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Performance Assessment of the ACQUITY UPLC H-Class PLUS Binary System

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GOAL

To assess the performance of the ACQUITY™ UPLC™ H-Class PLUS Binary System.

BACKGROUND

Liquid chromatography methods often need to balance many factors, including the speed of analysis or throughput, sensitivity, selectivity, and robustness. Methods may also require the use of long, shallow gradients to ensure increased resolution of all analytes. This is often the case for peptide mapping analysis, as well as other profiling types of applications.

THE SOLUTION

With these challenges in mind, the solution combines the ACQUITY UPLC Binary Solvent Manager (BSM) PLUS with the ACQUITY UPLC H-Class PLUS Sample Manager – Flow Through Needle (SM-FTN). Taking advantage of the binary pump's low dwell volume and high-pressure mixing, and the ACQUITY UPLC H-Class PLUS SM-FTN's larger bore internal tubing, further increases robust operation.

The ACQUITY UPLC H-Class PLUS Binary System accommodates challenging gradients, providing an increase in robust method throughput when used with BSM PLUS.

Materials and Experimentation

Analytical standards of lidocaine HCl, naphazoline HCl, amitriptyline HCl, loperamide HCl, and tolazamide were sourced from Sigma-Aldrich (Poole, UK). A 1 mg/mL solution of all five compounds was prepared as a mixture in methanol as a stock solution. This stock solution was used to prepare a final working solution of 100 ng/mL in 50:50 methanol-water. The samples were analyzed using the method conditions detailed in Table 1.

The mixture was injected on the ACQUITY UPLC H-Class PLUS Binary System directly and also using the isocratic hold (300 µL) recommended by the Waters Column Calculator (Figure 1).

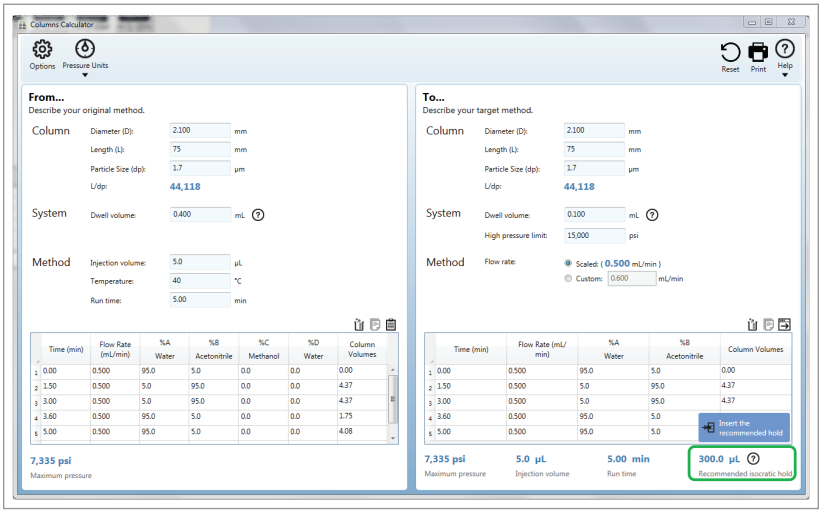


Figure 1. Waters Column Calculator with gradient modification for use with the BSM PLUS.

To assess within-day retention time reproducibility on the ACQUITY UPLC H-Class Binary System, six injections were performed with and without the isocratic hold. This was repeated on four occasions over a seven-day period to assess between-occasion robustness.

The mixture was also run on the ACQUITY UPLC H-Class PLUS Quaternary System on Day 7 for comparison.

Table 1. LC-MS method details.

