EVALUATING TRANSFERABILITY OF METHODS BETWEEN LIQUID CHROMATOGRAPHY INSTRUMENTATION

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INTRODUCTION

A majority of methods existing today in compendia and internal SOPs were developed on instrumentation dating back as far as 20 years ago. The more commonly used legacy High Performance LC (HPLC) instruments were generally quaternary low-pressure mixing systems. System evaluations would indicate large system volumes, a milliliter or more, as well as excessive extra-column volume contributing to wider peaks. As column technologies evolved to smaller particle sizes, theoretical benefits were not realized using these legacy instruments. As a result, LC instrumentation has evolved to reduce such band broadening effects in the form of UltraPerformance LC (UPLC). Today's technology now provides chemists with the system performance and flexibility to aid method transfer of legacy methodology.

Although these advances in LC technology are tremendous for the advancement of chromatographic science, the implication of fostering the new technology for the pharmaceutical industry becomes a challenge. Direct transfer of these methods to the newer technology may result in retention time and selectivity differences that may be related to decreases of system volume, different implementations of temperature control, or gradient mixing mechanisms used by today's instrumentation. Because of these differences, method transfer has been considered labor intensive, challenging and expensive. The pharmaceutical industry realizes the benefits of adopting today's instrumentation; however, the instrumentation must be able to provide a dual purpose of performing legacy methods and sub-2-µm methodology in their QA/QC environment without complications for increased asset utilization.

In this presentation, various compendia methods are used as examples highlighting a new method transfer calculator to facilitate the transfer of methods to and from any LC-based instrumentation with ease.

SYSTEM VOLUME PROCEDURE

Materials

- Capillary flow restrictor that gives 1500-2000 psi at 0.75 mL/min acetonitrile. (Waters P/N 430002180), approximately 30 cm of 50 mm I.D. fused silica capillary
- Low dead volume union: (2 required) (Waters P/N 700002636)
- Detector inlet tubing: 0.004 in. I.D. x 1/16 in. OD, (Waters P/N 430001783)

Test solutions

Solvent A: Acetonitrile
Solvent B: Propyl paraben, 11.0 mg/L in acetonitrile
Blank Sample: Acetonitrile
Gradient Table

Time	Flow	%A	%B	%C	%D	Curve
Init	0.75	100	0	0	0	*
5	0.75	100	0	0	0	6
15	0.75	0	100	0	0	6
25	0.75	0	100	0	0	1
35	0.75	100	0	0	0	1

Procedure

Run 10 min 100%A before injection
Make a 1 µL injection of the blank sample to start a 35 min acquisition

Calculating System Volume

Determine baseline absorbance between 4-5 min
Determine absorbance at 100%B between 24-25 min
Determine baseline absorbance between 34-35 min

Graphically from Display: Zoom in on absorbance trace between 4 and 5 min; record approximate absorbance as equal to 0%B. Zoom in on absorbance trace between 24 and 25 min; record approximate absorbance as equal to 100%B. Zoom in on absorbance trace between 34 and 35 min; record approximate absorbance as equal to 0%B. Subtract average of Absorbance 4-5 min and Absorbance 34-35 min from Absorbance 24-25 min. This result is the absorbance equal to 100%B. Multiply Absorbance 100%B by 0.5. This result is the absorbance at 50% B. Zoom in on absorbance at 50% B; record time as equal to Delivered 50%B. Subtract Programmed 50%B from Delivered 50%B; this is the dwell time. Calculate the system volume multiplying the dwell time and flow rate. Use this system volume in calculating method transfer parameters.

