further using custom MATLAB (Mathworks, Natick, MA, USA) scripts to calculate signal-to-noise ratios, analyte-to-standard ratios or simply average results across replicates. For this study data visualization was carried out using OriginPro 2021 (OriginLab Corporation, Northampton, MA, USA) from the MATLAB output.

We assessed the evolution of the high throughput DESI-MS analytical performance using two systems: (i) a Prosolia DESI 2D stage (Zionsville, IN, USA) coupled to a LTQ ion trap mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA), and (ii) a Waters DESI 2D stage equipped with a Waters prototype sprayer (identical to the Waters DESI XS stage sprayer) and a prototype heated transfer capillary, coupled to a Synapt G2-Si quadrupole time-of-flight mass spectrometer (Waters Corporation, Milford, MA, USA). For the sake of simplicity, these two systems will be referred to as the *Prosolia stage* and the *Waters stage* throughout the manuscript.

In both platforms, the analysis conditions were kept identical for the different cases evaluated. Methanol was used as DESI spray solvent in all cases. The *Prosolia stage* used a flow rate of 2.75 μL/min and 150 psi of nitrogen as nebulizing gas. DESI was performed using ± 4.5 kV, with the transfer capillary temperature set at 300°C. In positive ion mode the capillary voltage was set to 38 V and the tube lens voltage to 65 V, whereas in negative ion mode these values were -24 V and -70 V, respectively. Analysis was performed with the automatic gain control on, with a maximum injection time of 150 ms. The *Waters stage* was operated with a flow rate of 2 μL/min and 15 psi of nitrogen as nebulizing gas. DESI was performed using ± 0.65 kV, with the heated transfer capillary set to 400°C and the source temperature to 150°C. The sampling cone voltage was 40 V in both polarities. The scan time was set to 100 ms. In both stages, the DESI spray angle was kept at 55°, the sprayer being 2 - 3 mm away from the surface and 4 - 6 mm away from the

capillary inlet, which was in turn allowed to gently touch the analysis surface. In the case of the *Prosolia stage* the emitter protrusion was ca. 1 mm.

Both mass spectrometers were fully calibrated following manufacturer guidelines prior to the analyses for this study. The DESI-MS signal was checked before the analysis of each plate using colored Sharpie® Permanent Markers (Newell Brands, Atlanta, GA, USA) on a DESI plate, as suggested by the user operation manuals of both DESI sources. This test is based on the detection of several species from the dyes within each marker, most commonly Rhodamine 6G (M^+ *m/z* 443) in the red Sharpie®.

Cases studied

Four cases were studied for this inter-platform assessment. The cases involved different types of small molecules, namely metabolites, peptides, lipids and pharmaceutical compounds. The metabolite study consisted of the quantification of steroid sulfates in a biological buffer (100 mM Tris buffer pH 7.4, 0.1% Triton, 1 mM $MgCl_2$ and 30 μM phosphoadenosine phosphosulfate), typically used in enzymatic assays involving steroid sulfotransferases. Pregnenolone and DHEA sulfate were each quantified using the other as internal standard, a less expensive but equally effective strategy compared to the use of deuterated standards. A concentration range from 100 nM to 40 μM was assessed in both cases, using the negative ion mode for analysis. The internal standard concentration was set at 10 μM. Three independent sets of calibration solutions were prepared and analyzed during a single day on different DESI plates. Four instrumental replicates were analyzed for each calibration solution. This entire analysis was repeated on four different days spaced throughout a month with freshly prepared calibration sets.

For the peptide case, we studied the detection of Leu-enkephalin in two traditional biological buffers: 0.1 M phosphate buffer (pH 8) 0.1% BSA, and 0.1 M Tris-HCl (pH 8) 0.1% BSA. A range of concentrations from 3.6 nM to 72 μM was explored using both the positive and negative ion modes. In the case of the lipid analysis, we assessed performance in the detection of 13 deuterated lipid standards in methanol over a range of concentrations between 1 ng/mL and 10 μg/mL. Species were detected in both the negative and positive ion modes. For both the peptide and the lipids, three independent sets of dilutions were analyzed on different plates during one day. The analysis was repeated a week later with a freshly prepared set of solutions. The commercial lipid mixture used as stock was kept at -20°C prior to use.

