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Application News

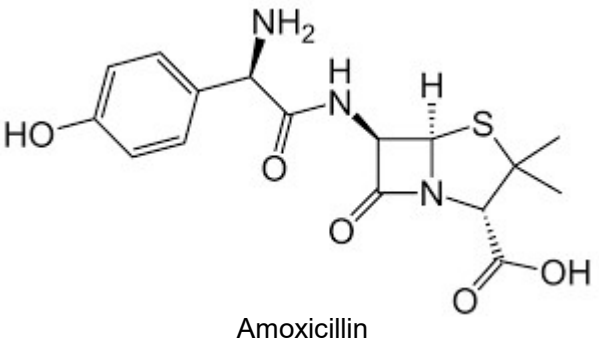
No. LC-16-ADI-038

Nexera UHPLC

USP method transfer on UHPLC is no more challenge for the analysis of Amoxicillin Oral Suspension

Introduction

Amoxicillin is a drug belonging to a class of compounds known as ß-lactam antibiotics. Amoxicillin, a member of the penicillin family, is used most often to treat a number of bacterial infections including H. influenzae, N. gonorrhoea, E. coli, Pneumococci, Streptococci, and some strains of Staphylococci. It is thought that these penicillin-derived compounds work to stop the bacteria from multiplying by inhibiting its cell wall synthesis [1][2].



Amoxicillin

USP 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 fast HPLC technique. In this study the USP monograph for amoxicillin oral suspension taken as an example to transfer the long HPLC method to a short UHPLC method using sub 2 micron column with modern stationary phase for high efficiency and high resolution.[3]

Experimental

The USP method was transferred from HPLC to UHPLC using method parameters mentioned in USP. The Samples and mobile phase were prepared according to the USP assay method guidelines for amoxicillin oral suspension. System suitability parameters checked as per requirements of USP method.

Buffer preparation : Dissolve 6.8 g/L of (50 mM) monobasic potassium phosphate in water. Adjust pH 5.0± 0.01 with 45% (v/v) solution of potassium hydroxide.

Standard preparation : 1.2mg/ml of USP amoxicillin prepared in buffer.

Sample preparation : The concentration of the sample specified in the USP monograph is 1 mg/mL. The sample was prepared from amoxicillin oral suspension as specified in the USP Monograph for amoxycillin: USP41-NF36. Amoxicillin oral suspension of 125 mg/ml used for assay test preparation and diluted with diluent to 1 mg/ml as per USP monograph. Test solution then filtered by passing through 0.22 µm nylon syringe filter.

UHPLC Method Transfer : The system, Nexera UHPLC was used for USP UHPLC method transfer. Nexera UHPLC developed as an all-round LC to handle diverse liquid chromatography requirements from promoting ultra fast, high-resolution analysis while reducing the burden on the environment. In addition to conventional analysis, the **130 MPa** system can withstand high pressure while reducing system volume to extend the range of application of ultra fast, high-resolution analysis. Method transferred by selecting column with same column chemistry i.e. L1 USP designation. Assay system suitability criteria including %RSD for peak area and USP tailing factor were checked as per USP monograph.

The USP method was transferred from HPLC to UHPLC using the Shimadzu Method transfer program. Details of USP, HPLC method parameters and UHPLC method parameters for amoxicillin oral suspension given in table 1 and table 2 respectively.

Table-1 : USP HPLC Measurement Conditions

Column	:	Shim Pack GIST AQ C18 4.6 x 250 mm, 5µm P/N: 227-30742-08
Mobile Phase	:	Acetonitrile and Buffer (1:24)
Separation Mode	:	Isocratic
Flow Rate	:	1.5 mL/min
Injection Volume	:	10 µL
Detection wavelength	:	230 nm
Run Time	:	20 minutes
Needle Wash	:	Water : Acetonitrile 90 : 10

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Table-1 : USP UHPLC Measurement Conditions

Column	:	Shim Pack GISS C18 2.1 X 100mm, 1.9µm P/N: 227-30048-02
Mobile Phase	:	Acetonitrile and Buffer (1:24)
Separation Mode	:	Isocratic
Flow Rate	:	0.4 mL/min
Injection Volume	:	0.8 µL
Detection	:	230 nm
Run Time	:	3 minutes
Needle Wash	:	Water : Acetonitrile 90 : 10

Results and Discussion

The USP method was transferred from HPLC to UHPLC using the Shimadzu Method transfer program. The separation conditions were transferred on the basis of column, resolution from the separation on LC-2030C into the method transfer program to generate UHPLC method conditions.

Separation on LC-2030 system as per USP method carried out by using USP L1 column, SHIM Pack GIST AQ 250 X4.6 mm, 5µm, chromatograms of amoxicillin standard and amoxicillin oral suspension sample shown in figure-1.

