Using a vacuum jacketed column with a multi reflecting time-of-flight MS to increase extractables analysis throughput whilst maintaining identification confidence

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INTRODUCTION

- **Liquid chromatography-mass spectrometry (LC-MS) for extractables analysis comprises of highly complex samples. Methods account for a range of compound chemistries, including non-polar compounds which require a lengthy high organic gradient component to ensure full elution.[1]**
- **This can be costly, time consuming, and heavy on solvent use. Reducing chromatographic gradient run times could decrease the impacts of these methods, however, it may affect peak capacity and feature detection.**
- **Vacuum jacketed columns (VJC) can significantly increase peak capacity, narrowing peak widths and correspondingly increasing peak heights, thereby allowing faster chromatography.[2]**
- **This can be combined with a multi reflecting time-of-flight mass spectrometer (MRT MS) that can acquire at fast scan speeds without information loss.[3] The Xevo MRT Mass Spectrometer is capable of scanning at 50 Hz while maintaining a mass resolution necessary for sufficiently profiling over decreasing peak widths.**
- **Here we report the advantages of a VJC coupled to the Xevo MRT MS for an extractables screening analysis.**

Figure 1. Waters Xevo MRT Mass Spectrometer and ACQUITY Premier System.

METHODS

Sample Preparation

Pharmaceutical packaging was extracted in isopropanol and then spiked with an extractables and leachables system suitability test mix (E&L SST). The initial method conditions are listed below. The waters_connect™ software platform was used for data acquisition and the UNIFI™ Application out of the waters_connect™ platform was used for data processing.

LC Conditions: ACQUITY™ Premier System

Column: ACQUITY CORTECS™ C18, 90 Å (1.6 µm, 2.1 x 100 mm Column)
Mobile Phase A / B: Water + 1 mM ammonium acetate + 0.1% formic acid / Methanol
Flow Rate: 0.3 mL/min
Column Temperature: 50 °C
Injection volume: 1 µL
Gradient : Mobile phase B was held at 2% for 0.5 minutes before it was ramped to 98% over 5 and a half minutes then held for 7 minutes. It was then dropped to 2% for 2 minutes.

MS Conditions System: Xevo™ MRT MS

Ionization mode: ESI+
Acquisition mode: MS^E
Source temp: 120 °C
Desolvation temp: 550 °C
Desolvation gas flow: 800 L/hr
Cone gas flow: 50 L/hr
Acquisition range: *m/z* 50-1200
Capillary voltage: 2.5 kV
Collision energy: Low: 6 eV
High ramp: 20-45 eV

Parameters Changed

Columns

ACQUITY CORTECS™ C18, 90 Å (1.6 µm, 2.1 x 50 mm)
Prototype VJC Cortecs (1.6 µm, 2.1 x 100 mm)
Prototype VJC Cortecs (1.6 µm, 2.1 x 50 mm)

Column Temp / Flow Rates

50, 55 °C / 0.3, 0.4, 0.5 mL/min

Gradients

15 minute down to 1 minute, and change in % B

Mobile Phase

B: Acetonitrile

MS Scan Speed

10, 20, 50 Hz

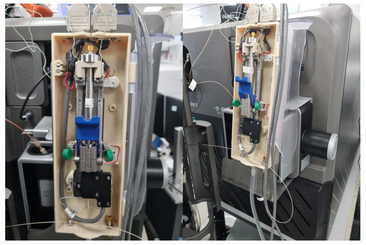

Figure 2. Prototype VJC mounted on the Xevo MRT MS.

VACUUM JACKETED COLUMN

The vacuum jacketed column (VJC) set up works in two ways to increase peak capacity and narrow peak widths[2]:

1. The column is significantly closer to the MS source which reduces post column volume. The emitter also has a narrower i.d. compared to standard flow electrospray ionization further reducing post column volume (Figure 2).

2.The vacuum jacket mitigates frictional heating and temperature differentials (Figure 3).

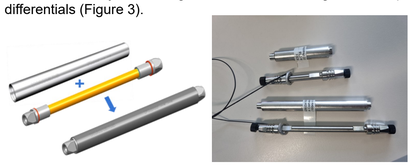

Figure 3. The vacuum jacket is placed around a traditional column.

In traditional UHPLC (ultra-high performance liquid chromatography) columns, frictional heating causes a temperature gradient across the column resulting in band broadening. Column ovens do help to mitigate this but a vacuum jacket provides near-adiabatic performance.

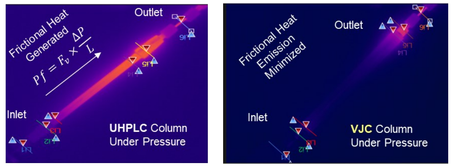

Figure 4. Frictional heating in the UHPLC column compared to the VJC column.

Increased peak capacity is achieved with VJC columns compared to UHPLC columns using the same column dimensions and chemistry, this is more pronounced at higher flow rates (Figure 5).

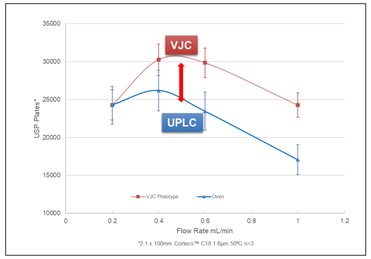

Figure 5. VJC improves LC efficiency. (Data from Dr Jason Hill, Waters Corporation.)