Finally, in the case of the pharmaceutical compounds, we screened a commercial library typically used in high throughput drug discovery campaigns. There were 318 compounds in the library, with molecular weights in the 150 - 500 Da range. The library had been stored at -20°C for a year prior to the analysis. All compounds in the library were received as 10 mM solutions in DMSO and used as such. Screening in this case was carried out using only the positive ion mode. Four replicates of each sample were analyzed from a single DESI plate.

No sample preparation or dilution was involved in any analysis. All the determinations were carried out directly from the matrices described.

Results and discussion

Quantification of steroid sulfates

Steroid sulfates were long considered to be inactive metabolites intended only for elimination [35,36]. However, increasing evidence has demonstrated that these sulfo-conjugates have biological importance, for example as an alternative source of steroids which can be controlled by local mechanisms (e.g. differential expression of sulfatases or sulfotransferases) that differ from those long known to be involved in hormone secretion [36,37]. Cholesterol sulfate for instance has been identified as an stabilizing agent in cell membranes, as well as an enzyme regulator involved in signal transduction.[38] Additionally, it has been found as a potential prostate cancer biomarker,[39] an identification backed up by the relationship found between the overexpression of cholesterol sulfotransferase and the promotion of this type of cancer [40,41]. Pregnenolone sulfate, on the other hand, exerts anti-inflammatory activity by promoting the degradation of key innate immune signaling proteins [42] and acts as an allosteric modulator of the N-methyl-D-aspartate receptor, having importance in neurological processes [43,44]. Similarly, DHEA sulfate is a neuroactive steroid that interacts with several relevant receptors in the brain (e.g. sigma, GABA-A), its decreased concentration being related to neuropsychiatric disorders.[45] Consequently, the quantification of these species in complex matrices is relevant both in a biochemical context, as well as in doping control, as the levels of these endogenous sulfates in urine can be used as biomarkers of anabolic androgenic steroids misuse [46,47].

Considering the importance of the analysis of these metabolites, we assessed the quantitative performance of the two high throughput DESI-MS platforms for the determination of both pregnenolone ($[M\text{-}H]^-$ *m/z* 395.2) and DHEA sulfate ($[M\text{-}H]^-$ *m/z* 367.2), using one species as internal standard for the other. The analysis was performed in the nM - μM range directly from a complex matrix (buffer, detergent, salt). These conditions were chosen as they are typical of biological assays involving this class of compounds (e.g. enzymatic assays of steroid sulfotransferases). **Figure 2** shows the calibration curve obtained using the *Waters stage* (top) and the *Prosolia stage* (bottom) for pregnenolone sulfate. As observed, both platforms provided satisfactory quantitative performance (R^2 coefficients were better than 0.99), but the results indicate that higher sensitivity and reproducibility is achieved using the *Waters* stage.

In terms of sensitivity, the *Waters stage* using the Q-ToF provided a limit of quantitation of 60 nM. The *Prosolia stage* on the LTQ gave a higher limit of quantitation of 1.79 μM (ca. 30 times higher). Note that the amount of analyte in the spotted samples (50 nL) is ca. 1 pg and 40 pg at the limit of quantitation of the *Waters* and *Prosolia systems*, respectively, with only 300 ms of analysis per sample in both cases. With regards to reproducibility, the *Prosolia stage* provided coefficients of variation (CVs) lower than 30%, with high relative variation at lower concentrations. The *Waters stage* achieved CVs better than 10% at all the concentrations explored, in most cases being below 5%. These results indicate that higher reproducibility is achieved with this system even at low (nM) analyte levels. Identical behavior was observed for the quantitation of DHEA sulfate (**Figure S1**). A summary of the figures of merit obtained for the high throughput quantitation of both steroid sulfates using the two DESI systems is included in **Table S1**.