Parameter	Value		
Mobile phase A (MPA)	2 mM ammonium formate/0.1% formic acid		
Mobile phase B (MPB)	Acetonitrile/0.1% formic acid		
Flow rate	0.5 mL/min		
Injection volume	5 µL		
Gradient conditions	Time (min)	%MPA	%MPB
	0	95	5
	1.5	5	95
	3.0	5	95
	3.6	95	5
	5.0	95	5
Column	ACQUITY UPLC BEH C_{18}, 1.7 µm, 2.1 x 75 mm		
ACQUITY QDa ESI positive mode	211 naphazoline HCl, 235 lidocaine HCl, 278 amitriptyline HCl, 312 tolazamide, 477 loperamide HCl		

RESULTS

Retention times for all compounds were consistent to two decimal places (Table 2).

The ACQUITY UPLC H-Class Binary System, without an isocratic hold, showed predictably shorter retention times for all compounds due to the system's reduced dwell volume when compared to the ACQUITY UPLC H-Class Quaternary System. The ACQUITY UPLC H-Class Binary System, with the calculated isocratic hold, showed a slight increase in retention times ranging between 6.7% and 10.5% (Table 3) due to volume differences between the ACQUITY UPLC H-Class Binary and the BSM PLUS volume used for the Waters Column Calculator.

Table 2. Summary of mean retention times (n=6) for all five components using the ACQUITY UPLC H-Class Binary System (with/without isocratic hold) on four occasions covering seven days, and the ACQUITY UPLC H-Class Quaternary System.

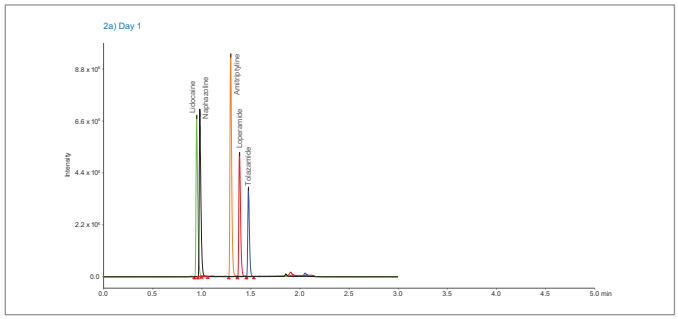
	Mean retention time (n = 6) of ACQUITY UPLC H-Class Binary								Mean retention time (n = 6)
	Without isocratic hold				With isocratic hold				ACQUITY UPLC H-Class Quaternary
	DAY				DAY				DAY
Compound	1	2	6	7	1	2	6	7	7
Lidocaine	0.96	0.96	0.96	0.96	1.54	1.54	1.54	1.54	1.40
Naphazoline	0.98	0.98	0.98	0.98	1.58	1.58	1.58	1.58	1.43
Amitriptyline	1.30	1.30	1.30	1.30	1.89	1.89	1.89	1.89	1.77
Loperamide	1.38	1.38	1.38	1.38	1.99	1.99	1.99	1.99	1.86
Tolazamide	1.48	1.48	1.48	1.48	2.08	2.08	2.08	2.08	1.95

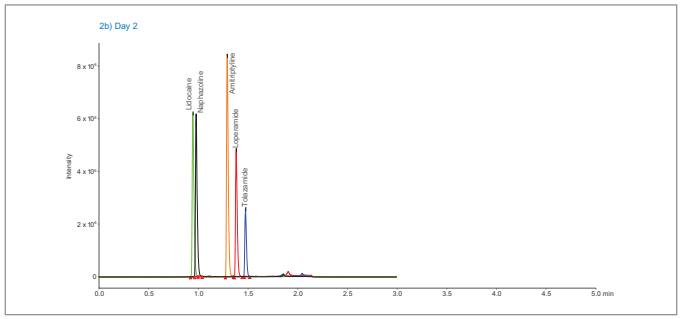
Table 3. Mean retention time differences (over four occasions) between the ACQUITY UPLC H-Class Binary System, with and without isocratic hold, and the ACQUITY UPLC H-Class Quaternary System.

