SPILL THE WINE: A RAPID MICROBORE METABOLIC PROFILING HILIC APPROACH COUPLED WITH ION MOBILITY HRMS FOR THE ANALYSIS OF ANTHOCYANINS IN RED WINE

Lauren Mullin[1], Adam King[2], Hernando Olivos[3], Robert Plumb[1]
[1] *Waters Corporation, 34 Maple St, Milford, MA 01757*
[2] *Waters Corporation, Stamford Avenue, Altrincham Road, SK9 4AX Wilmslow UK*
[3] *Waters Corporation, 100 Cummings Center Suite 407N, Beverly, MA 01915*

INTRODUCTION

- **Anthocyanins are phenolic pigment flavonoid compounds which are present in various plant derived food stuffs, including red wine[1],associated with red, blue and violet pigmentation**
- **Due to their polymerization behavior with tannins, they play a contributing role to the wine aging process[4], and exhibit numerous forms due to differing degrees of glycosylation and acylation[1].**
- **Recent studies from this group have investigated anthocyanin analysis through the use of HILIC coupled to HRMS[1,2], showing some improvements over conventional RPLC**
- **The presented study demonstrates the use of a rapid HILIC separation achieved through the use of microbore fluidics[3] in the analysis of eight different store bought red wine samples for the analysis of selected anthocyanins.**
- **Additionally, the use of travelling wave ion mobility separation (TW-IMS) coupled with HRMS acquisition provides a gas-phase separation which resolves some chromatographic co-elutions.**

METHODS

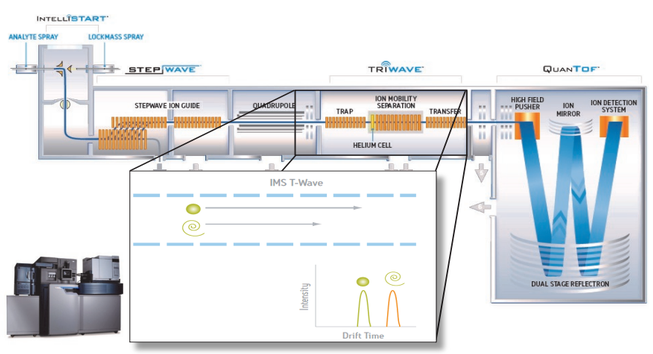
UPLC Conditions:

System: Waters ACQUITY I-Class
Column: BEH Amide 1.7µm 1.0 x 50 mm
Column Temp.: 50 ˚C
MP A: water + 0.1% formic acid
MP B: acetonitrile +0.1% formic acid
Injection Volume: 0.2µL
Gradient:

	Time (min)	Flow (mL/min)	%A	%B	Curve
1	Initial	0.200	1.0	99.0	Initial
2	0.03	0.200	1.0	99.0	6
3	2.33	0.200	50.0	50.0	6
4	2.37	0.200	1.0	99.0	6
5	3.33	0.200	1.0	99.0	6

HRMS Conditions:

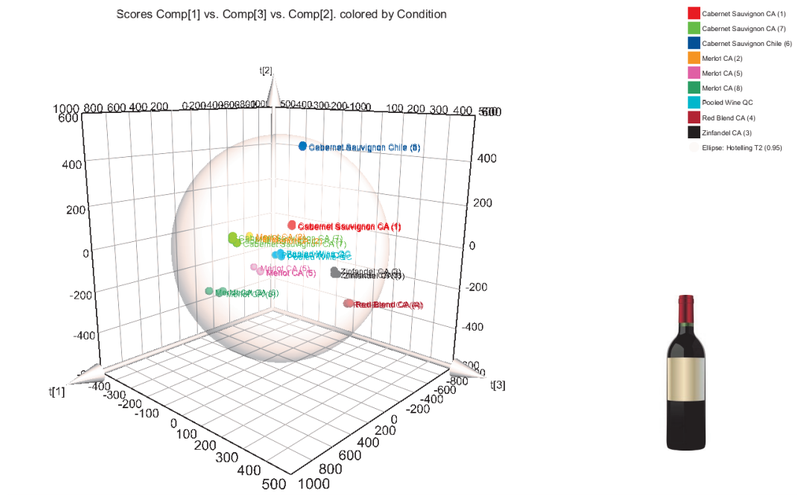
System: Synapt G2-Si with low-flow ESI source probe
Polarity: ESI^+
Capillary Voltage: 1.5 kV
Cone Voltage: 30 V
Mass Range: 50-1200 Da
Scan Time: 0.1 sec
Mobility Wave Height: 40 V
IMS Wave Velocity: 650 m/s
Mobility Gas: N_2
Tubing ID interfacing with MS (direct from column): 0.0025 in.