Detection of Leu-enkephalin

Endogenous peptides are another class of compounds whose detection in complex matrices is increasingly relevant for biological studies. [48–50] For instance, endogenous peptides in plants have been identified as regulators of the immune response,[51] whereas in mammals they have been associated with important roles in metabolic homeostasis, gene expression, signaling, immune defense, and pain modulation [48,52–54] So much biological importance has been linked to these compounds that many therapeutics have been developed as analogs of endogenous peptides [53,54]. An extensively studied class within these peptides are the opioid peptides, which are classified as such because they interact with opioid receptors in a similar manner to opioids (e.g. morphine, fentanyl), having in turn, similar nociceptive action. Opioid peptides can be classified in endorphins, enkephalins, dynorphins and endomorphins.[52] Enkephalins, which include Met-enkephalin and Leu-enkephalin, are well characterized endogenous peptides with activity on the μ and δ opioid receptors, as well as roles in neurotransmission and calcium influx modulation.[55] Several synthetic enkephalins have been developed as analogs in the context of analgesic

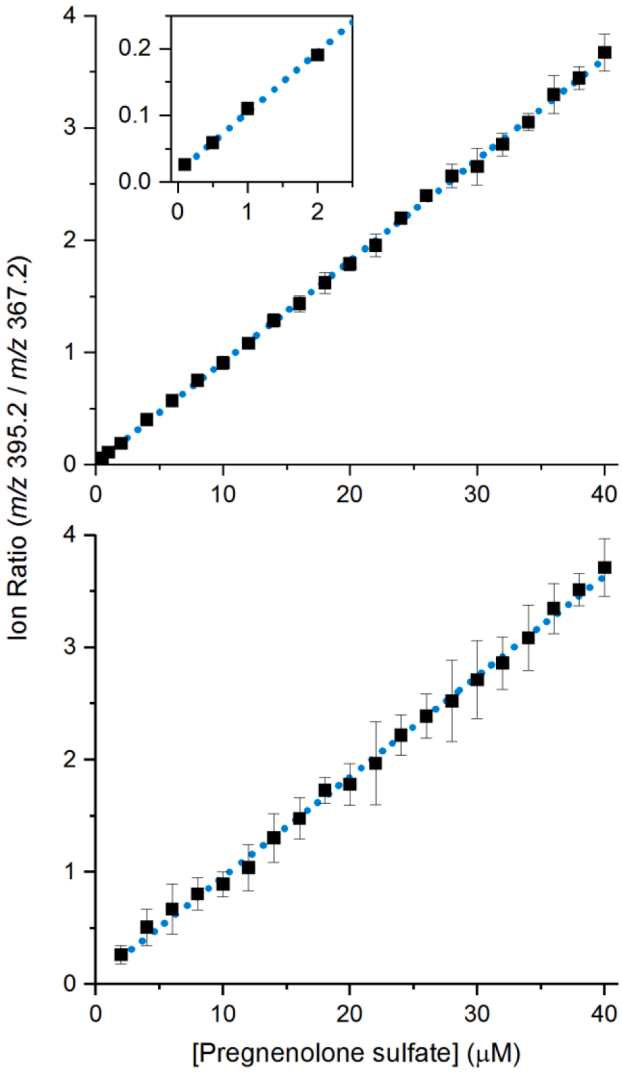

Figure 2. Calibration curves of pregnenolone sulfate (*m/z* 395.2) using the *Waters* (*top*; $R^2 = 0.9994$; CVs < 10%) and *Prosolia* (*bottom*; $R^2 = 0.9972$; CVs < 30%) DESI stages. Samples were all analyzed directly from 100 mM Tris buffer (pH 7.4), 0.1% Triton, 1 mM $MgCl_2$ and 30 μM phosphoadenosine phosphosulfate. All data points represent averages from 12 independent calibration solutions, each with four instrumental replicates, analyzed on different days. DHEA sulfate (10 μM; *m/z* 367.2) was used as internal standard. Inset (*top*) shows a magnification of the low-concentration range analyzed using the Waters DESI stage.

drug development.[56] Accordingly, the high throughput determination of such analytes in either biological fluids or bioassay matrices gives results relevant to both biochemical studies and to drug discovery efforts.

