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アプリケーションノート

Implementation of Methods Translation between Liquid Chromatography Instrumentation

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Abstract

In this application note, various U.S. Pharmacopeia (USP) compendial methods are used as examples to highlight a new method translation strategy to facilitate the transfer of methods to and from any LC-based instrument with ease.

Benefits

- Future proof your laboratory
- “Equivalent” vs. “Equal” column selectivity
- Increase productivity while decreasing costs
- Maximize asset utilization
- Understand the importance of L/dp
- Discover software tools to facilitate method translation

Introduction

Pharmaceutical research and development (R&D) organizations were early adopters who recognized the many benefits of UltraPerformance LC (UPLC) Technology including resolution, sensitivity, throughput, and productivity as compared to HPLC. Today, the number of projects involving new drug entities are increasingly performed utilizing UPLC.

Adopting UPLC for R&D activities is less complex than for laboratories involved with routine analysis, where its use requires consideration about the need to re-file methods for existing products. Routine analysis areas such as Quality Control (QC) laboratories own a vast supply and variety of HPLC instrumentation. Asset procurement regarding new technologies within these groups often requires convincing financial as well as scientific justification.

Although information illustrating UPLC’ s return on investment (ROI) for solvent consumption and analysis per unit time can be convincing for R&D, the QC environment requires key practical-use considerations. Managers and end users within QC laboratories require new instrumentation to provide dual purposes: first, the ability to perform both legacy methods and, second, the ability to use sub-2-µm particle columns and methodology in a routine analytical environment without complications. UPLC’ s adoption must also strategically provide seamless integration within current laboratory practices and decrease learning curves of the end users.

In this application, various U.S. Pharmacopeia (USP) compendial methods are used as examples to highlight a new method translation strategy to facilitate the transfer of methods to and from any LC-based instrument with ease.

Experimental

United States Pharmacopeia reference standards

- USP Monograph Galantamine Hydrobromide
  - USP Galantamine Hydrobromide RS and USP Galantamine Hydrobromide Related Compounds Mixture RS
- USP Dietary Supplement: Powdered Soy Isoflavone Extract Method
  - USP Apigenin RS, USP Diadzein RS, USP Diadzin RS, USP Genistein RS, USP Genistin RS, USP Glycitein RS, USP Glycitin RS, and USP Defatted Powdered Soy RS
- USP Monograph Loratadine
  - USP Loratadine RS, USP Loratadine Related Compound A RS, and USP Loratadine Compound B RS, Claritin

LC conditions

References to LC conditions are addressed as per USP Monographs, whereas specific utilization of LC instrumentation for each application is discussed in the figure captions.

