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# USP Method Transfer of Lamotrigine from HPLC to UPLC

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## Abstract

The simple workflow provides an approach for QC laboratories to transfer compendial methods from HPLC to UPLC. In this application note, the USP method for lamotrigine was successfully transferred to UPLC technology.

### Benefits

- Over 3000 injections achieved for drug formulation analysis
- 4x increase in throughput for routine sample analysis
- 89.5% reduction in solvent consumption

## Introduction

The pharmaceutical industry struggles with solving method transfer challenges. Implementation of UPLC increases overall profitability and as a result, pharmaceutical companies are transferring their legacy USP HPLC methodology to UPLC to increase efficiency within the QC environment. Therefore, simplified approaches supplemented with proven examples are essential to assisting their method translation goals with experience.

The compendial method for lamotrigine is a good representation of typical conditions described in other USP HPLC methodology. Lamotrigine is an anticonvulsant drug approved by the FDA and marketed by GSK as Lamictal primarily to treat seizures of specific diagnosis experienced by patients with epilepsy. For the treatment of bipolar depression, one-third of surveyed psychiatrists identify lamotrigine as the therapy with the greatest overall efficacy when compared to other currently available treatments.[1] Since the patent expiration in 2008, a report published by Allbusiness.com titled “GlaxoSmithKline (GSK) Q1- 2010” identified competition from generic pharmaceuticals accounted for a 26% y-o-y decrease of Lamictal sales since 2008.[2]

The goal of transferring the USP Lamotrigine HPLC methodology to UPLC was achieved by following the workflow illustrated in figure 1 derived from the strategy described in a previous application note.[3] A drug formulation analysis procedure was developed based on the drug substance methodology found in the USP for lamotrigine.[4] Method feasibility was determined through routine use studies designed to assess applicability within quality control laboratories.

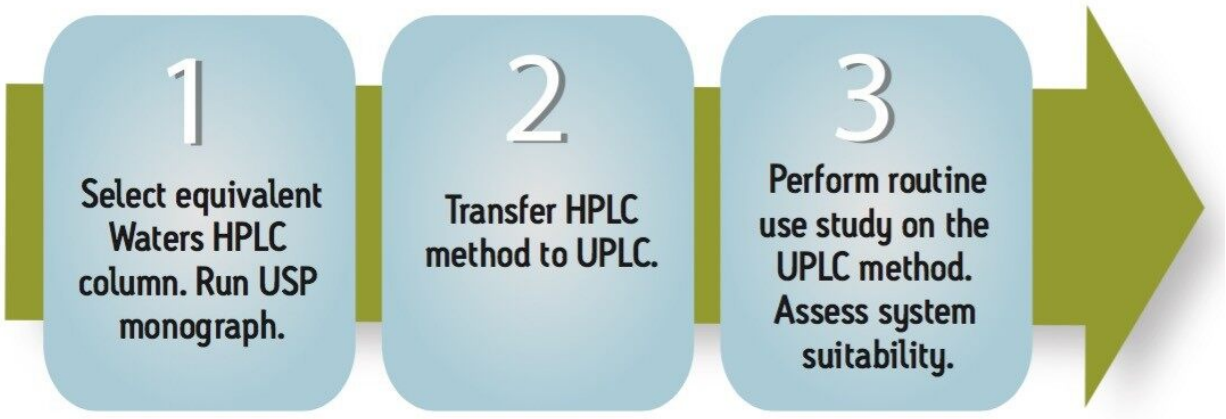


*Figure 1. Workflow for transferring methods from HPLC to UPLC.*

## Experimental

### Alliance 2695 HPLC Conditions

| | |
|---|---|
| Buffer: | 20 mM potassium phosphate monobasic |
| Mobile Phase: | A: 150:1 buffer:triethylamine adjusted to pH 2.0 with phosphoric acid<br>B: acetonitrile |
| Detection: | UV at 270 nm |
| Column: | XBridge $C_{18}$ , 4.6 x 150 mm, 5 µm (USP designation: L1), part number 186003116 |
| Needle Wash: | 95:5 water:acetonitrile |
| Seal Wash: | 95:5 water:methanol |

| | |
|---|---|
| Sample Diluent: | 0.1 M hydrochloric acid |
| Flow Rate: | 1.0 mL/min |
| Column Temp.: | 35 °C |
| Injection Volume: | 10 µL |
| Detection: | UV at 270 nm |

| Time (Minutes) | Solvent A (%) | Solvent B (%) | Column Volumes |
|---|---|---|---|
| Initial | 76.5 | 23.5 | - |
| 4.00 | 76.5 | 23.5 | 2.43 |
| 14.00 | 20.0 | 80.0 | 6.08 |
| 15.00 | 76.5 | 23.5 | 0.61 |
| 19.00 | 76.5 | 23.5 | 2.43 |

