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Future-Proofing the QC Laboratory for UPLC While Enabling the Faithful Analysis of Legacy HPLC Methods

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APPLICATION BENEFITS

- The capability to faithfully and robustly run legacy HPLC methods whilst providing access to true UPLC® technology when desired
- No modifications required to provide seamless development, transfer and implementation of UPLC methods
- Unprecedented efficiency savings along with solvent usage costs and corresponding solvent disposal costs

WATERS SOLUTIONS

ACQUITY UPLC H-Class System

ACQUITY UPLC Tuneable UV (TUV) Detector

Empower® Chromatography Data System Software

ACQUITY UPLC BEH Column

KEY WORDS

UPLC, QC, efficiency, legacy, validation, H-Class, future-proofing

INTRODUCTION

In this application note, we describe how AstraZeneca's QC department in Macclesfield, UK (a global center for developing QC new technologies) has successfully transferred and run all their registered QC methods on an ACQUITY UPLC® H-Class System. Three high throughput products having been successfully developed and validated for UPLC. In an effort to update and modernise their labs, it was critical for AstraZeneca to ensure the new technology would be efficient, easy to adopt, and cost effective. AstraZeneca updated their LC platforms by implementing Waters® UPLC in their pharmaceutical development department, with the intention of developing all new products on UPLC. While future-proofing the QC department to receive newer UPLC methods, it was critical to retain the ability to faithfully and robustly run legacy chromatography methods. The technology of choice was the Waters ACQUITY UPLC H-Class System, which has now been deployed through the AstraZeneca QC department.

Here, we will give an example of a high profile compound 'B' legacy HPLC method that is transferred from an Agilent 1100 to an ACQUITY UPLC H-Class System, along with the newly developed UPLC method, validated on the same instrument.

EXPERIMENTAL

The new UPLC method for compound B degradant products was created using the ACQUITY UPLC Columns Calculator to simplify transfer and scale HPLC methodology quickly to UPLC conditions with equivalent performance (and significantly reduced run times and solvent savings), ensuring it satisfied the system suitability criteria stated in the legacy HPLC method.

Impurities 1 and 2 of compound B were validated over a range of 50–200% of their respective specification limits in the presence of the main compound B.

HPLC conditions

HPLC system:	Agilent 1100 or ACQUITY UPLC H-Class
Column:	C_8, 5 µm, 25 cm x 4.6 mm
Flow rate:	1.3 mL/min
Injection volume:	50 µL
Run time:	30 min
Detection:	UV

UPLC conditions

UPLC System:	ACQUITY UPLC H-Class equipped with TUV detector
Column:	ACQUITY UPLC BEH, C_8, 1.7 µm, 10 cm x 2.1 mm (P/N 186002878)
Flow rate:	0.3 mL/min
Injection volume:	4.2 µL
Run time:	6.86 min

Data management

Empower 2 Chromatography Data System (CDS) Software

RESULTS AND DISCUSSION

For the ACQUITY UPLC H-Class System to be a successful forward facing platform in the quality control environment, it must first be able to faithfully and robustly reproduce the chromatography being generated on the laboratory's existing HPLC platform. Figure 1 shows the comparison of compound B's system suitability sample (SST) run on the Agilent 1100, the ACQUITY UPLC H-Class System in HPLC mode, and the ACQUITY UPLC H-Class System again using the newly developed UPLC method.

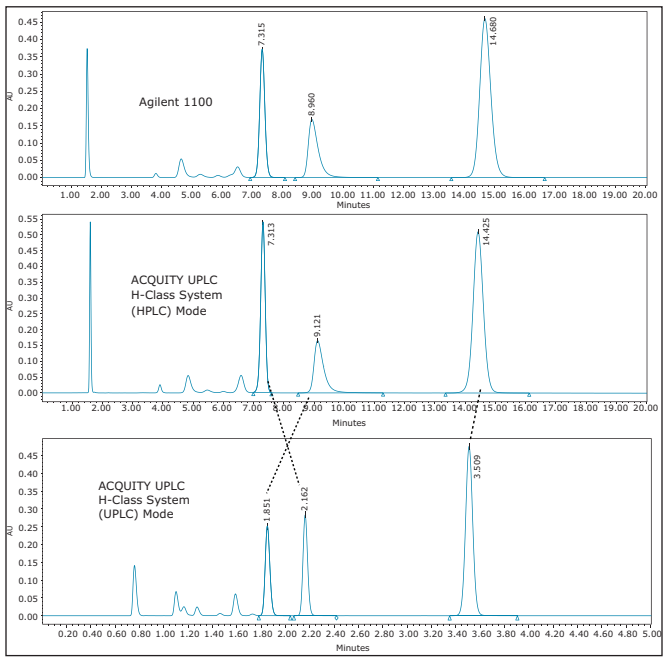

Figure 1. Comparison of compound B SST sample run on Agilent 1100 HPLC (top), ACQUITY UPLC H-Class System in HPLC mode (middle), and ACQUITY UPLC H-Class System in UPLC mode (bottom).