Data management

Empower 2 CDS

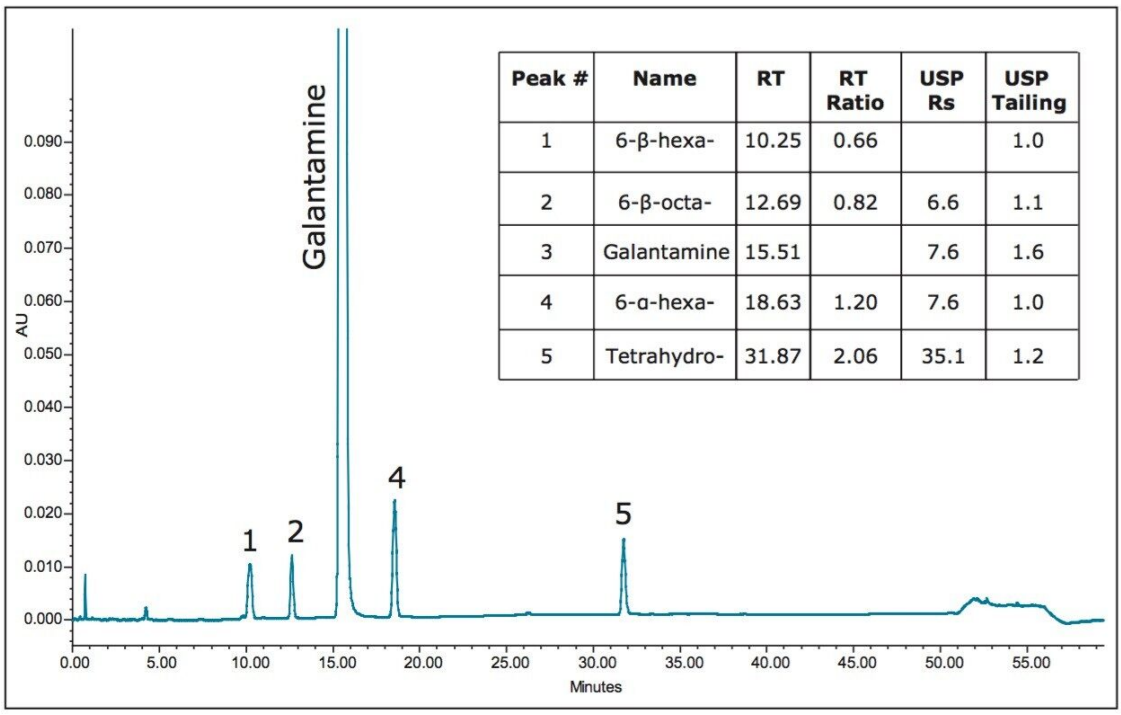
Results and Discussion

Successful method translation requires understanding three key chromatographic attributes before implementation. The analyst must consider the differences between LC instrumentation, column selectivity, and the resolving capability of the original methodology versus the target methodology. By understanding these three essential aspects of method translation, the benefits of increasing productivity and decreasing costs while maximizing asset utilization of present and future instrumentation can be realized.

Future-proofing your laboratory:
Translating HPLC methodology between LC instrumentation

