Employing ballistic gradients, vacuum jacketed columns and prototype benchtop multi reflecting time-of-flight (MRT) to increase lipidomic throughput whilst maintaining highly confident identifications

Authors: Matthew E. Daly[1], Nyasha Munjoma[1], Rob S. Plumb[2], Jason Hill[2], Nick Tomczyk[1], Lee A. Gethings[1], Richard Lock[1]
Affiliations: [1]Waters Corporation, Wilmslow, UK, [2]Waters Corporation, Milford, UK

INTRODUCTION

A common theme between all omics applications is the ever-increasing size of patient cohorts, driven by the need for identification of novel disease biomarkers and an increase in the power of the studies. However, as these studies scale to thousands of patient samples throughput becomes the limiting factor.

One such possible method is to decrease separation time using methods such as Rapid Microbore Metabolic Profiling (RAMMP) that scale down column dimensions and chromatographic separation time.[1] However, this can come at the cost of peak capacity and feature detection.

One way to maintain narrow peak widths is to reduce post-column volume by moving the column significantly closer to the source, and in combination with a vacuum jacket to mitigate frictional heating and temperature differentials, vacuum jacketed columns (VJC) can significantly increase peak capacity and narrow peak widths.[2]

Reliable identification of lipids in short analysis times requires high resolution mass spectrometers capable of fast scanning without loss of resolution. Multi reflecting time of flight utilises gridless mirrors to prevent ion loss while increasing flight length, and therefore, mass resolution. The benchtop Xevo™ MRT Mass Spectrometer is capable of scanning up to 100 Hz while maintaining a mass resolution of up to 100,000 full width half maximal (FWHM), necessary for sufficiently profiling ever decreasing peak widths (Figure 1).

As such, vacuum jacketed columns and the Xevo MRT MS were applied to the analysis of the lipidome of healthy controls and patients with different cancers and compared with conventional chromatography.