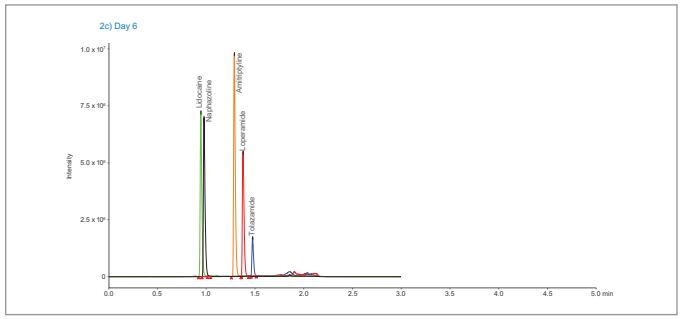
	Overall mean retention time ACQUITY UPLC H-Class Binary		Mean retention time (n = 6)	% RT difference (with RT ACQUITY UPLC H-Class Quaternary)	
	Without isocratic hold	With isocratic hold	ACQUITY UPLC H-Class Quaternary	Without isocratic hold	With isocratic hold
Lidocaine	0.96	1.54	1.40	-31.4	+10.0
Naphazoline	0.98	1.58	1.43	-31.4	+10.5
Amitriptyline	1.30	1.89	1.77	-26.6	+6.8
Loperamide	1.38	1.99	1.86	-25.8	+7.0
Tolazamide	1.48	2.08	1.95	-24.1	+6.7

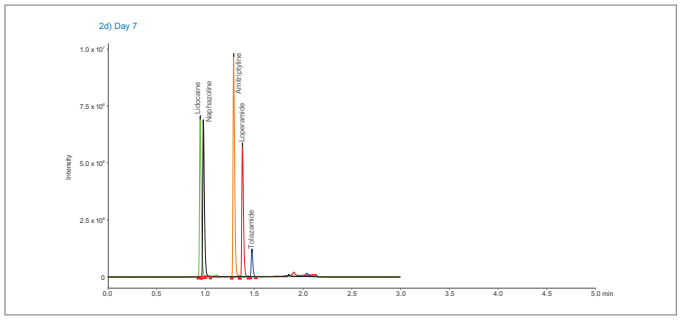
Figures 2a–2h show that the ACQUITY UPLC H-Class Binary System displays consistent chromatography within a single run over the four days tested.