As a prototype compound within this class, we studied Leu-enkephalin in order to assess the small peptide detection capabilities of the two high throughput DESI-MS systems. The analysis was carried out directly from two common buffers, phosphate and Tris, both identically adjusted (pH 8) and containing 0.1% of bovine serum albumin (BSA), a typical component of buffers in biochemical assays. The results of the analysis in both the positive and negative ion mode for the concentration range between 4 nM and 7.2 μM are summarized in **Figure 3**. In the negative mode, both platforms successfully allowed detection of the peptide (signal/blank ratio > 3) at concentrations equal to or higher than 0.72 μM. However, the *Waters stage* allowed for lower detection limits, providing successful and reproducible detection of Leu-enkephalin at concentrations as low as 7.2 nM directly from Tris buffer (**Figure S2**, top panel). This limit of detection is equivalent to 4 ppb, or 200 fg of analyte deposited on each spot (not all of which was sampled). The analysis in phosphate buffer showed poorer sensitivity, something expected due to the negative effect of high levels of phosphate ions when analyzing using the negative ion mode. Using the same platform, the samples in phosphate buffer yielded detection limits an order of magnitude higher than in Tris (72 nM). Nonetheless, the responses obtained in both matrices using the *Waters stage* outperformed those from the *Prosolia stage*, which allowed successful detection only above 720 nM (**Figure S2**, bottom panel; for comparison, the spectrum under identical conditions using the *Waters stage* is shown in the middle panel). Interestingly, no differences were observed between the two buffers using the *Prosolia stage* in negative ion mode.

With regards to positive ion mode, the *Waters stage* again outperformed the *Prosolia stage* platform in terms of sensitivity. The predominant species under the analysis conditions chosen was the sodium adduct of Leu-enkephalin instead of the protonated molecule. No differences in the performance between the two buffers were observed within each platform for the detection of the $[M+Na]^+$ species. The *Waters stage* allowed detection of the adduct at concentrations as low as 36 nM (20 ppb, or 1 pg of analyte deposited on each spot), whereas the *Prosolia stage* system achieved successful detection only at or above 7.2 μM, a 200 times higher detection limit. The protonated species was severely suppressed in favor of the sodium adduct in almost all cases. The $[M+H]^+$ ion was only detected successfully using the *Waters stage* from Tris buffer (≥72 nM), which is reasonable considering the high amount of sodium present in the phosphate buffer. Note that overall, the negative mode was identified as the most sensitive option for the analysis of this peptide, in contrast to the findings of studies which use LC-MS, where the positive ion mode is preferred for the determination of enkephalins in complex samples.[55]

Analysis of lipid standards

The study of lipids is a remarkably active research field, lipidomics having become one of the most significant branches of the omic sciences.[57] This development is a response to the importance that lipids have in living organisms, with major roles in signaling, energy storage and metabolism, as well as membrane formation and structure.[58–60] Lipidomics largely relies on mass spectrometry for the identification and quantification of individual lipid species [59,61], with DESI being one of the ionization techniques used for analysis, together with MALDI, and ESI after direct infusion of samples or when coupled with liquid chromatography [57,60]. Novel approaches, such as multiple reaction monitoring profiling, have also proven valuable at determining changes in the lipidome through lipid monitoring at the functional group level, instead of relying on specific molecular identifications.[62]

Due to the increasing relevance of lipid analysis, as well as the particular implications of high throughput lipidomics, for example for the rapid analysis of large clinical cohorts,[63] we explored the performance of the two high throughput DESI-MS platforms using a commercial mixture of 13 deuterated lipid standards. The mixture contained diverse functional groups common in lipidomics, namely: glycerophosphoethanolamine (PE), glycerophosphoglycerol (PG), glycerophosphocholine (PC), glycerophosphoserine (PS), glycerophosphoinositol (PI), sphingomyelin (SM), cholesterol ester, ceramide, triacylglycerol (TAG), diacylglycerol (DAG), and monoacylglycerol (MAG). All the compounds and corresponding *m/z* values detected ($[M+H]^+$, $[M+Na]^+$, $[M+NH_4]^+$, and $[M-H]^-$) are given in **Table S2**.

