

# EMPLOYING BALLISTIC GRADIENTS, VACUUM JACKETED COLUMNS AND A NOVEL MRT TO INCREASE LIPIDOMIC THROUGHPUT WHILST MAINTAINING HIGHLY CONFIDENT IDENTIFICATIONS

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## INTRODUCTION

One 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 <sup>1</sup>. However, this does come at the cost of peak capacity and feature detection.

One way to maintain narrow peak widths is to move the column significantly closer to the MS 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 (Figure 1) <sup>2</sup>.

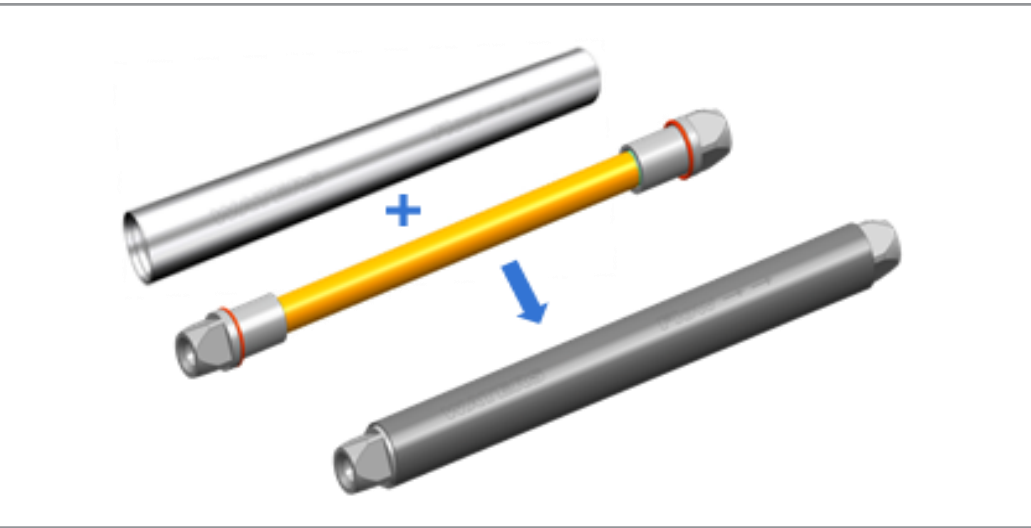


Figure 1. A vacuum sleeve is placed over a conventional column to produced a vacuum jacketed column.

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<sup>TM</sup> MRT Mass Spectrometer is capable of scanning at 100Hz while maintaining a mass resolution of 100,000 full width half maximal (FWHM), necessary for sufficiently profiling ever decreasing peak widths (Figure 2).

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.

## METHODS

Lipids from serum samples of healthy controls and patients with colon or rectum cancer were extracted using IPA and spiked with EquiSPLASH<sup>TM</sup> 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<sup>TM</sup> UPLC<sup>TM</sup> column over a four minute gradient at 0.8 ml/min. Solvent A was ACN:H<sub>2</sub>O: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 3 min. Column wash at 99.9 % B at 3.2 min for 0.5 min before column re-equilibration at 1 % B until 4 mins. A corresponding column in VJC format (2.1 mm x 50 mm CSH) was also applied for the separation.