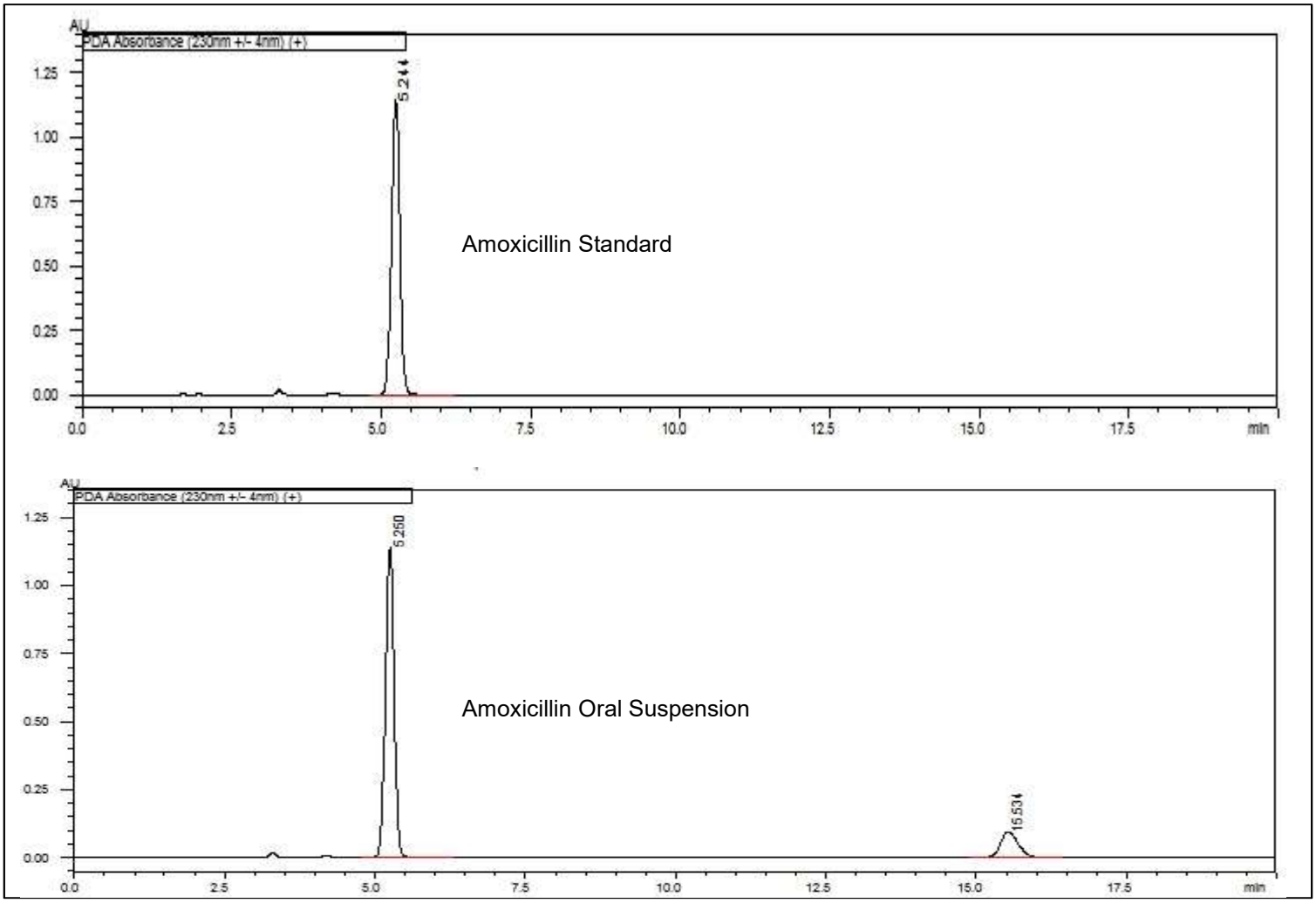

Figure 1. USP HPLC chromatograms of amoxicillin standard and amoxycillin oral suspension using Shim Pack GIST AQ 4.6 x 250 mm, 5 µm column on LC-2030 Prominence-i system

UHPLC Method

UHPLC method transfer were performed accounting for partical size with Shimadzu Shim Pack GISS C18, 2.1 x 100 mm, 1.9 µm column maintaining similar column chemistry. Five replicate injections of amoxicillin standard and amoxicillin oral suspension were analysed and assay suitability suitability criteria checked for %RSD for peak area and USP tailing factor. The run time of the UHPLC method is 3.0 minutes compared to a 20-minutes of HPLC method as per USP monograph, which saves approx. 85% analysis time and 96% savings of solvent and sample consumption. The UHPLC chromatogram of amoxycilline oral suspension shown in figure 2.