RESULTS

USP Methodology

HPLC to HPLC

Galantamine Hydrobromide USP

Column: L1 4.6 x 100 mm, 3.5 µm
MP A: 95% phosphate buffer solution: 5% Methanol
MP B: Acetonitrile
Injection Volume: 20 µL
Column Temperature: 55 °C
Detection: 230 nm

Gradient table

Time	Flow	%A	%B
0	1.5	100	0
6	1.5	100	0
20	1.5	95	5
35	1.5	85	15
50	1.5	80	20
51	1.5	40	60
55	1.5	40	60
56	1.5	100	0
60	1.5	100	0

System Suitability Criteria:

Assay:
% RSD of galatamine: NMT 1.0%

Related Substances:
USP Tailing of galantamine: NMT 2.0
Resolution of galantamine and 6-alphagalantamine: NLT 4.5

HPLC to UPLC

Powdered Soy Isoflavones Extract USP

Column: L1 3.0 x 250 mm, 5 µm
MP A: 0.5% Phosphoric acid
MP B: Acetonitrile
Injection Volume: 5 µL
Column Temperature: 40 °C
Detection: 260 nm

Gradient table

Time	Flow	%A	%B
0	0.65	90	10
60.0	0.65	70	30
60.5	0.65	10	90
63.5	0.65	10	90
64.0	0.65	90	10
74.0	0.65	90	10

System Suitability Criteria:
Diadzin Tailing (T): 0.8 < T < 1.2
Genistin %RSD: NMT 2.0%

Correlation coefficient (R^2) for working standards 1-5 is not less than 0.999.

**USP compendia method specifies more criteria, however due to limited space, malonyl/acetyl results not shown*

IMPLEMENTATION

Loratadine USP *(related substances assay test 2)*

Scenario 3: Implement approaches demonstrated in scenarios 1 and 2
Goal: Demonstrate seamless use of both ACQUITY UPLC instruments

Column: L1 4.6 x 250 mm, 5 µm (XBridge C18)
MP A: 0.96 g of 1-pentanesulfonic acid in 1 L adjusted to pH 3.00 + 0.05 with 10% phosphoric acid
MP B: Acetonitrile
Injection Volume: 20 µL
Column Temperature: 35 °C
Detection: 254 nm

Gradient table

Time	Flow	%A	%B
0	1.2	75	25
20	1.2	50	50
30	1.2	40	60
35	1.2	30	70
45	1.2	30	70
50	1.2	75	25

System Suitability Criteria:

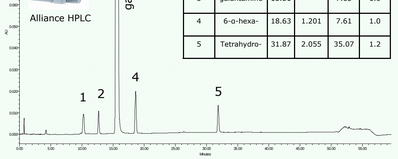
Assay:
Std Solution NMT 4.0% RSD

Related Substances:

- Rs between Loratadine rel. com A and rel. com. B is NLT 1.5
- %RSD of loratadine peak response NMT 10%

Starting Instrument and Chromatogram

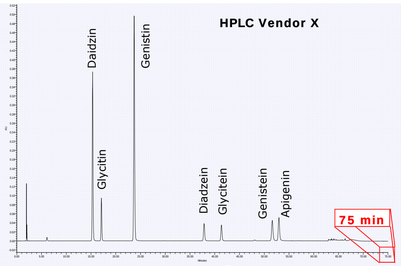
Alliance HPLC



Peak #	Name	RT	RT Ratio	USP Rs	USP Tailing
1	6-β-hexa-	10.25	0.661	18.97	1.0
2	6-β-octa-	12.69	0.818	6.64	1.1
3	galantamine	15.51		7.63	1.6
4	6-α-hexa-	18.63	1.201	7.61	1.0
5	Tetrahydro-	31.87	2.055	35.07	1.2

Measured Dwell: 950 µL
Heating: Convection Oven

USP Assay Results
Area %RSD =0.5%
(not shown)

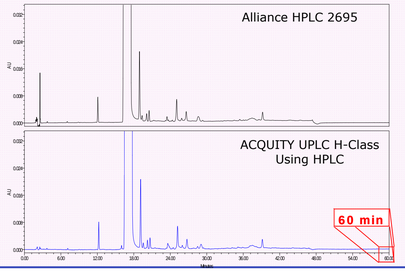


Injection: Working Std #3
Measured Dwell: 1.3 mL
Heating: Passive (2 sections)

