Boosting Discovery and Early Development of Drug Candidates with New MALDI Technology

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A golden rule of R&D in pharma is if you are to fail, fail early and fail cheap. This mantra has guided scientists for many years and this principle has been shown to drive innovation in a wide range of industries. The practice commonly adopted in pharma – using technology plus automation to rapidly screen compound libraries looking for 'hits', together with integrating ADME/TOX (Absorption, Distribution, Metabolism, Excretion/Toxicity) investigations as early as possible in the discovery process, to understand essential details of drug distribution and metabolism – has undoubtedly made a contribution to the rise in productivity in recent years. There has been an estimated 11.5% year-on-year drug pipeline growth (2015 – 2016), with the biggest rise being seen in the pre-clinical phase. Here, the 6,061 compounds reported to be in this phase in 2015 has grown to 6,861 in 2016 [1]. This article will discuss recent innovations in MALDI (Matrix-Assisted Laser Desorption Ionisation) mass spectrometry related to how two detection schemes that utilise these technology advances are being applied to accelerate pre-clinical drug discovery, one in ultra-high throughput screening (uHTS) programmes, the other in drug tissue distribution MALDI-imaging studies.

Context

There has been much written about desirable characteristics of an ideal analytical tool for small molecule R&D, for example, label-free, no probes, and with the ability to measure target analytes directly and quantitatively. Rapid, robust, easy-to-use, cost-effective and automation-ready are also requirements for most companies. Mass Spectrometry (MS) inherently delivers on many of these criteria, and has opened up a new arena for MALDI MS within the last years. MALDI is utilised with various MS analysers, the most common being axial TOF (Time of Flight) detectors, but others, such as orthogonal TOF or FT ICR (Fourier Transform Ion Cyclotron Resonance) are employed, depending on the analytical needs.

Today the technique is established across a range of applications in drug discovery and development. MALDI-MS is helping researchers identify the most promising small molecule leads, and is now expanding into the ultra-high throughput compound screening programmes that 'big pharma' relies on. Likewise, MALDI mass spectrometry imaging (MSI) is helping developers understand the spatial distribution and tissue physiology of a candidate drug and related metabolites before quantitative whole body autoradiography (QWBA) experiments, thereby permitting informed decisions whether to move the candidate to the next development stage.

It is also important to note that many candidate compounds generate metabolites which possess biological activity. These active metabolites may have different pharmacology and PK properties than the parent drug. A thorough understanding of the properties of active metabolites is central to estimating toxicity, which is the number 1 reason for withdrawal of a drug. Early information about the enzymes involved in the drug metabolism is very useful in the design of drug-drug interactions studies.

Application Example: Technology in Practice

When configured for HTS, as with the rapifleX MALDI PharmaPulseTM instrument, the result is a system that is up to 20 times faster than a traditional instrument, with improved robustness, sensitivity and extended mass range (MS/MS). Dr Peter Marshall et al (GlaxoSmithKline, Stevenage, UK) used the MALDI-TOF MS coupled with nanolitre liquid handling to enable them to screen more than 1 million samples per week [2]. The work was performed on a MALDI-TOF instrument with a 10 kHz laser. Mass spectra were acquired in the range of either m/z 80-400 or 700-3500, with 200 laser shots per sample. Under these experimental conditions, the system processed a 1536 well plate in 7.36 minutes. With a vast in-house collection of candidate compounds, simple scale-up calculations indicate that the analysis of 2 million compounds would require 7.85 days. An assessment of the robustness of the system and the methodology were made, with good correlation of results before and after 108 measurements.

They conclude that the technology and approach for uHTS was robust and can deliver very fast analysis times. The group has measured more than 1 million samples a week and found that it has been possible to measure more than 2 million samples without having to clean the instrument lens stack. Finally, looking forward to even higher throughput, the group achieved similar assay performance using 6144 well plates. If adopted into routine, this would cut the time required to screen 2 million compounds to 2.39 days.

In contrast, where MALDI MSI is being used to understand the distribution of a drug and its metabolites in model tissue, features such as those seen on the solariX system are ideal. Traditionally, quantitative whole-body autoradiography (QWBA), and/or liquid chromatography coupled to mass spectrometry LC/MS, have been the methods used to obtain drug distribution and metabolism. Both have challenges. QWBA is a robust technique, and the data generated is accepted by regulatory bodies around the world, however, QWBA presents a composite of the total radioactivity present – it may include any combination of parent drug, metabolites, impurities and degradation products. Thus, it has severe limitations for researchers looking for insight into biochemical pathways and mechanisms.