## ACQUITY UPLC Conditions

| | |
|---|---|
| Buffer: | 20 mM potassium phosphate monobasic |
| Mobile Phase: | A: 150:1 buffer:triethylamine adjusted to pH 2.0 with phosphoric acid<br>B: acetonitrile |
| Detection: | UV at 270 nm |
| Column: | ACQUITY UPLC BEH $C_{18}$, 2.1 x 50 mm, 1.7 µm, part number 186002350 |
| Weak Wash: | 95:5 water:acetonitrile |

| | |
|---|---|
| Strong Wash: | 50:50 acetonitrile:water |
| Diluent: | 0.1 M hydrochloric acid |
| Flow Rate: | 0.61 mL/min |
| Column Temp.: | 40 °C |
| Injection Volume: | 0.7 µL (partial loop using needle overfill mode) |
| Data Management: | Empower 2 CDS |

## USP System Suitability Criteria

| | |
|---|---|
| Tailing Factor for Lamotrigine: | NMT 1.5 |
| Area %RSD for five replicate injections: | NMT 1.5% RSD |

| Time (Minutes) | Solvent A (%) | Solvent B (%) | Column Volumes |
|---|---|---|---|
| Initial | 76.5 | 23.5 | - |
| 0.45 | 76.5 | 23.5 | 2.43 |
| 1.59 | 20.0 | 80.0 | 6.08 |
| 1.70 | 76.5 | 23.5 | 0.61 |
| 2.15 | 76.5 | 23.5 | 2.43 |

## Sample Preparation

The samples were prepared by transferring an appropriate pooled number of tablets to a 1 L volumetric flask to obtain a concentration equivalent to 1.0 mg/mL lamotrigine. Tablets were dissolved in 200 mL water and 800 mL methanol. This solution was mechanically shaken for 20 minutes followed by centrifugation at 4000 rpm for 20 minutes. Aliquots from the dissolved tablet sample solution were diluted with diluent to obtain a working sample concentration of 0.2 mg/mL.

## Results and Discussion

The USP HPLC method for lamotrigine and related compounds was validated using a Hypersil BDS $C_{18}$ 4.6 x 150 mm, 5 µm column (USP designation: L1), which does not have a commercially available sub-2 µm column configuration. The Waters Reversed-Phase Column Selectivity Chart identified XBridge $C_{18}$ as an equivalent L1 stationary phase scalable to sub-2 µm particles. The HPLC methodology was performed as written on the XBridge $C_{18}$ column (Figure 2a) meeting the system suitability requirements stated in the USP.

The HPLC method was transferred to an ACQUITY UPLC BEH $C_{18}$ column using the Waters ACQUITY UPLC Column Calculator. The calculator specified two options for transferring to UPLC methodology: “accounting for particle size” and “disregarding particle size”. The conditions indicated for the option “disregarding particle size” is a direct geometric scaling of the chromatography. The “accounting for particle size” conditions scales the flow rate to the optimum potential of the particle size while maintaining the gradient segment column volumes providing increases in throughput without sacrifices in efficiency. For the purposes of this application, the method transfer was performed using the optimal method conditions which account for particle size to realize the full potential of savings when transferring to UPLC. The resulting chromatograms compared in Figure 2 show that the selectivity and resolution was maintained.