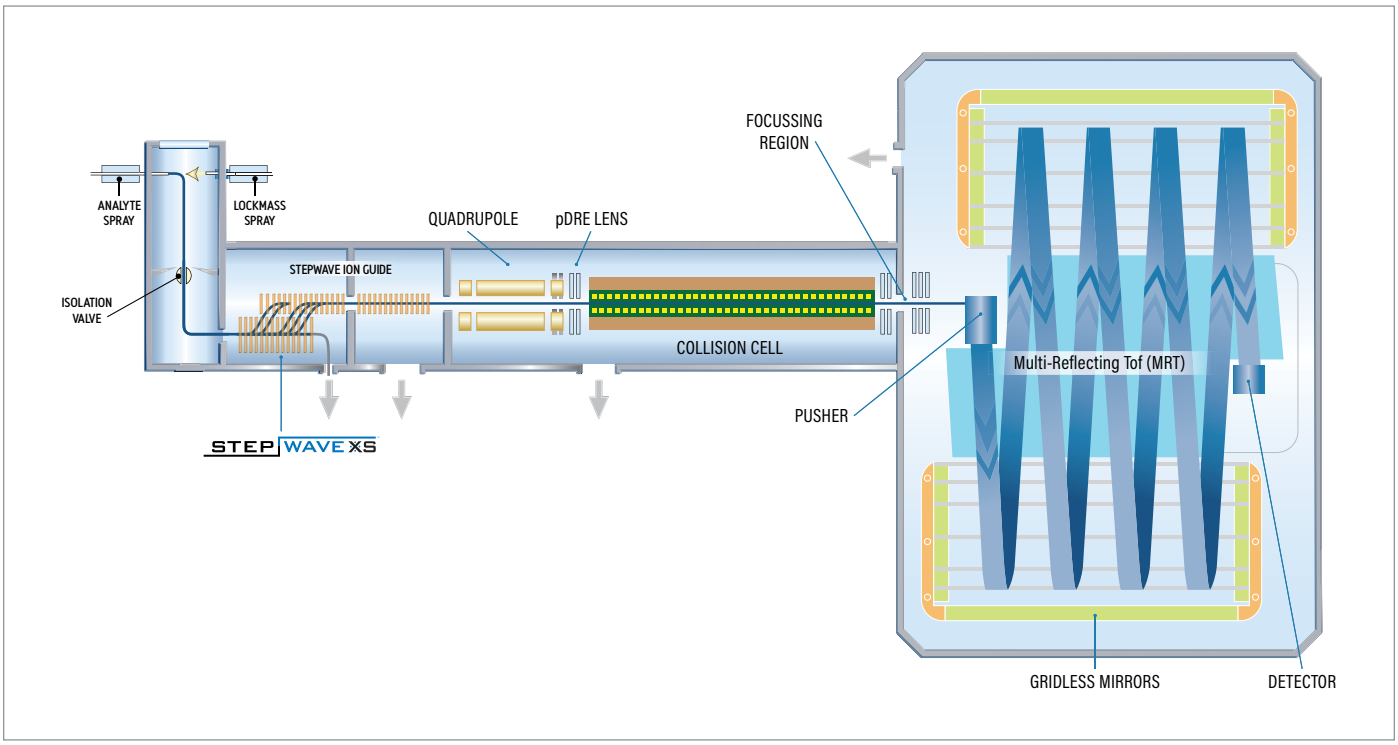

Figure 1. Schematic of the Xevo MRT MS

METHODS

Lipids from serum samples of healthy controls and patients with colon or rectum cancer were extracted using IPA and spiked with EquiSPLASH™ Standard at 100 ng/ml to act as an internal control.

Initially, extracted lipids were separated on a 2.1 mm x 50 mm diameter CSH™ UPLC™ Column over a four minute gradient at 0.8 ml/min. Solvent A was ACN:H_2O:1M aqueous ammonium formate (600:390:10 v/v) and B was IPA:ACN:1M aqueous ammonium formate (900:90:10 v/v). The gradient was as follows; start at 1 % B, then a linear increase to 30 % B at 0.1 min before 90% B at three minutes. Column wash at 99.9 % B at 3.2 minutes for 0.5 minutes before column re-equilibration at 1 % B until four minutes. A corresponding column in VJC format (2.1 mm x 50 mm CSH) was also applied for the separation.

For VJC configurations, the flow rate was scaled down to 0.5 ml/min while maintaining chromatographic gradient over four minutes, before a shortened equivalent gradient of two minute and one minute, respectively, was applied. The eluate was directed .towards the Xevo MRT MS operating at 20Hz (four minute and two minute gradient) or 30 Hz (one minute gradient) scan speed. Data were collected in a data-independent mode of acquisition (MS^E) with alternate scans of low collision energy (6 eV) and high collision energy (20-40 eV ramp) .

Generated data were converted to the .mzML format using DATA Convert Application as the injection finished acquiring before import into Lipostar2 Software (Mass-Analytica.com) for peak picking and identification of features and subsequently lipids using the LIPID MAPS® structure database [3]. Exported compound measurements from Lipostar2 were further analysed using MetaboAnalyst version 6 to generate PLS-DA plots.

Analysis of retention time and chromatographic peak width was completed within LC-MS Toolkit, an application available through the waters_connect™ Software Platform.

RESULTS

Initial experiments using a conventional UPLC Column revealed a complex chromatogram as expected and clear differences between the healthy controls and cancer samples when PLS-DA was applied to the data. Switching configuration to a VJC format while maintaining gradient time similarly adequately revealed differences between the groups however provided significantly a greater number of features and subsequently identified lipids.

A reduction in base peak width of chromatographic peaks was observed when using a conventional column vs VJC (Figure 2) coupled with an increase in peak height resulting from increased ionisation efficiency from a lower flow rate. The trend was observed regardless of retention time of the compound, indeed, Figure 2 demonstrates elution of phosphatidylserine, one of the earliest eluting compounds during lipid analysis.

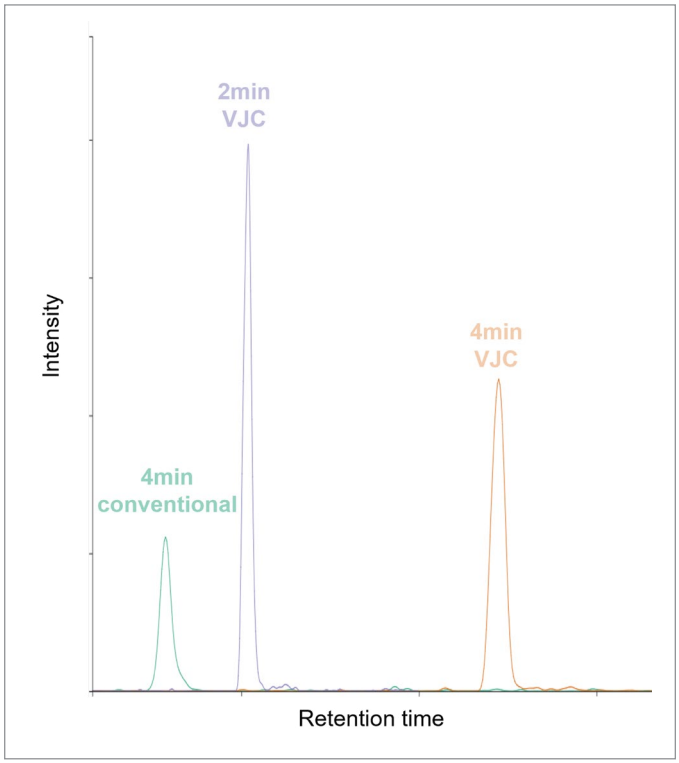

Figure 2. Extracted ion chromatogram of phosphatidylserine with m/z 755.5562 using a 2.1mm conventional column (green), 2.1 mm VJC (4 min method; orange) or 2.1 mm VJC (2 min method; blue).

