[TECHNOLOGY BRIEF]

Analysis of Nucleosides by UltraPerformance Convergence Chromatography (UPC^2)

Christopher Knappy,[1] Norman W Smith,[1] Arundhuti Sen,[1] Paul D Rainville,[2] and Robert S Plumb[2]
[1]King's College, London, UK; [2]Waters Corporation, Milford, MA, USA

GOAL

To demonstrate the advantages of utilizing UltraPerformance Convergence Chromatography™ (UPC$^{2®}$) to address the analysis of highly polar compounds.

BACKGROUND

Biological fluids contain a wealth of information regarding the metabolic state and health of an individual or sample cohort. While the analysis of a significant percentage of small molecule analytes contained in biological fluids can be readily achieved by reversed-phase liquid chromatography (RPLC), the analysis of highly polar analytes, such as nucleosides, can be a particular challenge due to the high polarity and resulting lack of retention that these molecules exhibit when analyzed by RPLC.

THE SOLUTION

Previous work by other investigators has shown the utility of UPC^2 for the analysis of polar pharmaceuticals contained in plasma.[1] In the study presented here, the use of UPC^2 to retain and separate nucleophilic bases was investigated. Four nucleosides (uracil, adenosine, cytosine, and cytidine) were selected because they represented some of the most challenging, highly polar, mammalian metabolites found in biofluids. Chromatographic retention, peak capacity, and resolution values were compared for these analytes using either silica- or organo-silica-based stationary phases

UPC^2 and Torus Column chemistry provides an improved method for the analysis of highly polar nucleophilic bases, an important class of biologically relevant metabolites.

Average critical pair resolution of adenosine and cytosine			
	35 °C	40 °C	45 °C
BEH	0.70	0.80	0.90
BEH 2-EP	0.28	0.36	0.31
CSH FP	0.98	0.95	0.87
HSS C_{18} SB	0.96	0.94	0.96
HSS Cyano	1.46	1.27	1.31
BEH HILIC	0.96	0.77	0.66
BEH Amide	1.20	1.21	1.00
DIOL	2.40	2.43	2.37
2-PIC	1.56	1.50	1.15
Torus DEA	4.68	4.48	4.58
1-AA	1.09	1.09	0.72

Table 1. Effect of UPC^2 parameters on average critical pair resolution.

in conjunction with a selection of bonded ligands and column temperature. The data displayed in Table 1 illustrates the effect of the stationary phase chemistry and column temperature on the chromatographic resolution of a critical pair of the nucleophilic bases: adenosine and cytidine. The average critical pair resolution was calculated using eleven columns, tested at three different temperatures (35, 40, and 45 °C). It is clear from the data shown in Table 1 that the Torus™ DEA chemistry exhibited the highest critical pair resolution for adenosine and cytosine compared to the other columns tested. Table 1 shows that the critical pair resolution values for these two nucleophilic bases was greater

than four for the Torus Column, an approximate two-fold increase compared to the DIOL column – which provided the second-best critical pair resolution value – and a greater than twelve-fold increase in the critical pair resolution with the 2-EP column – which is the most commonly utilized column chemistry in super critical fluid chromatography.

Figure 1 shows the separation of the four nucleoside bases and illustrates the increased resolution of the critical pair of adenosine and cytosine on the Torus Column as compared to the 2-EP column. Figure 1 further illustrates the improved retention for all of the nucleophilic bases separated on the Torus versus 2-EP columns. In addition to the evaluation of critical pair resolution and retention time, the peak capacity obtained from the different column chemistries and column temperatures was calculated (Table 2). As can be seen, the Torus Column again performed well in the analysis of these analytes, having average peak capacities in the order of 45 to 55 for a five-minute separation. Although higher peak capacities were obtained from the 2 PIC, 1 AA, and DIOL columns, the Torus Column exhibited a higher resolution of the critical pair of adenosine and cytosine, and a significantly higher peak capacity than the other seven columns tested.

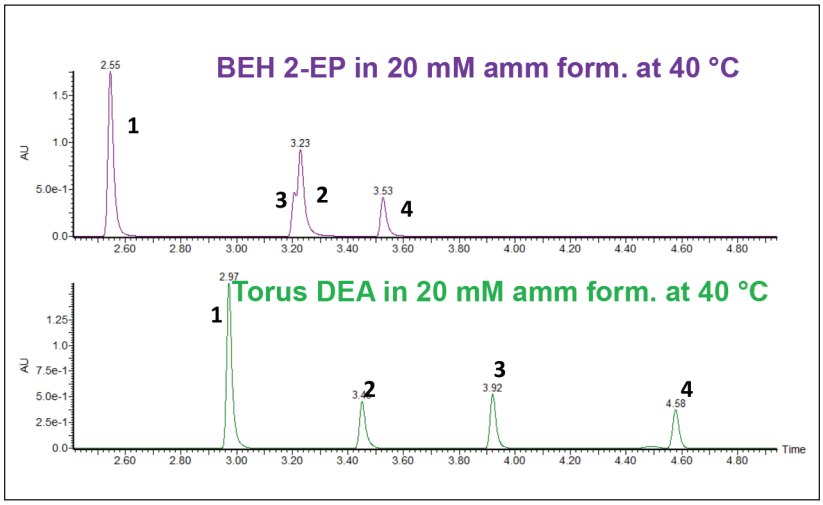

Figure 1. UPC² separation of Uracil (1), Adenosine (2), Cytosine (3), and Cytidine (4) on BEH 2-EP and Torus columns at 40 °C with 20 mM ammonium formate utilized as the co-solvent.

Average peak capacity			
	35 °C	40 °C	45 °C
BEH	38.57	45.60	47.87
BEH 2-EP	40.39	44.97	42.44
CSH FP	25.14	24.97	26.64
HSS C_{18} SB	37.39	39.51	37.57
HSS Cyano	23.27	23.28	20.88
BEH HILIC	29.94	28.14	28.32
BEH Amide	22.31	22.69	23.76
DIOL	61.20	62.36	63.00
2-PIC	66.51	66.53	68.23
Torus DEA	54.68	51.73	45.54
1-AA	52.27	53.74	50.33

Table 2. Average chromatographic peak capacities for column chemistries and temperatures utilized in the study.

SUMMARY

The data shown here illustrates the ability of UPC² for the analysis of highly polar, biologically-relevant metabolites, such as nucleosides. Four nucleosides – uracil, cytidine, adenosine, and cytosine – were all retained well by the UPC² separation. Furthermore, the utilization of the Torus DEA UPC² Column provided increased chromatographic performance in terms of peak capacity and critical pair resolution, as compared to other columns tested in this study.

Lastly, future work in coupling of the attributes of UPC² to retain highly polar, biologically-relevant metabolites to mass spectrometry may provide a useful analytical method for profiling and targeted analysis of biological samples.

References

1. Simeone, J. and Rainville, P. Direct Injection of Polar Compounds in Highly Organic Protein Precipitated Plasma by UPC2/MS/MS. Waters Application Note. 2013 720004754EN.

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