Application of Vacuum Jacketed UHPLC Columns Coupled with Mass Spectrometry in High Throughput Pharmaceutical Analysis

Paul D. Rainville, Thomas McDonald, Jason Hill, and Robert S. Plumb Waters Corporation 34 Maple Street Milford, MA 01757

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Introduction

Increased sensitivity, resolution, and speed of analysis have long been the hallmarks resulting from the use of sub 2 µm chromatographic particles. However, the use of these small chromatographic particles also results in deleterious frictional heating as the effluent moves through the column at pressures up to 18, 000 psi. This heating causes chromatographic band broadening and losses in analyte resolution. The use of vacuum technology and novel column housings has been shown to overcome these challenges (1,2) . Here we show the application of this technology coupled with mass spectrometry for high throughput analysis of verapamil metabolism produced via microsomal incubations.

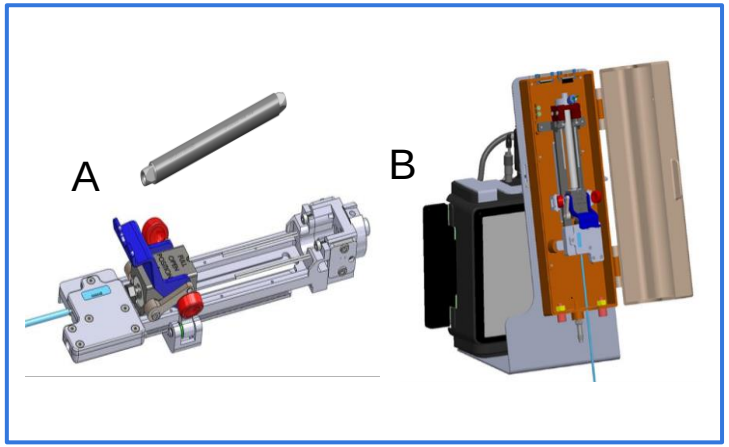

Figure 1A. Schematic drawing of VJC and novel column housing. Figure 1B. Schematic drawing of the novel column housing located near the source of the mass spectrometer.

Results/Discussion

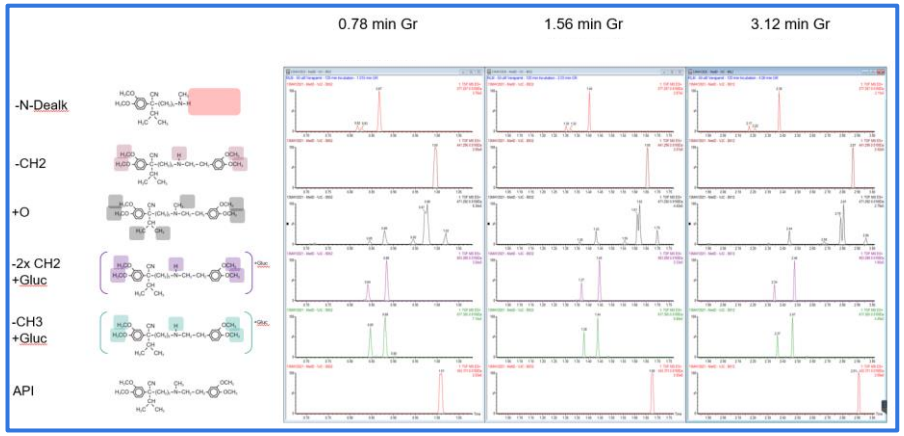

Figure 2. Example total ion chromatogram of the separation of Verapamil and associated metabolites under various gradient (Gr) duration with the VJC/MS.

