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Application Note

USP Method Transfer of Amoxicillin Oral Suspension from HPLC to UPLC

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Abstract

The USP compendial method for amoxicillin oral suspension was successfully transferred from HPLC to UPLC using the Waters Column Selectivity Chart and ACQUITY UPLC Columns Calculator. The UPLC method is approximately 70% faster than the HPLC method and affords a 92% savings in sample amount injected and mobile-phase solvent consumption. Routine column use using a phosphate buffered mobile phase at pH 5 and formulated oral suspension sample was evaluated on the ACQUITY UPLC BEH Shield RP18 column. A column wash alleviated an increase in pressure during the study and incorporating a routine wash is advocated to extend the lifetime of columns in general. After 2400 injections, the ACQUITY UPLC BEH Shield RP18 column still passed all USP suitability specifications for amoxicillin oral suspension, demonstrating that UPLC can be used to perform routine QC analysis of amoxicillin oral suspension samples without compromising column stability or separation performance.

Benefits

- Updating USP Methods from HPLC to UPLC using sub-2 µm columns
- 70% decrease in analysis time, faster throughput for routine sample analysis
- 92% reduction in solvent usage and sample injected

Introduction

USP compendial methods are often used as a basis for routine analysis of generically manufactured drugs. Often these methods do not take advantage of modern techniques such as sub-2 µm particle columns and UPLC. Many USP methods were also developed on older column technology, limiting efficiency of the analysis. Updating these methods to run on more current systems allows for more efficient batch analysis by maximizing sample throughput while retaining resolution.

Amoxicillin is a commonly used antibiotic that is produced generically throughout the world. Formulations vary and in this example, an oral suspension formulation is analyzed according to its USP monograph.[1] The method transfer from the USP compendial method to a more modern stationary phase and subsequent analysis by UPLC is demonstrated. The robustness of the updated method is evaluated by performing a routine use evaluation study, assessing the assay suitability criteria described in the USP method to ensure long-term column stability.

Experimental

Alliance 2695 HPLC conditions

Diluent:	50 mM potassium phosphate, monobasic in water - pH 5.0 with potassium hydroxide
Mobile Phase:	98:2 diluent:acetonitrile
Separation Mode:	Isocratic
Detection:	UV at 230 nm
USP Column:	XBridge Shield RP18, 4.6 x 250 mm, 5 µm (USP designation L1), part number 186003010
Needle Wash:	90:10 water:acetonitrile
Sample Purge:	90:10 water:acetonitrile
Seal Wash:	90:10 acetonitrile:water
Flow Rate:	1.5 mL/min
Injection Volume:	10 µL

Acquity UPLC H-Class conditions

Diluent:	50 mM potassium phosphate, monobasic in water - pH 5.0 with potassium hydroxide
Mobile Phase:	98:2 diluent:acetonitrile
Separation Mode:	Isocratic
Detection:	UV at 230 nm
Column:	ACQUITY UPLC BEH Shield RP18, 2.1 x 100 mm, 1.7 µm (USP designation L1), part number 186002854
Needle Wash:	90:10 water:acetonitrile
Sample Purge:	90:10 water:acetonitrile
Seal Wash:	90:10 acetonitrile:water
Flow Rate:	0.4 mL/min
Injection Volume:	0.8 µL

Sample Preparation

Amoxicillin oral suspension powder, reconsituted in water (50 mg/mL), made up to 1 mg/mL in diluent.

Amoxicillin standard made up to 1 mg/mL in diluent.

Samples were filtered though a 0.2 µm nylon filter (part number WAT200522), prior to analysis.

Data Management

Empower 2 CDS

Results and Discussion

Samples were prepared according to the USP assay method guidelines for amoxicillin oral suspension. The assay preparation for amoxicillin oral suspension specifies filtering the sample through a 1 µm or finer porosity filter. Care was taken to filter samples through a 0.2 µm nylon filter to remove any fine particulates. The USP method for amoxicillin oral suspension designates the use of an L1 column and the suggested column is µBondapak C_{18}. Using the Waters Column Selectivity Chart, a more modern L1 column, XBridge Shield RP18, was selected where direct scalability to the same UPLC column chemistry can be demonstrated. The USP compendial method was first run as described on an Alliance HPLC system using five replicate injections of both amoxicillin standard and amoxicillin oral suspension. Assay suitability criteria described in the monograph were monitored for both samples and found to be within specification (Table 1).

Amoxicillin Standard

	USP Criteria	HPLC	UPLC
Retention Time (min.)	none	5.24	1.45
%RSD Area*	NMT 2.0%	0.33	0.16
USP Tailing	NMT 2.5	1.02	0.94
USP Plate Count	NLT 1700	6632	15500
K Prime	1.1 to 2.8	1.63	1.29

Amoxicillin Oral Suspension

	USP Criteria	HPLC	UPLC
Retention Time (min.)	none	5.24	1.45
%RSD Area*	NMT 2.0%	0.27	0.47
USP Tailing	NMT 2.5	1.02	0.93
USP Plate Count	NLT 1700	6622	15504
K Prime	1.1 to 2.8	1.63	1.29

** 5 replicate injections*

Table 1. Assay suitability results comparing HPLC to UPLC for five replicate injections.