In the negative ion mode, detection of PE, PG, PI, PS and ceramide lipids was achieved. Using the *Waters stage* all compounds belonging to these classes were detected at 500 ng/mL (equivalent to 0.6-1.4 μM) with signal-to-blank ratios higher than 3 (**Figure 4A**), whereas with the *Prosolia stage* the 15:0-18:1(d7) PE and the C15 ceramide-d7 were not detected (**Figure 4B**), even at 10 μg/mL. Note that some species

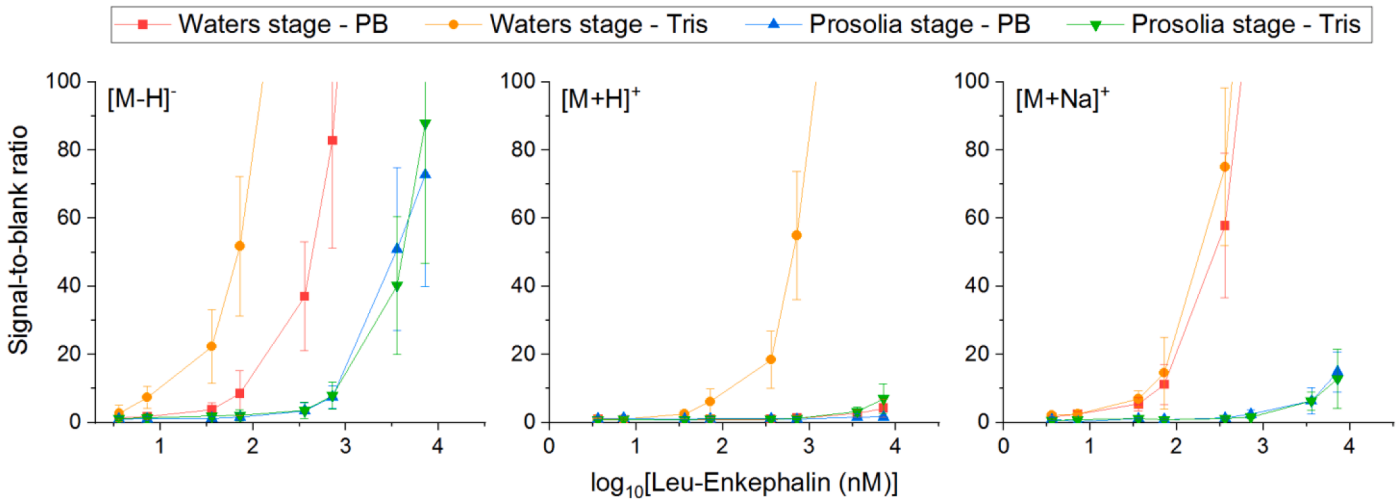

Figure 3. Comparison of the signal-to-blank ratios obtained for the analysis of leucine enkephalin in two common biological buffers: 0.1 M phosphate buffer (PB, pH 8) 0.1% BSA, and 0.1 M Tris-HCl (pH 8) 0.1% BSA. All data points represent averages from six independent samples, each with four instrumental replicates, analyzed on two different days. Results for the analysis in negative ion mode ($[M-H]^-$, *m/z* 554.3, *left*) and positive ion mode ($[M+H]^+$, *m/z* 556.3, *center*; $[M+Na]^+$, *m/z* 558.3, *right*) are shown.

provided lower detection limits using the *Waters stage*, down to 10 ng/mL (10-30 nM), as shown in **Table S3**. Interestingly, the profiles (relative intensities amongst sets of analytes) seemed consistent across the two systems.