Scaling down to two minutes gradient time with VJC decreased base peak width further and also lead to a corresponding increase in peak height. Subsequently, increased peak height resulted in improved signal to noise using VJC and therefore sensitivity of the method was also increased. Applying the increase in sensitivity to analysis of cancer patient samples allowed for an increase of 3.5 times in the number of features identified using VJC when compared to a conventional column (Figure 3). Continuing on the path to shorter analysis times, the applicability of a shorter gradient was investigated. As such, shortening the gradient by 50% using a VJC also yielded more features compared to a four minute method with a conventional column, leading to a 100% increase in throughput and ~70% reduction on solvent consumption. Of note, the decrease in gradient time did not impact the ability to sufficiently profile the chromatographic peak owing to the fast scanning capabilities of the the Xevo MRT MS without any impact on mass resolution. No loss of diagnostic power was observed, as analysis of a full study cohort of patients with colorectal cancer vs healthy control demonstrated clear separation using PLS-DA with study QCs accurately located on the plot.

When utilising a one minute VJC method, PLS-DA again showed clear differences between the control vs disease group. This offered a 300% increase in throughput and ~85% decrease in solvent consumption compared to the four minute method with a conventional column.

DISCUSSION

Analysts are increasingly required to improve throughput, which poses challenges to maintain data quality. Further, there is a drive for analysis methods to become more environmentally friendly and reduce the use of organic solvents. Vacuum jacketed columns combined with the high resolution and fast scanning capabilities of the Xevo MRT MS facilitate unparalleled throughput with no compromise in data.

Transferring a fast 2.1 mm conventional column method to a VJC format realised potential gains in feature detection of 3.5 times. An increase in peak capacity using VJC also allowed a reduction in flow rate and therefore solvent consumption, resulting in a more environmentally friendly analysis that also provided better results compared to conventional chromatography.

Dependent on the final purpose of the analysis, methods can be scaled down for increased throughput, or kept the same if a deeper investigation into the lipidome is required. Indeed, Plumb *et al.* (2022) demonstrated that VJC performed better in all metrics measured when compared to a conventional column.

As the drive for increased throughput continues, utilising a mass spectrometer with a scan speed capable of sufficiently profiling ever decreasing chromatographic peaks is of paramount importance. The Xevo MRT MS has demonstrated that capability without any detriment to resolution, making it the ideal instrument to perform high-throughput studies.