The ACQUITY UPLC H-Class System has reliably replicated the chromatography from the Agilent 1100 and reproduced the relative retention times (RRT's) of impurities 1 and 2, with respect to the main peak (Table 1). In HPLC mode the H-Class also consistently reproduces the peak areas of Compound B and it's related impurities compared to those obtained on the legacy LC system (Table 2).

Retention time comparability

Compound B	Main peak RT (mins)	Impurity 1 RT (mins)	Impurity RRT wrt main peak	Impurity 2 RT (mins)	Impurity RRT wrt main peak
Agilent 1100	14.680	7.315	**0.50**	8.960	**0.61**
ACQUITY UPLC H-Class System **HPLC** mode	14.425	7.313	**0.51**	9.121	**0.63**
ACQUITY UPLC H-Class System **UPLC** mode	3.509	2.162	**0.62**	1.851	**0.53**

Table 1. Retention times/relative retention times of compound B and impurities 1 and 2 generated using the legacy method on the Agilent 1100 and the ACQUITY UPLC H-Class System, along with the newly developed UPLC method results obtained from the ACQUITY UPLC H-Class System.

Peak area comparability

Compound B	Main peak area	Impurity 1 peak area	Relative peak area wrt main peak	Impurity 2 peak area	Relative peak area wrt main peak
Agilent 1100	11891513	4462703	**0.38**	3896619	**0.33**
ACQUITY UPLC H-Class System **HPLC** mode	11094170	4436024	**0.40**	3477557	**0.31**

Table 2. Relative peak areas with respect to the main peak of impurities 1 and 2. Results show consistent relative areas between the Agilent 1100 and the ACQUITY UPLC H-Class System in HPLC mode.

The UPLC method has a runtime of just under under 7 minutes, compared to the legacy method runtime of 30 minutes. There is also a marked improvement in peak symmetry. Impurities 1 and 2 have switched elution order, although this has not compromised system suitability criteria (Figure 1 bottom trace).

Validation

Once the newly developed UPLC method for compound B degradants had satisfied system suitability criteria, the method was subject to a partial validation based on ICH Guidelines Q2[1] covering linearity, recovery, repeatability, and limit of detection (LOD) and quantitation (LOQ).

The range of the the validation covered 50–200% of the impurities' respective specification limits, this exceeds the ICH Guideline's suggestion of 70–130% for added assurance of method robustness.

Table 3 summarises the validation data obtained. Raw linearity data is presented in Figure 2 and Table 3, method precision data in Table 4 and impurity 1 and 2 recovery raw data is presented in Table 5 and 6 respectively.

Test		Result	Acceptance criteria	Pass
Linearity	Imp 2	R^2 – 0.9999	>0.99	Y✓
		y-intercept – 0.03%	±5%	
	Imp 1	R^2 – 0.9992	>0.99	Y✓
		y-intercept – 0.30%	±5%	
Recovery	Imp 2	50% level: 98.8%	70.0–130.0%	Y✓
		100% level: 100.8%		
		200% level: 101.6%		
	Imp 1	50% level: 98.8%	70.0–130.0%	Y✓
		100% level: 100.8%		
		200% level: 101.6%		
Repeatability	Imp 2	4.9% RSD	<10.0%	Y✓
	Imp 1	1.21% RSD	<10.0%	Y✓
LOD		0.011% (1.918×10^{-2} µg/mL)	n/a	n/a
LOD		0.033% (5.755×10^{-2} µg/mL)	n/a	n/a

Table 3. Validation data for compound 'B' degradants method.

Linearity

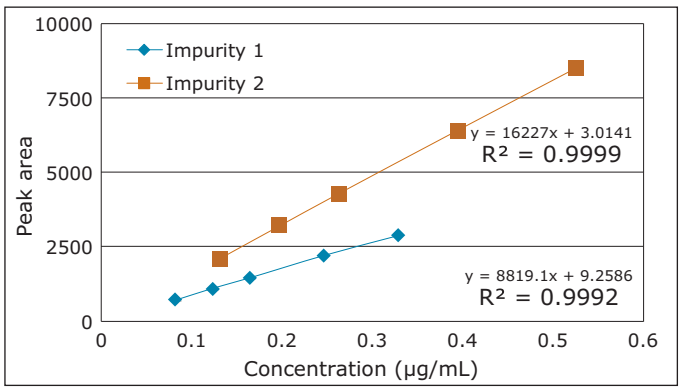

Figure 2. Linearity - The UPLC method for compound B degradants comfortably satisfied the acceptance criteria with linearity. Linearity was performed over the range of 50–200% of respective specification limits of impurities 1 and 2.

Conc impurity 1 (µg/mL)	Conc impurity 2 (µg/mL)	Peak area impurity 1	Peak area impurity 2
0.0822	0.1315	709	2114
0.1233	0.1972	1111	3205
0.1644	0.2630	1463	4295
0.2466	0.3944	2216	6418
0.3288	0.5260	2884	8520

Table 3. Linearity raw data.