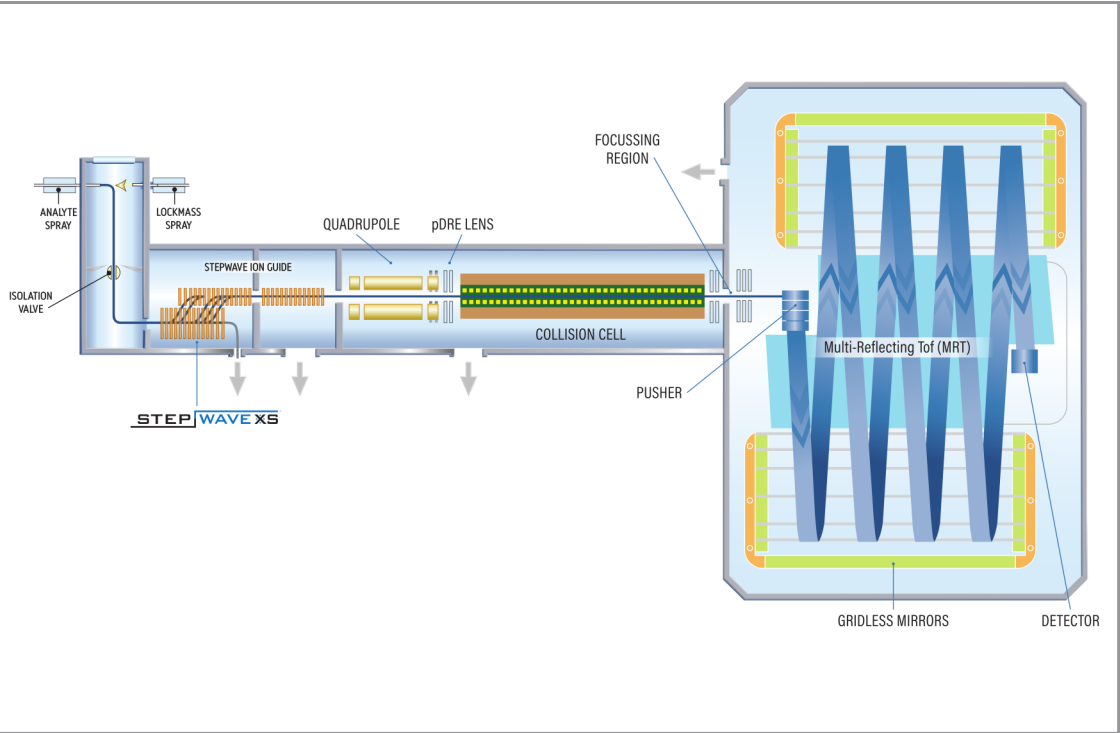


Figure 2. Schematic of the Xevo MRT MS

For VJC configurations, the flow rate was scaled down to 0.5 ml/min while maintaining chromatographic gradient over 4 mins, before a shortened equivalent gradient of 2 minute and 1 minute, respectively, was applied. The eluate was directed towards a prototype benchtop multi reflecting time-of-flight (MRT) mass spectrometer operating at 20 Hz (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<sup>E</sup>) where alternate scans of low collision energy (6 eV) and high collision energy (20-40 eV ramp) are recorded.

Generated data were converted to .mzML format using DATA Convert as the injection finished acquiring before import into Lipostar 2<sup>TM</sup> for peak picking and identification of features and subsequently lipids using the LIPID MAPS<sup>®</sup> structure database <sup>3</sup>. Exported compound measurements from Lipostar were further analysed using MetaboAnalyst vers 6 <sup>4</sup> to generate PLS-DA plots.

Analysis of retention time and chromatographic peak width was completed within LC-MS Toolkit, an application available through waters\_connect<sup>TM</sup>.

## 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 more number of features and subsequently identified lipids.

- Maintenance in base peak width of chromatographic peaks when using a conventional column vs VJC (Figure 3) but did increase peak height due to increased ionisation efficiency resulting from lower flow
- Scaling down to 2 minutes gradient time decreased base peak width and lead to a further corresponding increase in peak height
- Increased peak height resulted in improved signal to noise using VJC and therefore sensitivity of the method was also increased
- Increase in sensitivity lead to 3.5x number of features identified using a 4 min VJC method compared to a conventional column (Figure 4)
- Shortening the gradient by 50% using a VJC also yielded more features compared to a 4 minute method with a conventional column, leading to a 100% increase in throughput and ~70% reduction on solvent consumption