Regarding positive ion mode, detection of PC, PE, TAG, DAG, MAG, SM and ceramide lipids were all successful. PEs, acylglycerols and ceramides, were mostly detected as adducts, commonly with sodium, and, in few cases, with ammonium (which is present in the commercial standard itself). Both platforms were successful at detecting all the expected species at 1 μg/mL, as shown in Figure 4**C** and Figure 4**D** for the *Waters* and *Prosolia* stages, respectively. Similar to the negative ion mode, using the *Waters stage* lower detection limits can be achieved for some species, down to 10 ng/mL, whereas with the *Prosolia stage* several compounds are already not detected below 1 μg/mL (**Table S3**). These results are consistent with the peptide analysis data, indicating that higher sensitivity is achieved overall in negative ion mode with the *Waters stage* when comparing both platforms.

Note that the 18:1(d7) cholesterol ester was not identified with either DESI system, in either ion mode. This is in agreement with literature reports on the difficulty of the direct analysis of cholesteryl esters, which typically require derivatization steps [64] or enhanced detection by the formation of ammonium or lithium adducts.[65] Both cases are compatible with DESI, as it easily allows the addition of a reactant to the spray solvent (reactive DESI), something already reported for the case of direct cholesterol analysis.[66]

Screening of small molecule libraries

The last case studied was the screening of a small molecule (150 - 500 Da) library, typically used for high throughput drug discovery. This kind of compound library has become increasingly popular, following the growth of high throughput screening technologies and facilities in both industry and academia.[67] Widely recognized key factors in screening campaigns using small molecule libraries include both the quality of the library as well as how is it maintained and verified over time.[68–70] High throughput methodologies are then required for rapid quality control of the libraries. However, it is recognized that traditional liquid chromatography based methods do not provide high enough throughput to analyze even modest-sized libraries, so that in many cases libraries are used only after random sampling of a few compounds, whose results are used as estimates for entire plates.[69]

For this reason, we assessed the capabilities of both DESI systems for the rapid screening of a commercial small molecule library containing 318 compounds in DMSO, a solvent typically too viscous for methods that require the sample to pass through capillaries [71,72]. The properties of the drug-like molecules in the library are summarized in **Figure S3**. The analysis was carried out in the positive ion mode, and the data analysis considered both the expected $[M+H]^+$ and $[M+Na]^+$ ions for each compound. The signal-to-blank ratio heat maps obtained, calculated using the maximum response between these two species, are shown in Figure 5. As observed, both DESI-MS systems were successful (signal/blank ratio ≥ 3) at detecting ions with the expected *m/z* values, with success rates of 93% and 90% using the *Waters* and *Prosolia* stages, respectively. All the negative results (signal/blank ratio < 3) obtained with the *Waters stage* correspond to negative results using the *Prosolia stage*, whereas all the negative results using the *Prosolia stage* relate to signal-to-blank ratios lower than 10 using the *Waters stage*, showing good agreement between both systems for the overall screening of the plate.

Upon further inspection of the nature of the compounds that were not detected successfully, we determined that most of them correspond to structures were nitrogen lone pairs occur in resonance structures, such as in aromatic amides, thioureas and nitro groups, particularly when there is extensive conjugation within the molecule (**Scheme S1**). Such structural features that might be associated with decreased proton affinities.

Additionally, in spite of the good agreement between data from both DESI stages, it is noteworthy that 70% of the compounds screened provided a higher signal-to-blank ratio using the *Waters system* compared to the *Prosolia system* (*see* **Figure 6**). These results are in agreement with the previous cases studied, and they indicate that higher sensitivity is achieved using the *Waters stage*. At the same time, they demonstrate the potential of high throughput DESI-MS for the rapid quality control of compound libraries, especially considering that using either of the platforms the analysis of a full 384 plate requires less than 7 minutes, and the typical sample consumption is only 50 nL.