Precision

Peak area impurity 1	Peak area impurity 2	
1446	4261	
1448	4271	
1453	4334	
1447	4286	
1493	4342	
1472	4276	
1460	**4295**	**Mean**
18.93	**34.36**	**SD**
1.3	**0.8**	**%RSD**

Table 4. Performed using six separate preparations of Compound B standard spiked with impurities 1 and 2 at their respective impurity limits (figures adjusted for background impurity content in standard).

Recovery

Impurity 1 recovery data

% of Nominal	Peak area	µg/mL	Mean peak area 100% (n=6)	% Recovery	Mean % (recovery)
50	717	0.0822	1460	98.219	
50	724	0.0822	1460	99.178	
50	722	0.0822	1460	98.904	98.8
100	1466	0.1644	1460	100.411	
100	1513	0.1644	1460	103.630	
100	1448	0.1644	1460	99.178	
100	1445	0.1644	1460	98.973	
100	1467	0.1644	1460	100.479	
100	1487	0.1644	1460	101.849	100.8
200	2926	0.3288	1460	100.205	
200	3034	0.3288	1460	103.904	
200	2944	0.3288	1460	100.822	101.6
			Overall mean	**100.5**	
			SD	**1.8**	
			%RSD	**1.8**	

Table 5. Recovery data for impurity 1 covering 50–200% of the range of specification limit. The 50% and 200% levels were prepared in triplicate, and the 100% level n=6 (100% data also used for precision).

Impurity 2 recovery data

% of nominal	Peak area	µg/mL	Mean peak area 100% (n=6)	% Recovery	Mean % recovery
50	2152	0.1315	4295	100.210	
50	2113	0.1315	4295	98.393	
50	2077	0.1315	4295	96.717	98.4
100	4261	0.2630	4295	99.208	
100	4271	0.2630	4295	99.441	
100	4334	0.2630	4295	100.908	
100	4286	0.2630	4295	99.790	
100	4342	0.2630	4295	101.094	
100	4276	0.2630	4295	99.558	100.0
200	8536	0.5260	4295	99.371	
200	8503	0.5260	4295	98.987	
200	8561	0.5260	4295	100.210	99.2
			Overall mean	**99.4**	
			SD	**1.2**	
			%RSD	**1.2**	

Table 6. Recovery data for impurity 2 covering 50–200% of the range of specification limit. The 50% and 200% levels were prepared in triplicate, and the 100% level n=6 (100% data also used for precision).

Efficiency and savings

The implementation of a faster UPLC method will have a significant positive impact on workflow efficiency, as well as the cost of solvent use and corresponding solvent disposal costs.

Compound B has had all associated legacy methods transferred to UPLC Technology, and was successfully validated. Table 7 shows calculated cost and efficiency savings based on AstraZeneca's batch throughput of compound B.

	Runtime/Month (hours)				Solvent Volume/Month (litres)				Solvent Cost/Month			
	HPLC	UPLC	% Save	Actual Saving	HPLC	UPLC	% Save	Actual Saving	HPLC	UPLC	% Save	Actual Saving
Content	120.0	27.4	**77.2**	**92.6**	9.36	0.49	**94.8**	**8.87**	£30.51	£1.60	**94.8**	**£28.91**
Dissolution	81.7	14.9	**81.8**	**66.8**	6.37	0.72	**88.7**	**5.65**	£20.77	£2.34	**88.7**	**£18.43**
Assay/ Related Substances	140.0	32.0	**77.1**	**108.0**	10.92	0.58	**94.7**	**10.30**	£35.60	£1.89	**94.7**	**£33.71**
Total				**267.4**				**24.82**				**£81.05**

Table 7. Estimated workflow efficiency and solvent cost savings with the implementation of UPLC Technology for all compound B methods, based on AstraZeneca's batch throughput.

CONCLUSIONS

The UPLC data detailed for compound B shows a time saving of 77–82%, equating to over 267 hours per month, with solvent savings of 89–95% per month. This not only impacts solvents costs associated with purchase and disposal, but also reduces the need for large storage volume, impacting space savings, and health, and safety.

The Waters ACQUITY UPLC H-Class Systems' success in transitioning legacy methods within the Quality Control environment of AstraZeneca exemplifies the instrument's ability to offer a seamless alternative to existing HPLC platforms, while uniquely offering the option of true UPLC technology when desired.

AstraZeneca now successfully runs all registered QC methods on an ACQUITY UPLC H-Class System, with three high throughput products successfully transferred to UPLC and validated.

The success of the Waters ACQUITY UPLC H-Class System in the Quality Control department of AstraZeneca Macclesfield has led to a wider adoption of the system by AstraZeneca globally.

References

1. ICH Guidelines : Validation of analytical procedures: Text and methodology Q2(R1).
2. USP General Chapter, <621> Chromatography, USP36-NF31, The United States Pharmacopeia Convention, official December 1, 2013.

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