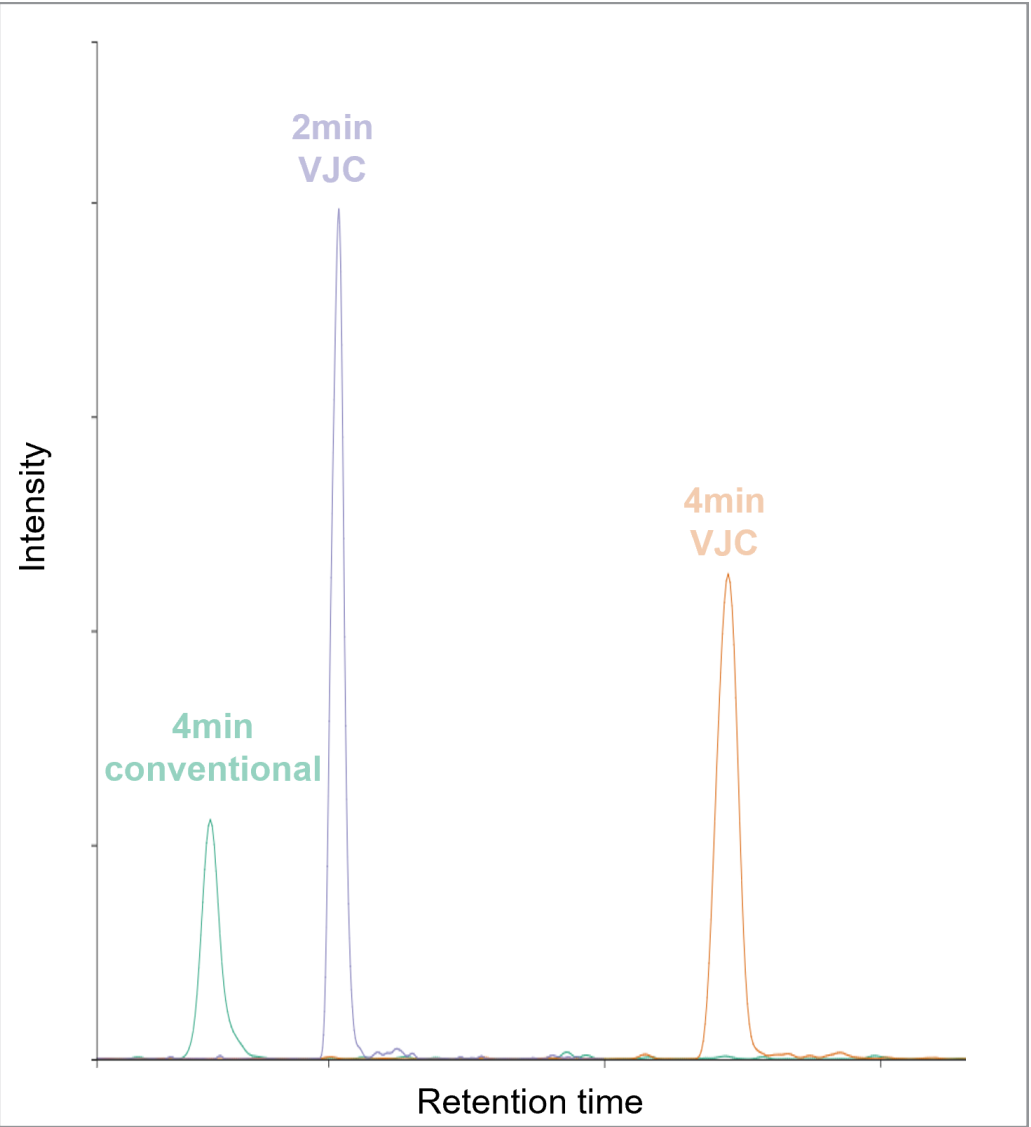


Figure 3. Extracted ion chromatogram of phosphatidylserine with m/z 755.5562 using a 2.1mm conventional column (green), 2.1mm VJC (4 min method; orange) or 2.1mm VJC column (2 min method; blue).

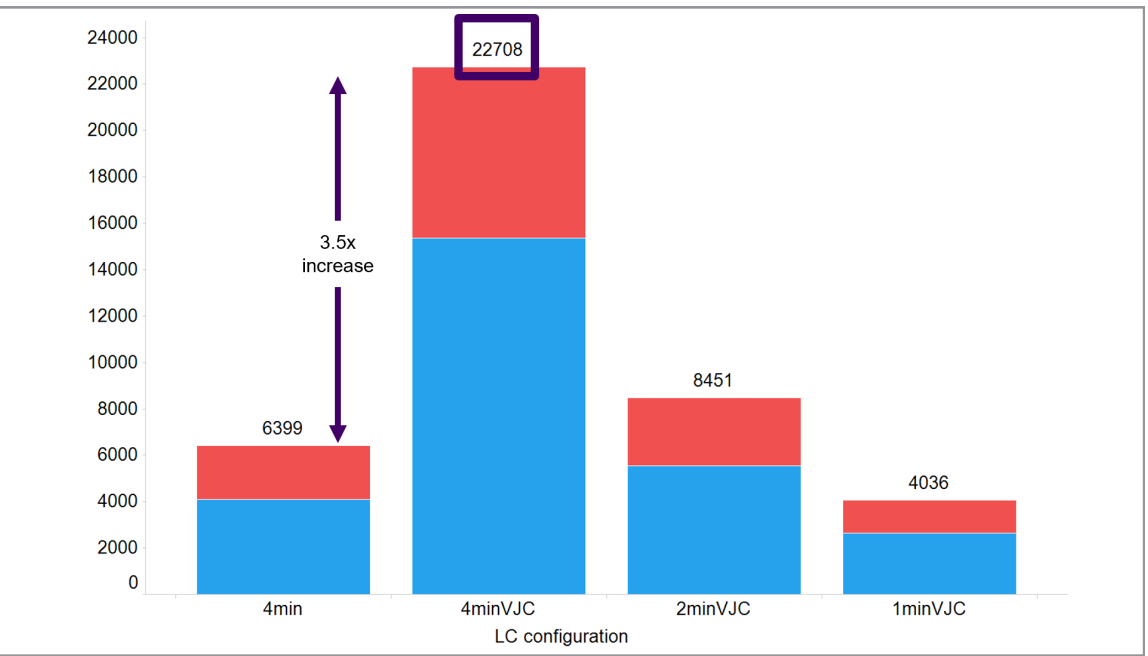


Figure 4. Stacked bar chart representing identified lipids (red) and unidentified features (blue) found using four different column and gradient combinations