RESULTS

Scaling the column length and gradient resulted in reduced peak capacity and band broadening. Using the VJC column produced narrower peaks, particularly for the early eluting compounds shown in Figure 6 for the E&L SST masses extracted from the sample chromatograms.

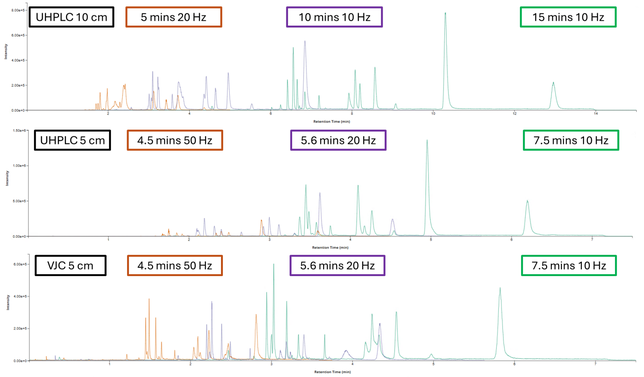

Figure 6. UHPLC 10 cm column with 15, 10, and 5 minute gradients. UHPLC 5 cm column with 7.5, 5.6, and 4.5 minute gradients. VJC 5 cm column with 7.5, 5.6, and 4.5 minute gradients.

The 10 cm VJC and the 10 cm UHPLC column with the same method conditions had comparable results but when reducing the gradient from 15 minutes the VJC demonstrated increased peak capacity with narrower and sharper peaks as can be seen here for Irganox 245 (Figure 8).

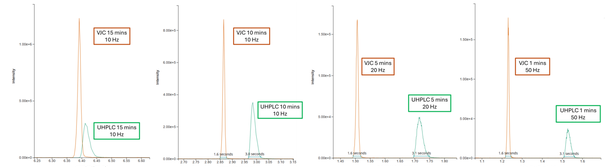

Figure 7. Comparing the VJC and UHPLC 10 cm columns for decreasing gradients for Irganox 245.

The BPI (base peak intensity) chromatogram from a 5 cm VJC and a 5 cm UHPLC column of the samples is shown in Figure 8. Particularly at the early stages of the gradient there is less band broadening and sharper peaks which on average are more intense.

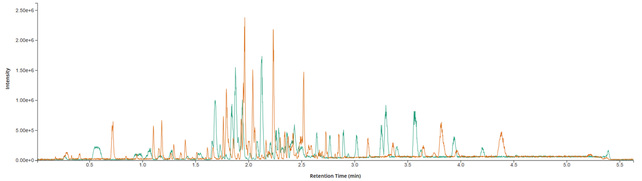

Figure 8. The BPI of the sample injections comparing the VJC and UHPLC 5 cm columns.

DISCUSSION

The samples were screened against the Waters E&L Library to find matches based on accurate mass of precursors and fragment ion matches.[4] Filters can be applied to significantly reduce the number of potential possible candidates increasing confidence in the identifications. The Xevo MRT MS has high mass accuracy (Figure 9) so a mass error filter of ≤1 ppm can be applied. All columns resulted in over 10000 features. Just looking for identified took them all to over 250 identifications. Applying the mass error filter and fragment ion match took them down to >10 identifications.

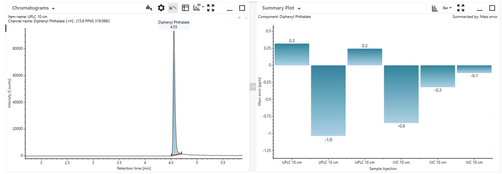

Figure 9. Mass error of ≤1 ppm for diphenyl phthalate for replicate injections on the UHPLC and VJC 10 cm columns.

CONCLUSION

- **Extractables screening methods are typically long and reducing the chromatographic gradient can reduce the burden on cost, time, and environmental impact of a method. Keeping the same column, however, can increase band broadening and reduce peak capacity.**
- **Using a vacuum jacketed column helps two fold by reducing the post column volume and minimizing frictional heating. This was demonstrated, for an extractables analysis, to reduce peak widths and increase peak capacity at shorter gradient times and shorter column lengths, particularly for the early eluting compounds.**
- **This can be combined with the fast scanning facilitated by the Xevo MRT MS to maintain the number of features found and identifications made. Therefore, the chromatographic gradient can be shortened >30%, increasing analysis throughput.**

References

1. Sanig R., Riba I. Analytical Considerations for Extractables Screening With Liquid Chromatography-High Resolution Mass Spectrometry. Application Note 720007492, January 2022.
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. Fox J., Qing Yu Y., Berger S J., Pittman N. Advanced Oligonucleotide Characterization Using Multi-Reflecting Time-of-Flight Technology. 720008764, April 2025
4. McCullagh M., Mortishire-Smith R. J., Goshawk J., Cooper J., Obkircher M., Sprecher H., Koehling R., Nold m., Jacobsen J., Sanig R., Extractables, Leachables, and Contact Materials: The Invaluable Benefit of Ion Mobility-Enhanced Mass Spectrometry Libraries, Application Note 720007655, June 2022.

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