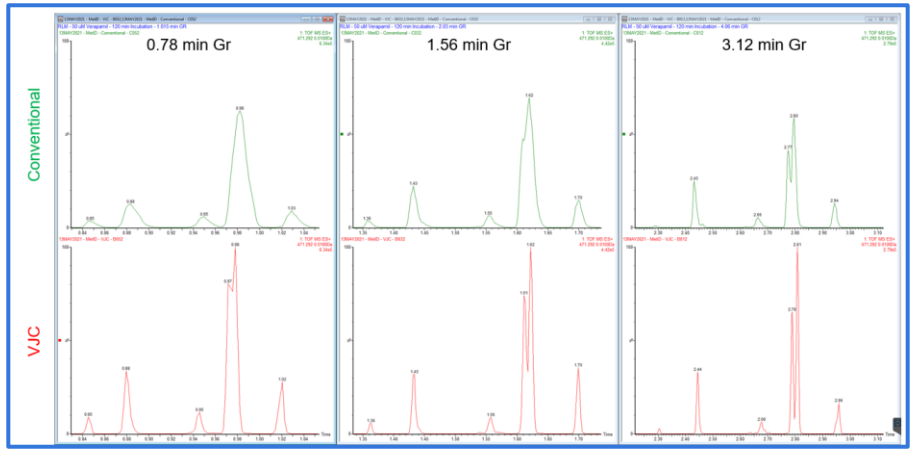

Figure 3. Zoomed in view of total ion chromatogram on hydroxylated metabolites of verapamil shown with conventional sub 2µm LC/MS and VJC/MS.

The VJC features flat face seals at the inlet and outlet of the column that reduces the need for conventional threaded fittings. The novel column housing enables the VJC to be incorporated in a "plug and play " fashion into the housing. The housing is located just outside of the ESI source as a means to reduce any post column dispersion that may occurs from the out let of the column to the inlet of the ESI source (Figure 1). Data produced from the VJC/MS system showed that rapid separations were possible of verapamil and associated metabolites with analysis times of under 1 minute (Figure 2). Comparisons of data generated by the VJC/MS system conventional LC/MS showed that narrow peak widths and increases in peak height were better with the VJC/MS system (Figure 3). The data produced from these studies shows that the promise and future application of VJC/MS technology to other high through put analysis in the pharmaceutical industry.

Experimental

LC System: Waters ACQUITY™
I Class SystemFlow rate:0.6 mL/min
Gradient: linear gradients with various end times
Mobile phase A: 0.1 % Formic acid/Water
Mobile phase B: 0.1 % Formic acid /Acetonitrile
Columns: 2.1 x 50 mm Conventional and VJC were packed with 1.7 µm bridged ethylene hybrid C18 packing material
Injection volume: 2 µL

Mass Spectrometry System: Xevo™ G2-XS Q-ToF
Capillary Voltage: 2 KV
Cone Voltage: 50 V
Source Temperature: 140 °C
Desolvation Temperature 600 °C
Desolvation gas: 1000 L/Hr
Cone gas: 50 L/Hr
Acquisition mode:50-1000 Da MSE mode

Sample preparation

Samples were preincubated in phosphate buffer with 1 mg/mL of microsomal protein and 50 µM of the enzymatic substrates for five minutes at 37 °C. Reactions were initiated via addition of UDPGA and NADPH (20 mM final concentration). Aliquots of the reaction mix were removed and immediately mixed with an equal volume of cold acetonitrile. Samples were centrifuged and the supernatant drawn off and mixed with an equal volume of water.

Conclusions

The use of VJC technology in conjunction with a novel column housing successfully enabled high throughput analysis of under one minute for the probe pharmaceutical, verapamil and associated metabolites.

The VJC/MS system showed improvement in chromatographic resolution and peak height enabling the reduction in analysis time compared to conventional LC/MS.

References

1. Vacuum-Jacketed Columns: Maximum Efficiency, Easy Deployment Without Oven, and Improved LC–MS Performance, Gritti , F., LCGC, Special Issue-05-02-2019, Volume 32, Issue 5, Pages: 8–13

2. High-Throughput UHPLC/MS/MS-Based Metabolic Profiling Using a Vacuum Jacketed Column, Plumb, R., *et al*., Anal. Chem. 2021, Volume 93, Issue 30, Pages: 10644–10652

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