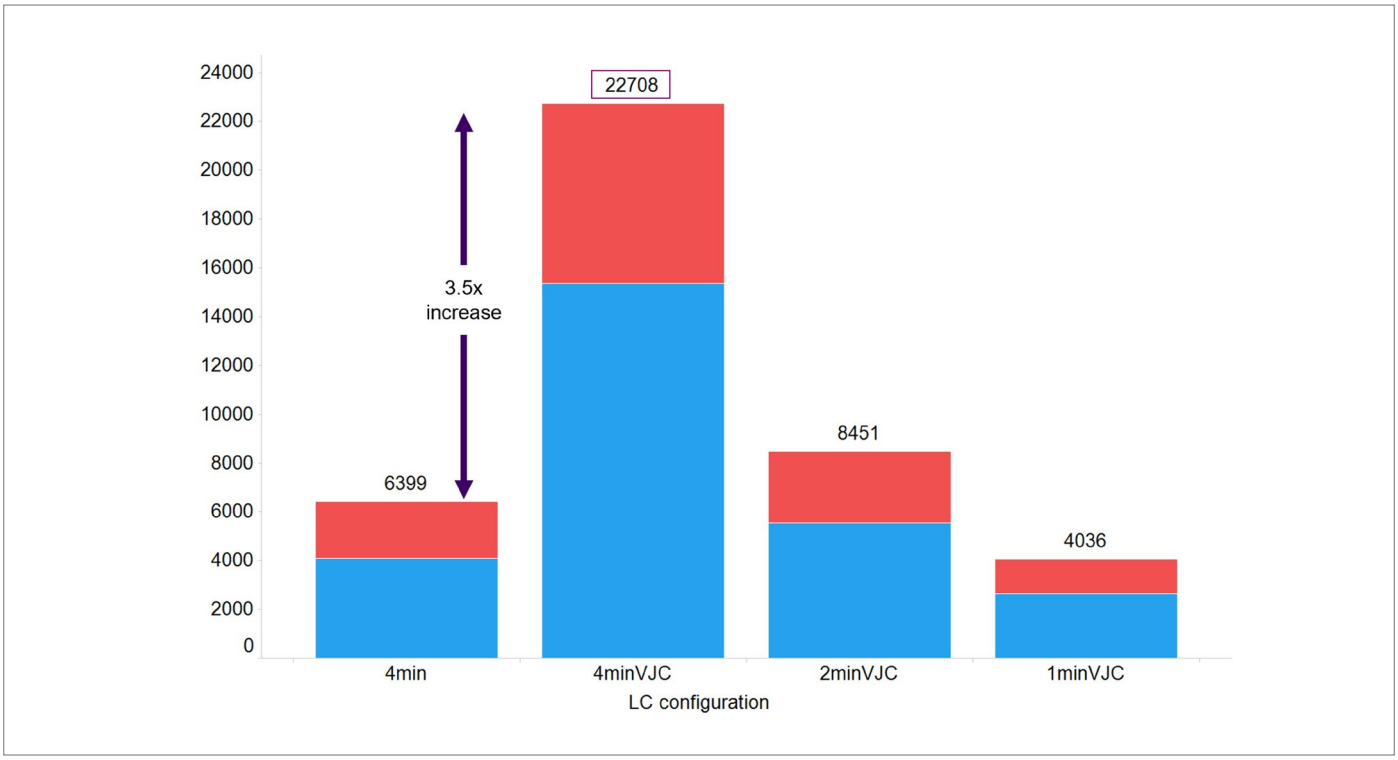

Figure 3. Stacked bar chart representing identified lipids (red) and unidentified features (blue) found using four different column and gradient combinations

CONCLUSION

Overall, applications of VJC for high-throughput analysis of lipids has been demonstrated using cancer patient samples. Transferring a standard method using conventional columns to VJC lead to 3.5 times more features identified and a ~37% decrease in solvent consumption. Whereas halving the gradient time while maintaining flow rate identified more features than a longer conventional method while also using ~70% less solvent. Employing an ultra-fast VJC method of one minute demonstrated sufficient profiling of the study cohort and clear separation between the healthy vs disease state using PLS-DA.

Finally, the Xevo MRT MS provided excellent and uncompromising mass resolution and when combined with fast scanning facilitated the high number of features identified from profiling narrow chromatographic peaks, a feat not possible with trapping mass spectrometers.

REFERENCES

1. *Gray, N., Adesina-Georgiadis, K., Chekmeneva, E., Plumb, R. S., Wilson, I. D., and Nicholson, J. K. Development of a Rapid Microbore Metabolic Profiling Ultraperformance Liquid Chromatography–Mass Spectrometry Approach for High-Throughput Phenotyping Studies. Analytical Chemistry 2016, 88 (11), 5742-5751. DOI: 10.1021/acs.analchem.6b00038*
2. *Plumb, R. S., Isaac, G. I., Rainville, P. R., Hill, J., Gethings, L. A., Johnson, K. A., Lauterbach, J. and Wilson, I. D. High Throughput UHPLC-MS-Based Lipidomics Using Vacuum Jacketed Columns. Journal of Proteome Research 2022 21 (3), 691-701. DOI: 10.1021/acs.jproteome.1c00836*
3. *Conroy, M. J., Andrews, R. M., Andrews, S., Cockayne, L., Dennis, E. A., Fahy, E., Gaud, C., Griffiths, W. J., Jukes, G., Kolchin, M., Mendivelso, K., Lopez-Clavijo, A. F., Ready, C., Subramaniam, S., and O'Donnell, V. B., (2023) LIPID MAPS: update to databases and tools for the lipidomics community. Nucleic Acids Research, DOI: 10.1093/nar/gkad896*
4. *Pang, Z., Lu, Y., Zhou, G., Hui, F., Xu, L., Viau, C., Spigelman, A., MacDonald, P., Wishart, D., Li, S., and Xia, J. (2024) MetaboAnalyst 6.0: towards a unified platform for metabolomics data processing, analysis and interpretation Nucleic Acids Research (doi: 10.1093/nar/gkae253)*

Waters™

Waters Corporation
34 Maple Street
Milford, MA 01757 U.S.A.
T: 1 508 478 2000
F: 1 508 872 1990
waters.com