- Decrease in gradient time did not impact the ability to sufficient profile the chromatographic peak owing the fast scanning on the Xevo MRT MS without any impact on mass resolution
- 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 (Figure 5). This offers a 300% increase in throughput and ~85% decrease in solvent consumption compared to the four minute method with a conventional column.

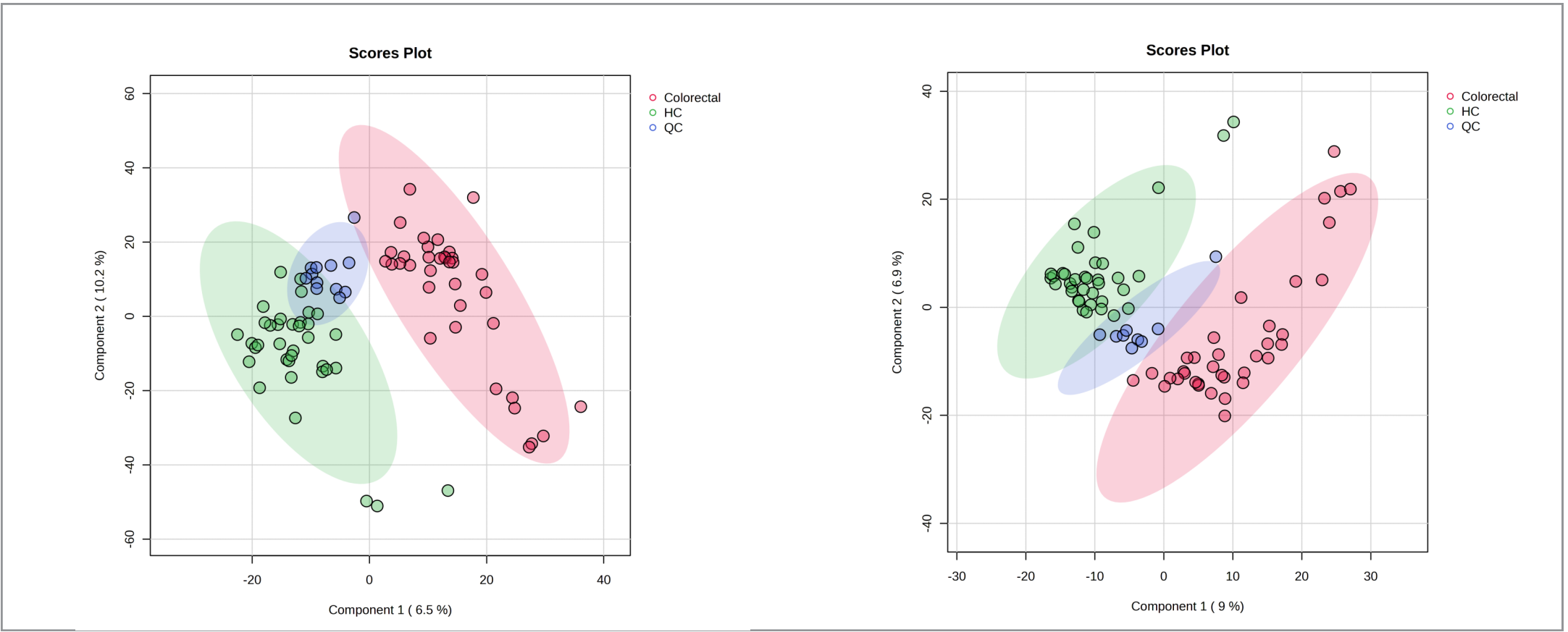


Figure 5. PLS-DA plot of features detected in all samples by 2min (left) and 1min (right) VJC method showing clear separation between the cancer, QC and control samples..

## 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 2.1mm 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 perfect instrument to perform high-throughput studies.

## CONCLUSION

- Transferring a standard method to VJC lead to 3.5x more features identified and a ~37% decrease in solvent consumption
- 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 1 minute demonstrated sufficient profiling of the study cohort and clear separation between the healthy vs disease state using PLS-DA
- 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

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