LC/MS analysis is performed on extracts from tissue homogenates. The technique can not indicate any spatial information and, equally importantly, can be misleading. For example, if an analyte in the tissue is highly localised, the extraction and homogenisation process will act as a dilution, masking this distribution and giving a relatively low concentration, sometimes even below limit of detection. Localisation of an analyte is often an indication of toxicity, and would be missed in this case. Alternatively, if an analyte is determined to have a high concentration from tissue homogenate, a researcher could draw incorrect conclusions about toxicity because the analyte is presumed to be evenly present throughout the tissue.

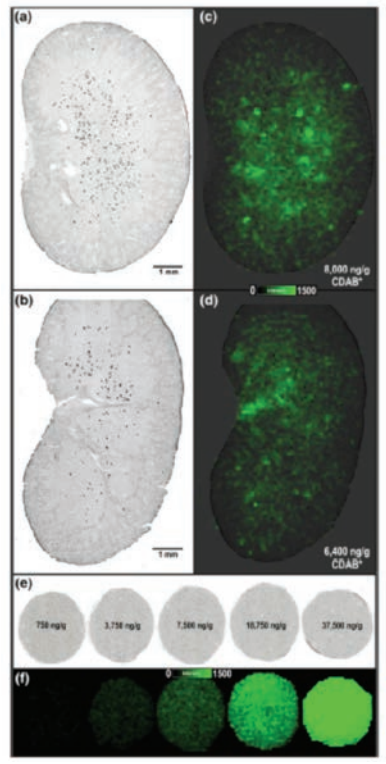


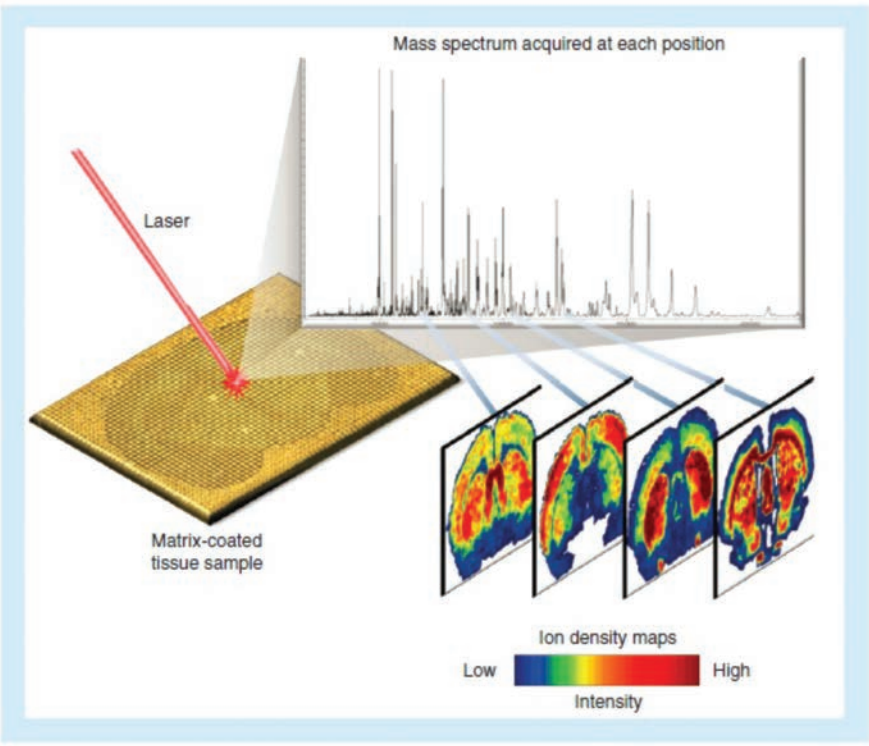
Figure 1. Optical scans of kidney tissue sections from PND 7-13 juvenile rats [4].

By comparison, MSI takes a conventional tissue section and coats it with a matrix, which extracts molecules from the tissue, but retains the spatial relationships found in the underlying tissue. Following preparation, the sample is measured in the spectrometer. The result is spatially resolved mass spectra. Because the laser only probes the matrix crystals which are on the surface of the section, the underlying cellular features are not disrupted and can be taken through a standard histological staining routine so that a high-quality histology image can be captured. By merging this scan with the molecular information from the MALDI mass spectrometer a histology-directed analysis of the tissue is possible.

Recent work by M. Reid Groseclose et al (ref) evaluated the additional information that MALDI MSI could provide over and above LC-MS in a nephrotoxicity study on the anti-cancer drug Dabrafenib (DAB) in rats. This work was in support of developing DAB for use in paediatric patients. Previous studies had identified some unexpected adverse kidney effects in juvenile rats. These effects had not been seen in adult studies [3].