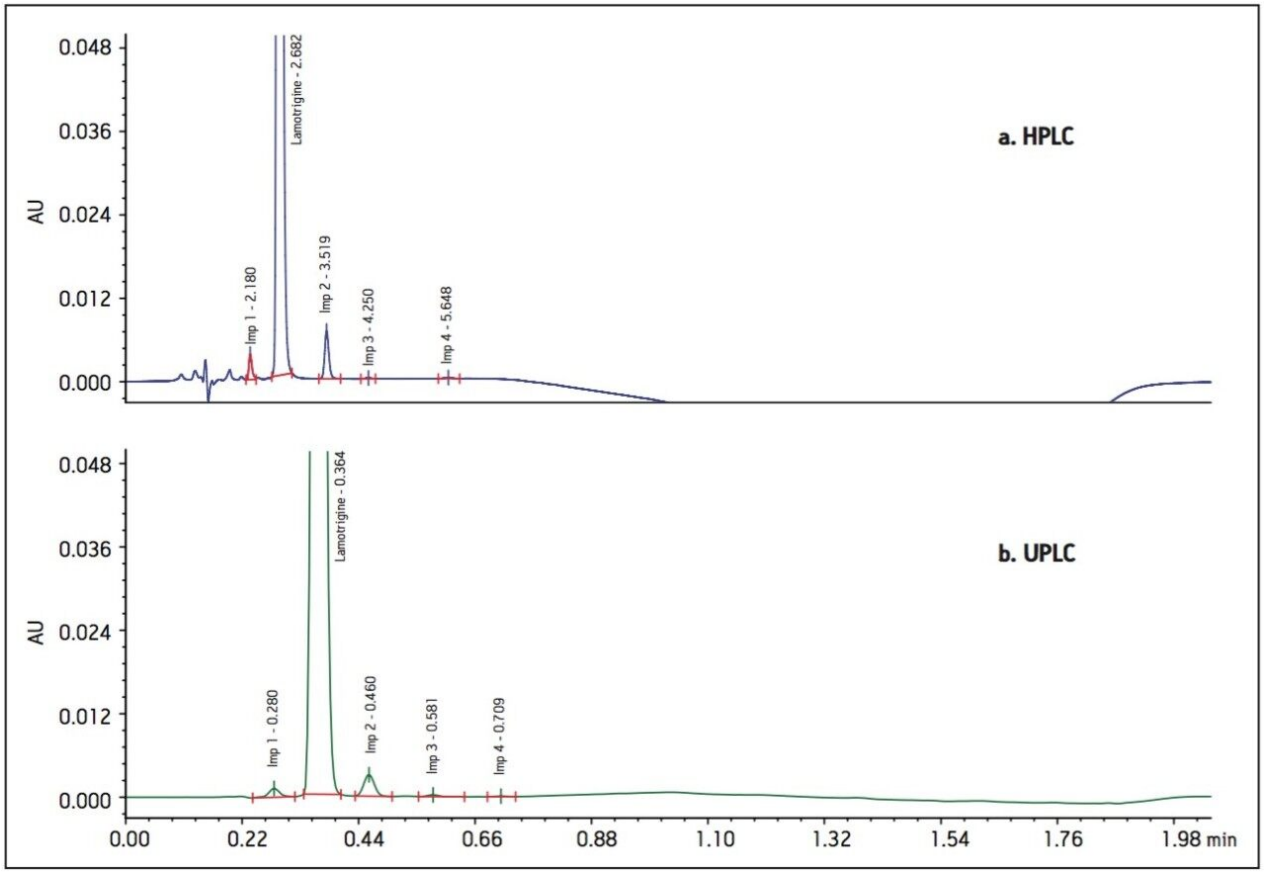


*Figure 2. Comparisons of HPLC and UPLC chromatograms for injections of prepared lamotrigine tablet samples.*

The methodologies were evaluated and compared to the HPLC results in Tables 1 and 2.

| | HPLC on Alliance | | UPLC Optimally Scaled | |
|---|---|---|---|---|
| Peak | RT Ratio | % Area | RT Ratio | % Area |
| Imp 1 | 0.81 | 0.45 | 0.77 | 0.51 |
| Lamotrigine | -- | 98.39 | -- | 98.21 |
| Imp 2 | 1.31 | 1.10 | 1.26 | 1.15 |
| Imp 3 | 1.58 | 0.02 | 1.59 | 0.10 |
| Imp 4 | 2.11 | 0.04 | 1.95 | 0.03 |

*Table 1. Comparisons of HPLC vs. scaled options for UPLC resulting in equivalent results regarding relative retention ratio and area % in respect to the API lamotrigine.*

| | HPLC | UPLC |
|---|---|---|
| Area %RSD X < 1.5% | 0.10% | 0.10% |
| Tailing X < 1.5% | 1.31 | 1.1 |

*Table 2. Lamotrigine assay suitability criteria comparing results for HPLC vs. UPLC methodology.*