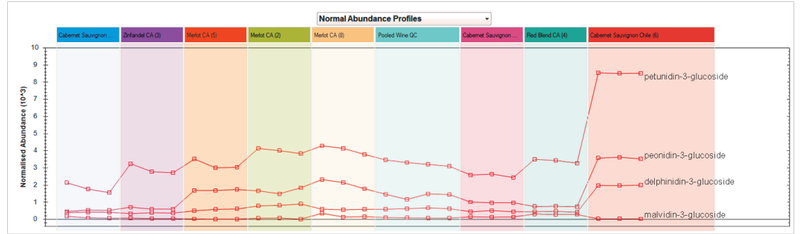
TW-IMS HRMS system from Waters, the Synapt G2-Si.

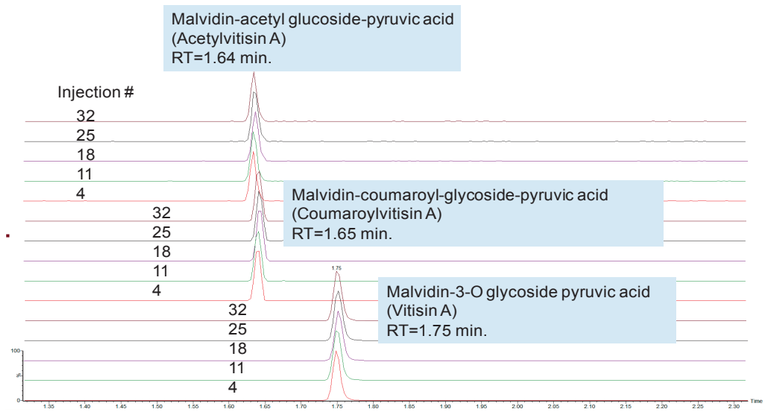
RESULTS AND DISCUSSION

Data was acquired in randomized, replicate injections of 1:1 diluted wine samples. Samples show consistent grouping per wine, as seen in the PCA plot.

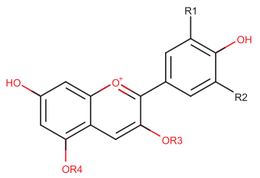


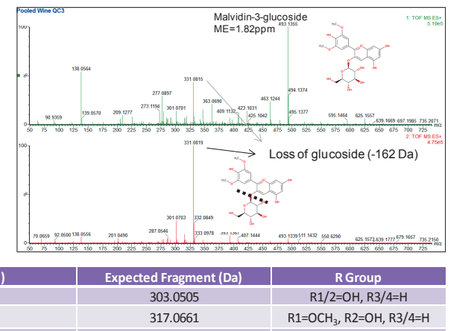
Tracking of the 3-glucoside anthocyanins in the trend plot below indicates stability of measurement across samples. Identifications were proposed based off accurate mass measurements of precursor and fragment ions, as displaced in the spectra. RT, fragmentation and R-group designation (as it relates to the anthocyanin basic structure) for each analyte is described in the adjacent table.