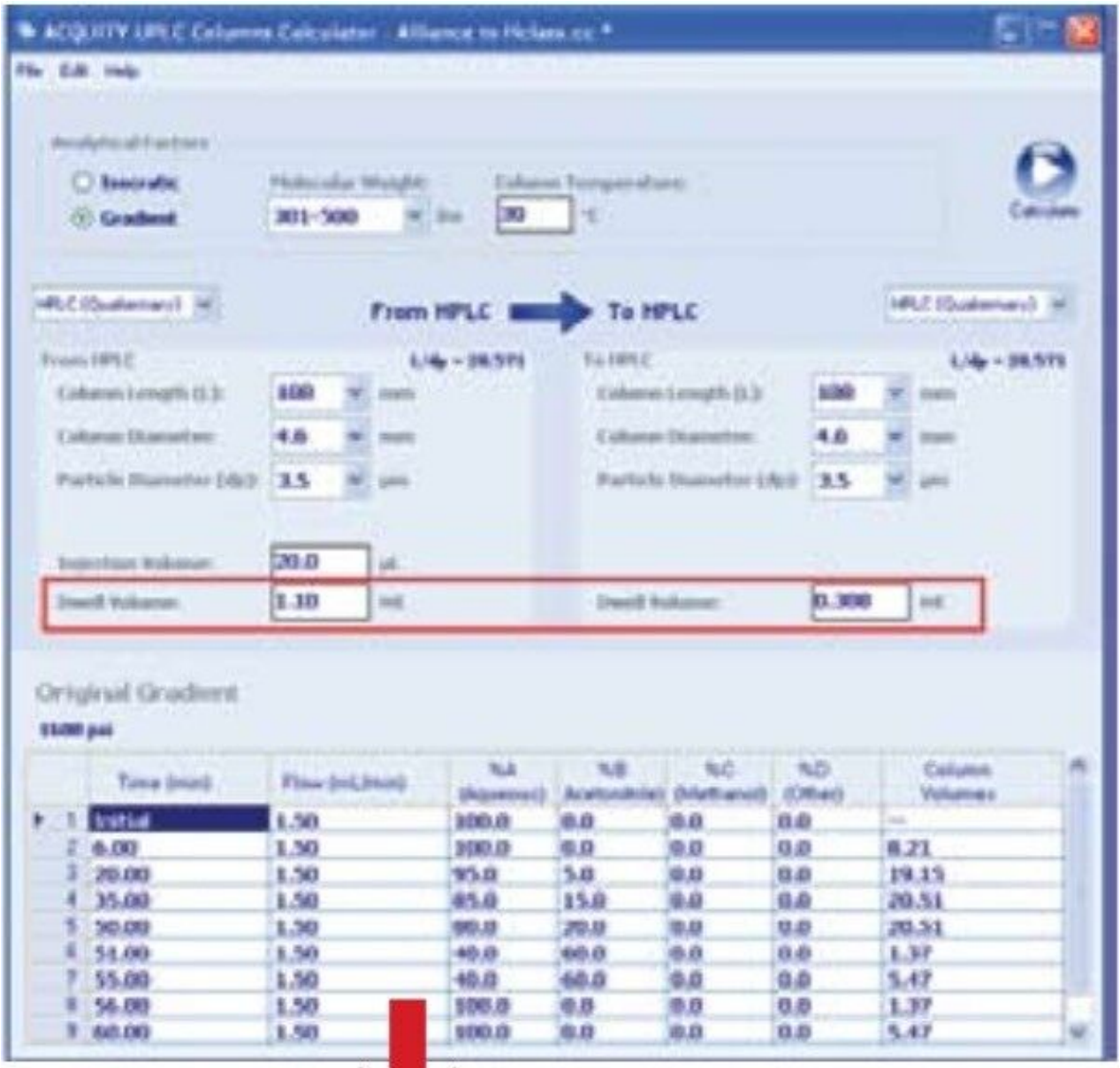
The QC laboratory frequently utilizes a variety of LC instruments for API and drug product analysis. Therefore, instrumentation flexibility is essential. Direct transfer of methods to newer technology may result in retention time and selectivity differences that may be related to decreases in instrument dwell volume.

