Novel High-Throughput MALDI-TOF MS Workflow for Screening of Different Analytes at Each Position on a Plate

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Introduction

MALDI-TOF mass spectrometry was established as a label-free ultrahigh-throughput screening approach for biochemical assays where substrates and products were small molecules, peptides and proteins [1, 2]. In this case the same list of analytes was monitored at every position on a MALDI plate. More recently, we extended this approach to screen synthetic chemical reactions, which required monitoring different small molecule starting materials and products at every position on a MALDI plate (Figure 1) [3]. This necessitated the development of a novel software workflow and optimization of data processing methods for a dramatically increased number of analytes. This work focuses on application of the new Synthesis Screening workflow not only to screening chemical reactions but also to screening a custom library of small molecule pharmaceuticals.

Methods

A custom library of 41 small molecule compounds was prepared on a MALDI plate in 384 format and each compound was spotted 8 times then the dried spots were overlaid with a 2 mg/ml solution of α-cyano-4-hydroxycinnamic acid matrix (Figure 2A).

The library was analyzed in automated mode on a rapifleX MALDI-TOF/TOF system equipped with a 10 kHz scanning beam laser. The automated runs were set up, monitored and visualized in MALDI PharmaPulse 2.2 software that included the novel Synthesis Screening module. The new software workflow allowed monitoring of up to 100 different analytes per position on a MALDI plate prepared in 384, 1536 or higher density formats.

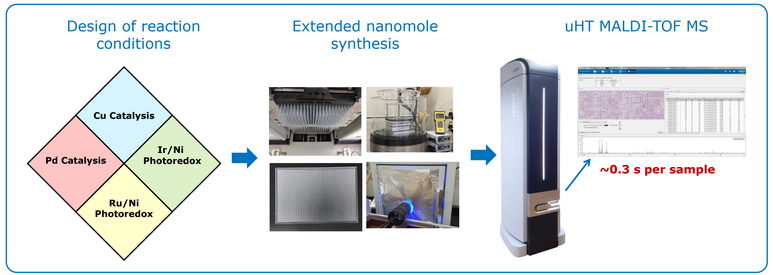

Figure 1. Synthetic reaction screening workflow

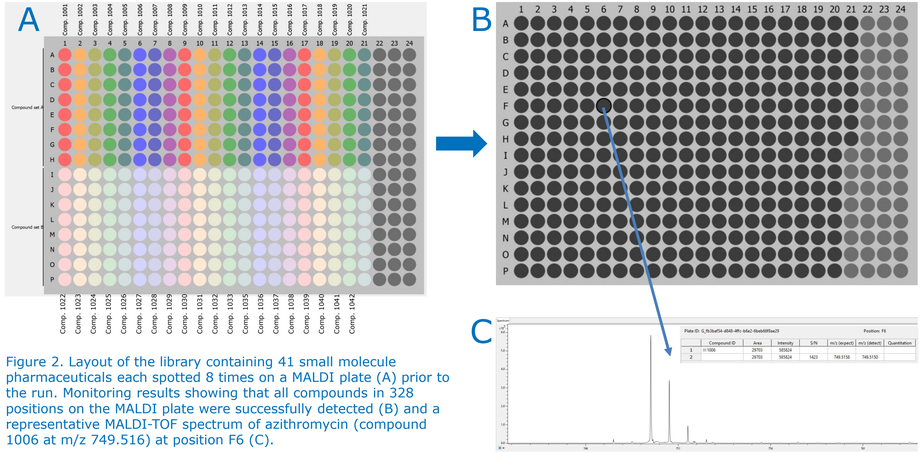

Figure 2. Layout of the library containing 41 small molecule pharmaceuticals each spotted 8 times on a MALDI plate (A) prior to the run. Monitoring results showing that all compounds in 328 positions on the MALDI plate were successfully detected (B) and a representative MALDI-TOF spectrum of azithromycin (compound 1006 at m/z 749.516) at position F6 (C).