Initially, MALDI MSI could determine the distribution of DAB and its metabolites in the kidney (*Figure 1*). Subsequent analysis of tubular deposits seen in the first experiments, provided the chemical composition at these locations and triggered a more complete risk assessment for paediatric treatment with Dabrafenib than would have been possible using LC-MS analysis alone.

The principles of MALDI Imaging (MALDI IMS)



MALDI Imaging Mass Spectrometer Experimental Workflow

The MALDI imaging experiment is initiated by mounting a tissue section onto a target, applying a matrix and rastering a laser across the surface of the tissue. At each discrete location within a virtual grid the laser is fired, and a mass spectrum is acquired. By plotting the ion intensities as a function of the x and y coordinates on the tissue, ion images are generated.

New Technology & Advanced Tools in MALDI Technology

MALDI has become an established and powerful technique for drug discovery and instrument manufacturers have switched their focus to certain key system components of the system. These have become the subject of intensive development as companies look to advance performance, reduce costs and streamline workflows for researchers. The goals for a user may be specific to each application. For example, speed, throughput and cost per sample in HTS vs. resolution and sensitivity in imaging applications. However, improving and optimising the critical components for an individual application can deliver improvements for all.

At the heart of a system is a high speed laser operating at 10 kHz. Stability is critical for both spatial and mass resolution such that improving the precision of the optical bench which carries the ion optics and the necessary lenses, reflectors and detector modules, performance can be significantly upgraded. The configuration of a typical TOF/TOF optical bench is featured in *Figure 2*.

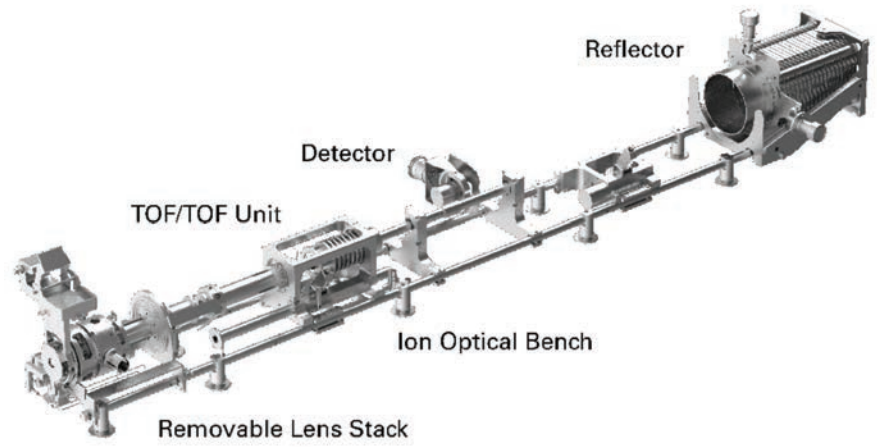


Figure 2. Ion path mounted on optical bench with TOF/TOF unit.

Importantly, expanding the performance envelope of a MALDI system requires a fast laser. The cutting-edge systems of today imply a 10 kHz laser, but accommodating a 10 kHz laser can raise many technical and mechanical issues that need to be solved. For example, traditional instruments reposition the sample relative to a fixed laser spot. The mechanics of moving a sample quickly and precisely enough to keep up with a fast laser are challenging. However, by reversing the traditional thinking and moving the laser relative to a fixed sample position, the potential of the 10 kHz laser has been fully realised.

In addition, developments in detector design, notably, incorporation of the academically acclaimed dynamically harmonised ParaCell™, developed by Professor Eugene Nikolaev and co-workers at the Russian Academy of Sciences in Moscow, into solariX™ XR system. This innovative design stabilises the ion cyclotron resonance signal over a broad mass range. Mass spectra can be acquired with 10 million resolving power when high-throughput is not required. The instrument also provides benchmark performance at faster acquisition rates, resolving power is greater than 250,000 at m/z 400 in one second at 7T. This and other recent improvements create a nearly ideal and yet unmatched analyser for complex mixtures like those found in MALDI imaging.

The challenge that fast analysis poses for control, capture and processing routines is also a significant consideration when an instrument is working at the speeds required for routine analysis. Unlike classical MALDI instruments, new-generation systems now use strategies such as parallel computing threads, each fast enough to keep up with the speed of the analysis.

Summary

Having emerged in basic research and subsequently proven its value in routine uHTS and MSI applications, forward-thinking researchers are investing in the latest instrumentation and expanding the application of the technique, anticipating additional insights and a deeper understanding of a candidate compound early in the development process. In order to facilitate a rapid route to market for new, safe and efficacious drugs pharmaceutical companies are seeking out innovative approaches and technologies [5]. MALDI technology is already making an impact in small molecule R&D, and many industry observers believe we will see this grow significantly over the coming years.

References

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