- R^2 for all compounds across 5 working stds concentrations > 0.999
- Daidzin tailing = 1.1
- Genistin %RSD = 0.6

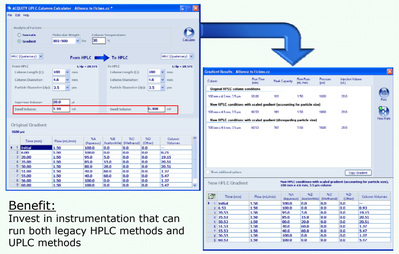
HPLC Methodology - Loratadine DS Lot 1 Sample Results

1. Transferred HPLC methodology to ACQUITY UPLC H-Class instrumentation.



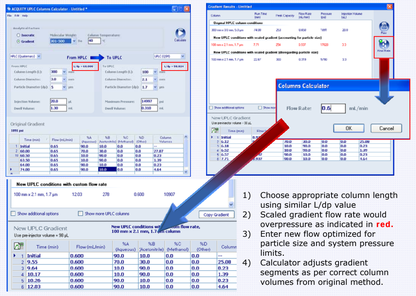
Translation Using ACQUITY UPLC Columns Calculator

Scenario 1: Future Proofing your Laboratory
Goal: Transfer existing HPLC method to a different LC system



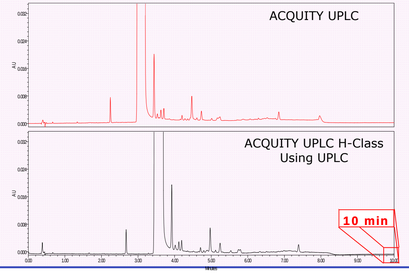
Benefit:
Invest in instrumentation that can run both legacy HPLC methods and UPLC methods

Scenario 2: Method Adjustment—Reduce Analysis Time
Goal: Improve method by taking advantage of sub-2-µm particles

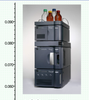


1) Choose appropriate column length using similar L/dp value
2) Scaled gradient flow rate would overpressure as indicated in **red.**
3) Enter new flow optimized for particle size and system pressure limits.
4) Calculator adjusts gradient segments as per correct column volumes from original method.

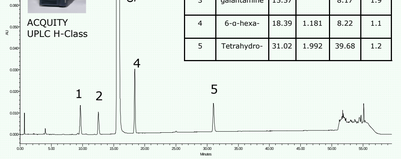
2. Method was then optimized for sub-2-µm particle size.
3. Translated ACQUITY UPLC H-Class methodology for use on traditional ACQUITY UPLC maximizing asset utilization with other UPLC instruments



Target Instrumentation and Chromatogram



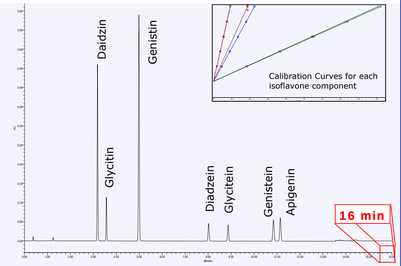
ACQUITY
UPLC H-Class



Peak #	Name	RT	RT Ratio	USP Rs	USP Tailing
1	6-β-hexa-	9.66	0.620	23.26	1.0
2	6-β-octa-	12.55	0.806	9.08	1.2
3	galantamine	15.57		8.17	1.9
4	6-α-hexa-	18.39	1.181	8.22	1.1
5	Tetrahydro-	31.02	1.992	39.68	1.2

Measured Dwell: 280 µL
Heating: Active preheating

Assay Results
Area %RSD =0.2%
(not shown)



Instrument: ACQUITY UPLC
Measured Dwell: 82 µL
Heating: Passive
Injection: Working Std #3