## Routine Use Study

The UPLC method was evaluated in a routine-use study to more closely simulate what would be expected in a quality control laboratory. The USP system suitability criteria was assessed periodically throughout the study. The results successfully met the USP specifications for tailing and %RSD throughout the study and reported in Table 3. The routine-use study was completed at 3000 injections with no issues. Liquid chromatography methods analyzing drug formulation methodology can occasionally result in increases in system pressure due to improper sample preparation resulting from excipients collecting at the inlet frit of the column. System pressure was monitored and plotted for the duration of the routine-use study. The pressure remained stable throughout the study, as shown Figure 3.

| | Injections 1-5 | Injections 1510-1515 | Injections 2058-2064 |
|---|---|---|---|
| Area %RSD | 0.2 | 0.2 | 0.1 |
| Tailing | 1.1 | 1.1 | 1.1 |
| Max Pressure | 7600 | 7577 | 7526 |

*Table 3. System suitability results during the routine use study.*

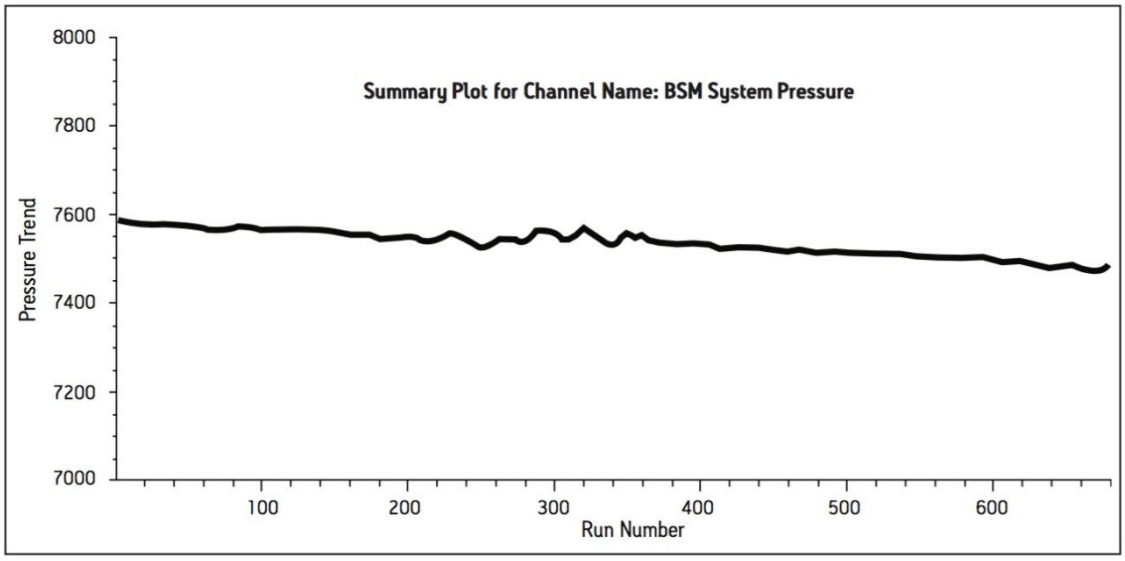


*Figure 3. A pressure trend plot for the UPLC method. The pressure was measured at the maximum pressure point for each injection, which was determined to be at 0.4 minutes for each injection.*

## Conclusion

The simple workflow provides an approach for QC laboratories to transfer compendial methods from HPLC to UPLC. The USP method for lamotrigine was successfully transferred to UPLC technology. The method was evaluated utilizing standards and tablet samples. Over 3000 injections were performed with no indication of reduction in UPLC methodology performance, as measured by the system suitability criteria specified in the

USP method for lamotrigine. The UPLC method provides almost a 5-fold reduction in run time while maintaining the integrity of the system suitability criteria of the USP method. The UPLC method, as calculated for accounting for particle size, provides approximately 89% savings in run time and solvent consumption (2 minutes UPLC vs. 19 minutes HPLC), allowing quality control laboratories synergistic improvements that provide benefits in cost reductions.

## References


1. www.desicionresourcesinc.com
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3. Jones M.D., Alden P., Fountain K.J., Aubin A. *Implementation of Methods Translation between Liquid Chromatography Instrumentation*, Waters Application Note [2010]; Part Number 720003721EN.
4. USP Monograph. Lamotrigine, USP34-NF29, 4631. The United States Pharmacopoeial Convention, official from May 1, 2011.


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