Conclusions

We assessed the analytical performance of high throughput DESI-MS, from the early and no longer commercialized Prosolia source to the new Waters DESI system. This evaluation was carried out using different compound classes, including steroid sulfates, endogenous peptides, lipids and pharmaceutical compounds. Our results indicate that, while

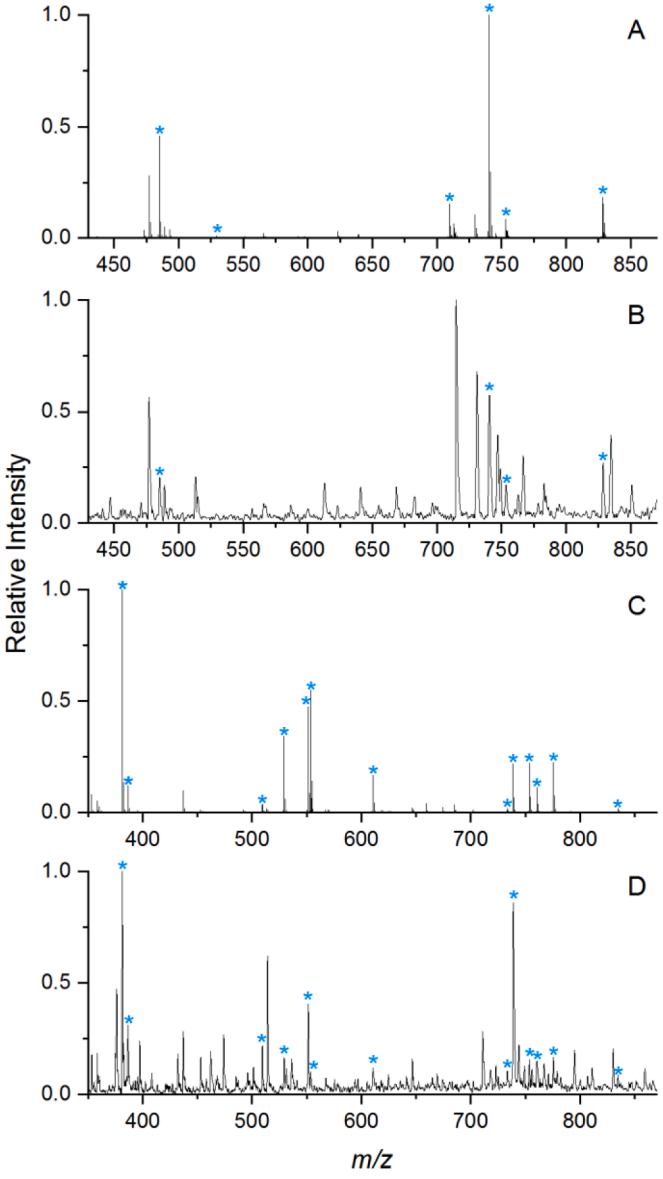

Figure 4. Representative mass spectra of a commercial mixture of lipid standards analyzed using the high throughput system. Analysis was performed in both negative (A and B; 500 ng/mL) and positive (C and D; 1 μg/mL) ion modes. Spectra A and C were acquired using the *Waters DESI stage*, whereas spectra B and D were acquired using the *Prosolia DESI stage*. Star symbols denote peaks at *m/z* values corresponding to the expected lipids. The difference in resolution is due to the choice of different mass analyzers.