- R^2 for all compounds across 5 working stds concentrations > 0.999
- Daidzin tailing = 0.99
- Genistin %RSD = 0.12

Peak	Relative Retention Time Ratios			
	Alliance HPLC 2695	H-Class HPLC	H-Class UPLC	Traditional UPLC
Imp. 1	0.72	0.70	0.74	0.71
Loratadine	-	-	-	-
Imp. 2	1.12	1.09	1.09	1.08
Imp. 3	1.15	1.12	1.11	1.11
Imp. 4	1.19	1.16	1.14	1.14
Imp. 5	1.22	1.18	1.16	1.16
Imp. 6	1.39	1.35	1.30	1.32
Imp. 7	1.49	1.44	1.36	1.41
Imp. 8	1.58	1.53	1.45	1.49
Imp. 9	2.32	2.24	2.05	2.16

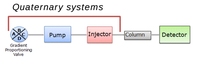
DISCUSSION

The compendia methods transferred for this presentation were facilitated by the columns calculator with good success without altering the chromatographic attributes and integrity of the originating methodology. It should be noted that each vendor's LC instrumentation may execute functionalities such as heating, gradient delivery, mixing, etc. in different ways. Preliminary discussions within the team had expectations of differing instrument functionalities potentially affecting the ease of transfer. Observations of the results showed very slight differences in retention time ratios, peak shape discrepancies which needed further investigation out of the scope of this presentation. It was also observed through other examples that originating HPLC methodology had insufficient re-equilibrations and gradient regeneration times affecting subsequent injections. Below are a subset of discussion points and results that should be addressed during method transfer experiments.

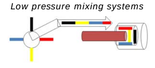
Differences in Column Chemistry: Choosing a compatible column chemistry was key when transferring from legacy HPLC to UPLC. The reversed-phase selectivity chart facilitated a proper stationary phase selection. Download at *www.waters.com/selectivitychart*

Differences in Gradient Delivery and Mixing Efficiency: Quaternary pumping systems (*low pressure mixing systems*) of different vendors may use different algorithms that "packet" the formation of the gradient. Some systems use a "ABBA" packeting, an "AB" packeting, an "ABA" packeting, or some variation. Mixer design and internal volumes will affect the blending/mixing efficiency of the mobile phase, hence affecting the gradient formation and gradient delivery. Binary pumping systems (*high pressure mixing systems*) begin mixing after the pump head as the mobile phase is introduced into the mixer, hence forming a "gradient-like" profile of mixing within the mixing device.

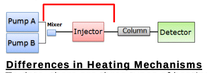
Quaternary systems



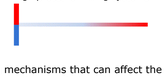
Low pressure mixing systems



Binary systems



High pressure mixing systems



Differences in Heating Mechanisms

To date, there are three types of heating mechanisms that can affect the peak selectivity of the separation. Experimental results to determine an apparent isoretention of two test compounds; 4-amino-2,6-dinitro toluene and 2,4-dinitro toluene, yielded the following temperature correlations:

Vendor	Model	Type	Apparent Iso-retention
Waters	Alliance	Convection	39.0
Waters	ACQUITY UPLC HTCH	Passive	44.1
Waters	ACQUITY UPLC H-Class CH-A	Active	40.5
Shimadzu		Convection	41.8
Agilent	A1200	Passive	46.8

CONCLUSION

- **Three USP compendial methods were successfully transferred to various LC configurations without compromising the integrity of the originating method.**
- **Methods were successfully translated to take benefit of sub-2-µm stationary phases.**
- **The new ACQUITY UPLC Columns Calculator accounted for differences within system volumes. Flow rates and injection volumes were scaled while compensating for appropriate column volumes per gradient segments.**
- **Observations determined that not all systems implement functions the same way, therefore additional method alterations may be necessary for transfer of less robust methods.**

References: USP32-N27 Supplement: No 2,

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