The USP method was then transferred from HPLC to UPLC using the ACQUITY UPLC Columns Calculator.[2] Scaling was performed accounting for particle size and the column was scaled to an ACQUITY UPLC BEH Shield RP18, 1.7 µm column, maintaining the same column chemistry. Five replicate injections of both amoxicillin oral suspension and amoxicillin standard were analyzed separately. Assay suitability criteria including %RSD for peak area, k prime, USP tailing factor, and USP plate count were compared between HPLC and UPLC. A comparison of both systems is shown in Table 1, where the UPLC transferred method passes criteria in all regards. Finally, the run time of the UPLC method is 4.5 minutes compared to a 15-minute HPLC method, affording an approximate 70% savings in analysis time and 92% savings in solvent consumption and sample injected (Figure

1).

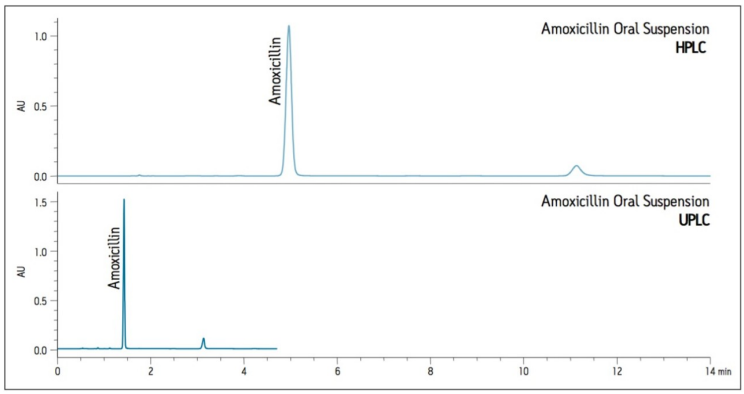

Figure 1. Chromatograms of amoxicillin oral suspension comparing HPLC to UPLC per the USP method.

Routine Use Study

In order to evaluate the effects of using common USP mobile phases such as phosphate buffer and formulated drug samples on newer column technology, a routine use evaluation was performed on the 1.7 µm ACQUITY UPLC BEH Shield RP18 column using the amoxicillin oral suspension sample.

Amoxicillin oral suspension was analyzed using amoxicillin standard as a bracketing standard, as might be seen in a typical quality control (QC) laboratory. Five replicate injections of amoxicillin standard were followed by twenty replicate injections of amoxicillin oral suspension and this cycle of injections was repeated continuously until assay suitability criteria no longer passed. Pressure, retention time, amoxicillin peak area, k prime, USP tailing factor and USP plate count were monitored throughout the study.

Pressure remained stable at approximately 8000 psi for 500 injections, after which the pressure began to increase gradually until the maximum system pressure was reached at 1700 injections (Figure 2). The system and column were washed with 90:10 water:acetonitrile for 2 to 3 hours. The column was re-equilibrated to the method starting conditions and the pressure returned to starting levels of 8000 psi, whereby the routine use evaluation was re-started.

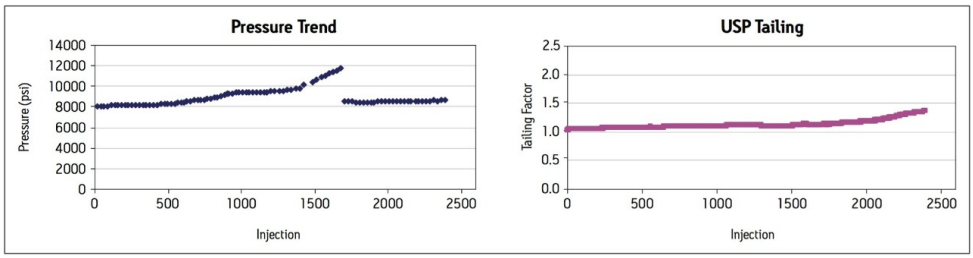

Figure 2. Pressure and USP tailing trend plots from the ACQUITY UPLC BEH Shield RP18 routine use evaluation.

Retention factor and plate count remained within the USP assay suitability criteria throughout the study. USP tailing for the amoxicillin peak increased slightly over 2000 injections but was still well within the USP criteria of NMT 2.5. The routine use evaluation was stopped at approximately 2400 injections and system suitability results were still well within passing criteria (Table 2).

Amoxicillin Oral Suspension

	USP Criteria	Routine Use Evaluation	
		Start	2400+ injections
Retention Time (min.)	none	5.24	1.50
%RSD Area*	NMT 2.0%	0.27	0.05
USP Tailing	NMT 2.5	1.02	1.39
USP Plate Count	NLT 1700	6622	6209
K Prime	1.1 to 2.8	1.63	1.39

** 5 replicate injections*

Table 2. Assay suitability results before and after 2400 injections from the routine use evaluation.

Conclusion

The USP compendial method for amoxicillin oral suspension was successfully transferred from HPLC to UPLC using the Waters Column Selectivity Chart and ACQUITY UPLC Columns Calculator. The UPLC method is approximately 70% faster than the HPLC method and affords a 92% savings in sample amount injected and mobile-phase solvent consumption. Routine column use using a phosphate buffered mobile phase at pH 5 and formulated oral suspension sample was evaluated on the ACQUITY UPLC BEH Shield RP18 column. A column wash alleviated an increase in pressure during the study and incorporating a routine wash is advocated to extend the lifetime of columns in general. After 2400 injections, the ACQUITY UPLC BEH Shield RP18 column still passed all USP suitability specifications for amoxicillin oral suspension, demonstrating that UPLC can be used to perform routine QC analysis of amoxicillin oral suspension samples without compromising column stability or separation performance.

References

1. USP Monograph. Amoxicillin for Oral Suspension, USP34-NF29 [1886]. T he United States Pharmacopeial Convention, official from May 1, 2011.
2. 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

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