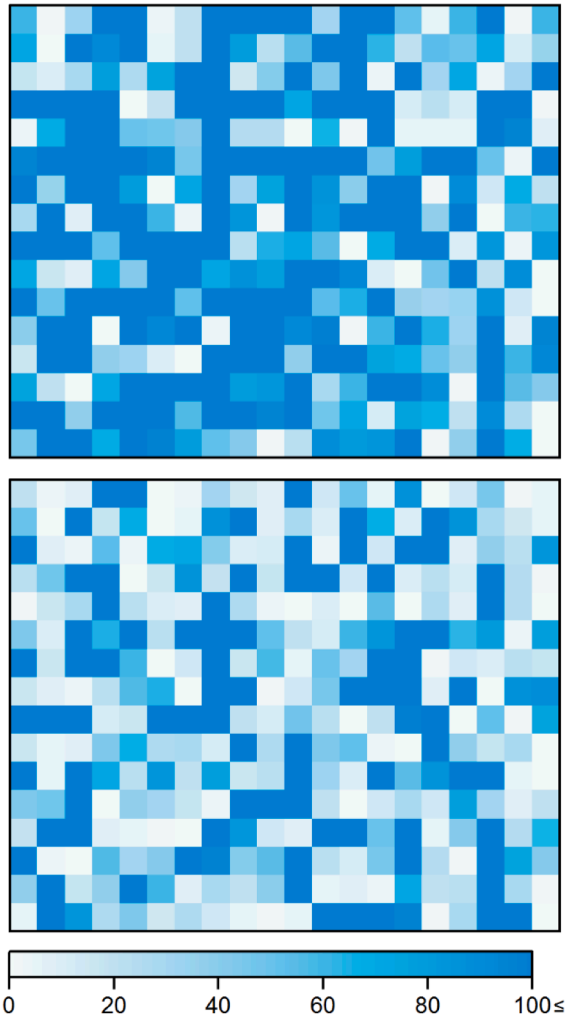

Figure 5. Heat maps of signal-to-blank ratio for the screening of a commercial compound library using the *Waters* (top) and *Prosolia* (bottom) DESI stages. Signal-to-blank is calculated using the average of 4 replicate spots for each analyte and 256 spots for the blank spectrum. All results (1,528 sampled spots) were acquired in ca. 25 minutes. The highest response whether $[M+H]^+$ and $[M+Na]^+$ is included in the heat map. Note that, despite the platform differences, in both cases the success at detecting the species of interest (signal-to-blank $\geq$ 3) is higher than 90% using only the positive ion mode for analysis.

both platforms allow for fast quantitative and qualitative analysis directly from complex matrices, the Waters DESI ion source represents an advance in terms of sensitivity and reproducibility from the original Prosolia stage. This is observed in the decrease of both limits of detection (between 1 and 3 orders of magnitude) and CVs (average reduction of around 10% for quantitation using an internal standard).

The improvement in analytical performance is driven by technological developments of the DESI stage and not the intrinsic differences between the mass spectrometers (ion trap vs. quadrupole time-of-flight) coupled to each stage. The advances on the Waters stage, compared to the early Prosolia version, include a robust prototype sprayer which operates under significantly lower nebulizing gas pressures (150 psi vs. 15 psi) and voltages (± 4.5 kV vs. ± 0.65 kV), as well as a prototype heated transfer capillary, which improves ion transmission through the 90° angle capillary bend required for the implementation of DESI on a Synapt instrument. The absence of either of these components reduces the overall signal intensity by around one order of magnitude, indicating that the DESI stage is mainly responsible of the observed system performance. This is also reflected in the lower limits of detection that can be obtained with nano-ESI (**Table S4**) using the same mass spectrometer. However, nano-ESI suffers from low salt tolerance and capillary clogging compared to DESI.

Advances in the DESI technology for high throughput analysis, should focus not only on data quality, but on speed, for instance faster stage control permits lower dead times (i.e. the time spent moving from one sample spot to the next). As shown in this study, diverse compounds were analyzed in different matrices, with excellent sensitivity and quantitative responses, using identical DESI conditions (geometry, solvent, gas, voltages). This contrasts with earlier DESI studies, where lengthy and rigorous optimization of multiple variables was common for every new sample or analyte. This increase in robustness, as here demonstrated, makes high throughput DESI-MS, and the use of DESI in general, attractive for rapid and simple analysis. The fact that no significant analytical method development is needed for a very wide range of applications is noteworthy.

It is also worth highlighting that, despite the observed improvement from the early to the new DESI source, all the results described in this manuscript originate from the analysis of *sub-nanogram amounts* of analytes, directly from complex matrices (such as typically non-mass spectrometry friendly buffers), with each sample being analyzed in under 300 ms (only 3 scans collected and averaged per sample). Additionally, the broad applicability and adequate analytical performance demonstrate the potential of high throughput DESI-MS analysis in fields ranging from enzymology and lipidomics to quality control and drug discovery.

Declaration of Competing Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.talo.2021.100046.

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