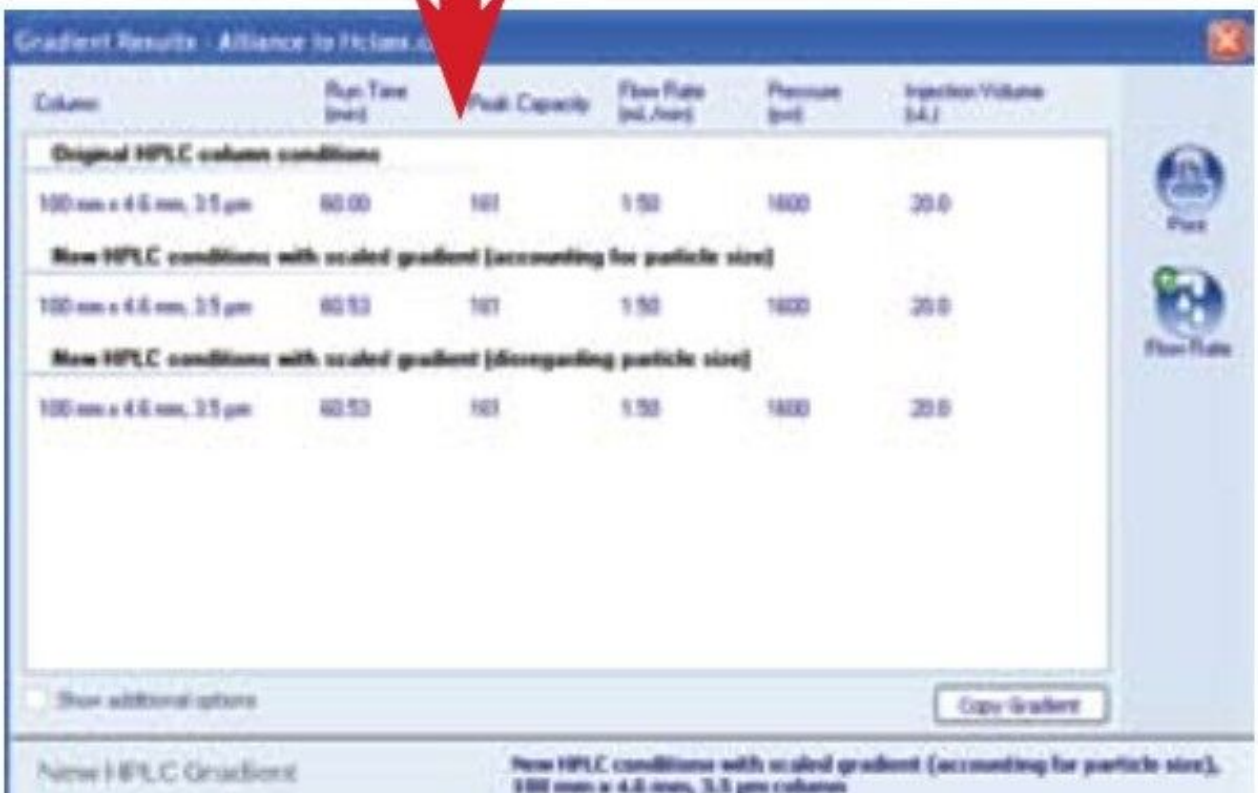
To illustrate the flexibility provided by the Waters ACQUITY UPLC H-Class System, the USP method for galantamine hydrobromide and related substances was performed on an HPLC instrument (Figure 1). USP system suitability requirements for the related substances assay specify USP tailing of galantamine NMT 2.0 and a resolution of galantamine and 6-alphagalantamine NLT 4.5. When utilizing the same HPLC column on each instrument, the ACQUITY UPLC Columns Calculator (Figure 2) can be used to calculate the differences in the instrument dwell volume. The resulting data yielded no compromise in chromatographic integrity during the translation of the method for use on a UPLC instrument of less dwell volume (Figure 3).



Peak #	Name	RT	RT Ratio	USP Rs	USP Tailing
1	6-β-hexa-	10.25	0.66		1.0
2	6-β-octa-	12.69	0.82	6.6	1.1
3	Galantamine	15.51		7.6	1.6
4	6-α-hexa-	18.63	1.20	7.6	1.0
5	Tetrahydro-	31.87	2.06	35.1	1.2

Figure 1. USP Method for galantamine and related substances performed on an Alliance HPLC 2695 with measured dwell volume of 1.1 mL. An XBridge C_{18} (L1) column with dimensions 4.6 x 100 mm, 3.5 µm was used.


From HPLC
To HPLC
Original Gradient


Original HPLC column conditions
New HPLC conditions with scaled gradient (accounting for particle size)
New HPLC conditions with scaled gradient (disregarding particle size)

- Three USP compendial methods were successfully transferred to various LC configurations without compromising the integrity of the originating method.
- Techniques were demonstrated to maximize global asset utilization and maintain lab productivity.
- Methods were successfully translated to take benefit of sub-2-µm stationary phases.
- Software tools are available to facilitate the scaling and column selection
  - The AC QUITY UPLC Columns Calculator accounts for differences within system dwell volumes. Flow rates and injection volumes are scaled while compensating for appropriate column volumes per gradient time segment.
  - The Reversed Phase Column Selectivity Chart facilitates the selection of equivalent column selectivity when an equal selectivity column is unavailable.

References

1. Galatamine Hydrobromide: USP32-NF27 Supplement:No.2, page 4245.
2. Powdered Soy Isoflavones Extract: USP32-NF27, page 1074.
3. Loratadine: USP32-NF27, page 2805.

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ACQUITY UPLC H-Class PLUS System <https://www.waters.com/10138533>

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