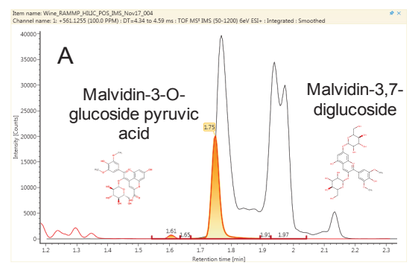


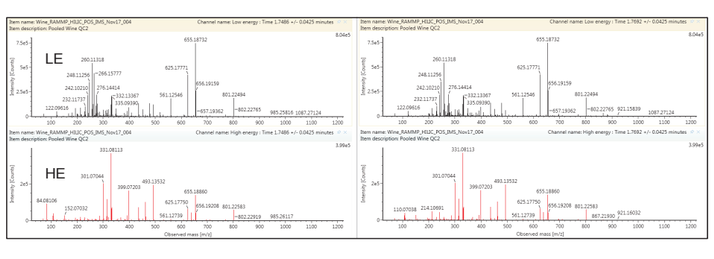
An example of chromatographic conservation is displayed above for the replicate injections. The total run time for the RAMMP HILIC method was 3.33 minutes.

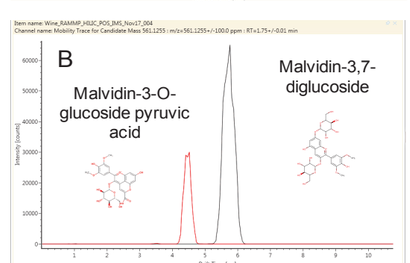


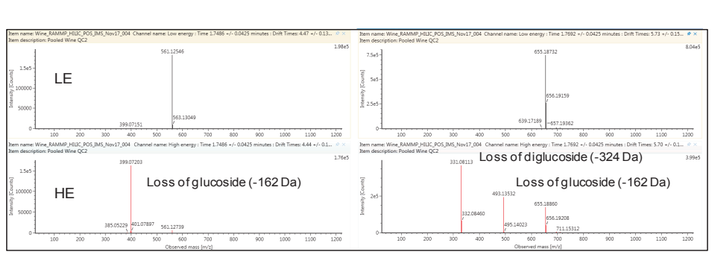


Anthocyanin	RT (min.)	Expected Fragment (Da)	R Group
Delphinidin-3-glucoside	1.95	303.0505	R1/2=OH, R3/4=H
Petunidin-3-glucoside	1.67	317.0661	R1=OCH_3, R2=OH, R3/4=H
Peonidin-3-glucoside	1.59	301.0712	R1=OCH_3, R2/3/4=H
Malvidin-3-glucoside	1.23	331.0818	R1/2=OCH_3, R3/4=H









In the case of some analytes, sub-optimal peak shape and co-elutions could be observed. One example is that of malvidin-3-O-glucoside pyruvic acid (Vitisin A) co-eluting with the unresolved malvidin-3,7-diglucose. Further resolution was afforded in the gas phase by the use of ion mobility. The mobility separation and of both malvidin-3-O-glucoside pyruvic acid and malvidin-3,7-diglucoside is evident. As a consequence of the mobility separation, spectral clarity is afforded through the removal of interferences that do not share the same drift time.

CONCLUSIONS

- **Various anthocyanins were found in red wine samples consistently using rapid HILIC separation**
- **Exact mass measurement of analytes provided structural information required for this complex mixture analysis**
- **Ion mobility separations greatly assisted in differentiation of co-eluting analytes, acting as a strong compliment of the rapid chromatography method**

References

1.Willemse C et al. (2013) *J. Chrom. A* 1319:127-140
2.Willemse C et al. (2015) *Anal. Chem.* 87:12006-12015
3.Gray G et al. (2016) Anal. Chem. 88:5742-5751
3.http://www.calwineries.com/learn/wine-chemistry/chemical-components-of-wine/anthocyanins

TO DOWNLOAD A COPY OF THIS POSTER, VISIT WWW.WATERS.COM/POSTERS