ACQUITY UPLC H-Class Binary System without isocratic hold

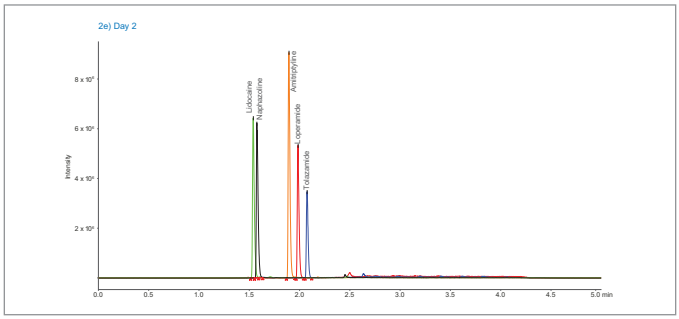


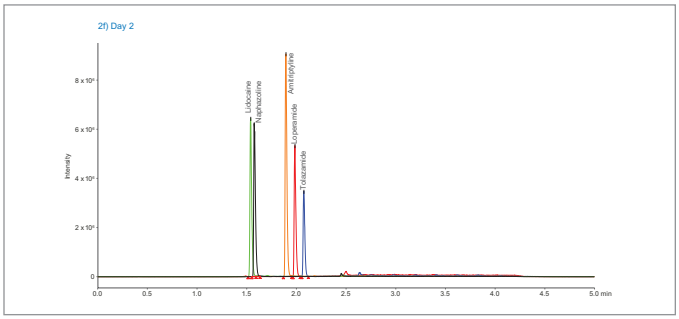


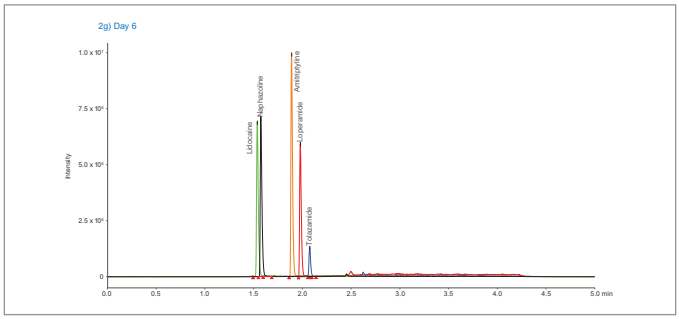


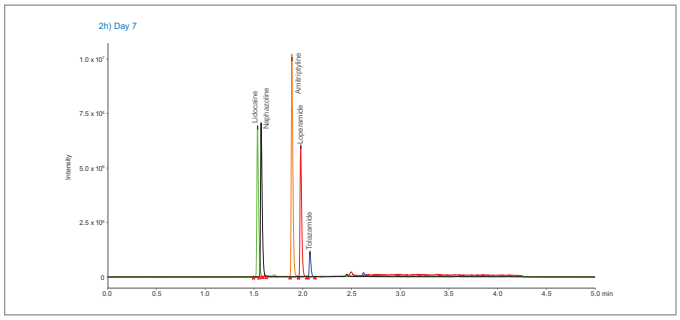


ACQUITY UPLC H-Class Binary System with isocratic hold

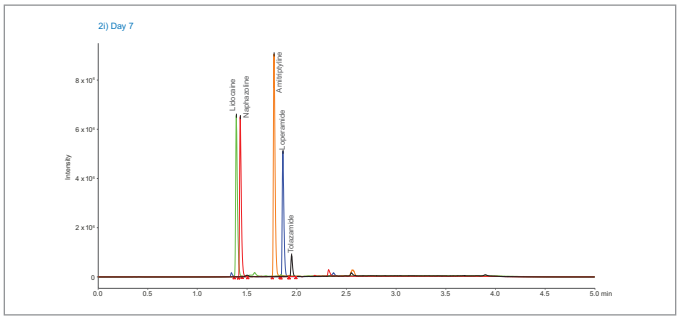








ACQUITY UPLC H-Class Quaternary System



Figures 2a–d. Injections of a 100 ng/mL sample mix using the ACQUITY UPLC H-Class Binary System with no isocratic hold.

Figures 2e–h. Injections of a 100 ng/mL sample mix using the ACQUITY UPLC H-Class Binary System with isocratic hold.

Figure 2i. Injection of a 100 ng/mL sample mix using the ACQUITY UPLC H-Class Quaternary System.

SUMMARY

The ACQUITY UPLC H-Class Binary System has shown to be a demonstrably robust platform for transferring the described method, exhibiting consistent retention times to two decimal places for all compounds tested both within- and between-day.

The difference in compound retention times, when directly transferring this method from the ACQUITY UPLC H-Class Quaternary to the ACQUITY UPLC H-Class Binary System (with no isocratic hold), ranges between a 24.1% and 31.4% reduction in retention time.

For direct method transfer from the ACQUITY UPLC H-Class Quaternary to the ACQUITY UPLC H-Class Binary System, with the intention of maintaining consistent retention times, the precise pre-column volume of the ACQUITY UPLC H-Class Binary would have to be ascertained to ensure an accurate transition.

Direct transfer without a gradient delay offers the opportunity for method improvement with the analysis time going from five minutes to three minutes, representing a 40% reduction in runtime.

REFERENCES

1. Henry, C.; Wrona, M.; Kirk, J.; Ladd, R. Waters ACQUITY UPLC H-Class Coupled with an ACQUITY QDa Detector to Provide a Highly Sensitive and Specific Solution for Cleaning Validation. Waters Application Note, 720005871EN (2016).

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