Results

- Ultrahigh-throughput automated runs of the MALDI plate with the custom library of small molecule pharmaceuticals resulted in less than one failed plate positions per run (averaged from 3 runs). One run had no failed positions while the other two runs had one failed position each (Figures 2A and 2B).
- Five compounds from the custom library were monitored as sodium adducts $[M+Na]^+$ while the remaining compounds were detected as $[M+H]^+$ ions. The software allowed monitoring of adduct ions selectively for specified analytes of interest or for all analytes.
- The plate view of monitoring results from the automated runs is linked to individual MALDI-TOF spectra and extracted signal parameters for each monitored compound. For example, MALDI plate position F6 is linked to the spectrum of azithromycin at m/z 749.516 (compound 1006) (Figures 2B and 2C).

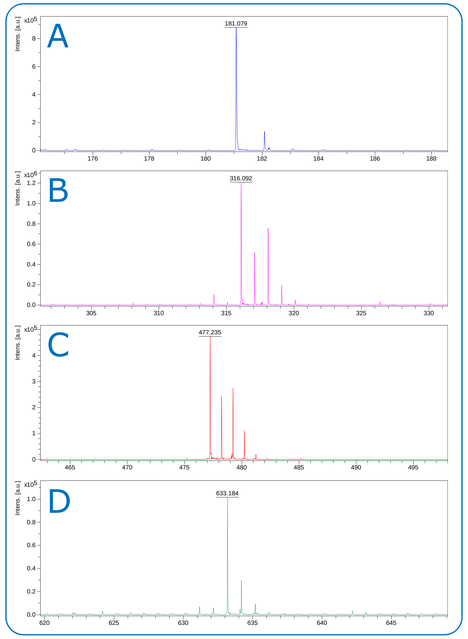

Figure 3. Representative spectra of theobromine at m/z 181.072 (compound 1040)(A), chlorprothixene at m/z 316.092 (compound 1016) (B), loperamide at m/z 477.230 (compound 1029) (C) and hesperidin at m/z 633.179 ($[M+Na]^+$ ion; compound 1025) (D) from uHT MALDI run of the compound library

- Representative MALDI-TOF spectra demonstrate the excellent data quality that was obtained in the automated runs for small molecule library compounds in the mass range of 155-760 Da. Each spectrum was acquired using 2000 laser shots (Figure 3).
- The Synthesis Screening module includes relative quantitation options using the same internal standard for the whole MALDI plate or a different internal standard for each position on the plate.

Summary

- The Synthesis Screening module in the MALDI PharmaPulse software was used to screen a custom library of 41 small molecule pharmaceuticals.
- The automated runs using a MALDI plate in 384 format had less than one failed plate position per run on average.

References

[1] Haslam C, Hellicar J, Dunn A, Fuetterer A, Hardy N, Marshall P, Paape R, Pemberton M, Resemann A, Leveridge M. *J. Biomol. Screen.* 2016; **21**: 176–186.

[2] Heap RE, Hope AG, Pearson LA, Reyskens KMSE, McElroy SP, Hastie CJ, Porter DW, Arthur JSC, Gray DW, Trost M. *SLAS Disc.* 2017, **10**: 1193–1202.

[3] Lin S, Dikler S, Blincoe WD, Ferguson RD, Sheridan RP, Peng Z, Conway DV, Zawatzky K, Wang H, Cernak T, Davies IW, DiRocco DA, Sheng H, Welch CJ, Dreher SD. *Science* 2018; **361**: eaar6236.

Conclusions

- The novel Synthesis Screening module in the MALDI PharmaPulse software was designed for all workflows that required monitoring of different compounds at each position on a MALDI plate such as screening synthetic chemical reactions.
- The Synthesis Screening module is applicable to screening and QC of small molecule compound libraries.
- Other potential applications of this module include multiplexed biochemical and cell-based assays.

MALDI-TOF HTS