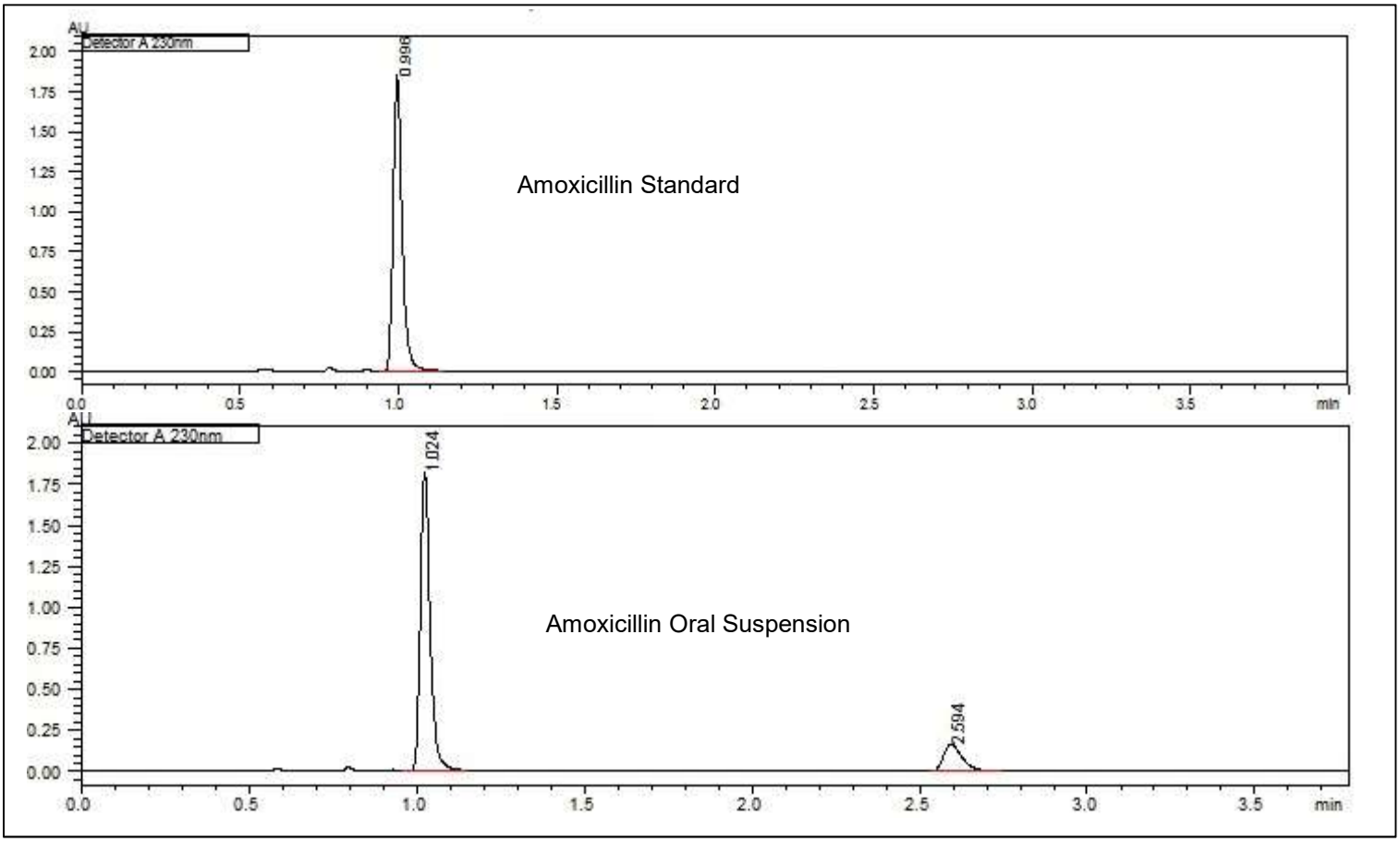

Figure 2. USP UHPLC chromatograms of amoxicillin standard and amoxycillin oral suspension using UHPLC column (Shim Pack GISS 2.1 x 100 mm, 1.9µm on NEXERA UHPLC system.

Amoxicillin Assay System suitability results comparing HPLC to UHPLC for five replicate injections

Table-3: Amoxicillin Standard

Parameter	USP Criteria	HPLC	UHPLC
Retention Time (Minutes)	none	5.244	0.998
%RSD Area	NMT 2.0%	0.145	0.198
USP Tailing	NMT 2.5	0.980	1.29

Table-4: Amoxicillin Oral Suspension

Parameter	USP Criteria	HPLC	UHPLC
Retention Time (Minutes)	none	5.250	1.024
%RSD Area	NMT 2.0%	0.145	0.198
USP Tailing	NMT 2.5	0.980	1.29

Conclusion:

USP method for Amoxicillin oral suspension, USP Monograph USP41-NF36, was successfully transferred from the original HPLC method to the UHPLC method by using modern sub-2 µm column chemistry. Method transferred from HPLC to UHPLC meets the system suitability of USP requirements i.e. %RSD of area of 5 replicate injections NMT 2.0 and USP tailing NMT 2.5 given in table 3 and table-4. The method transfer was facilitated by use of the Shimadzu method transfer program with open column chemistry.

The Shim Pack GISS, 1.9 µm column demonstrated robust and reliable performance. Other benefits realized by transferring this methodology are 85% reduction in run time, 96% reduction in solvent consumption and sample consumption. The ease of transfer using the simple method transfer program helps in the seamless adoption of UHPLC technology which enhances the overall laboratory efficiency and productivity for running USP monographs.

References:

1. Shimadzu LC World Talk International edition spring, C190-E097, 2006.
2. Dhanusha Thambavita et al, *Journal of Pharmaceutical Sciences,* Vol-106, 2017.
3. USP Monograph. Amoxicillin for Oral Suspension, USP41-NF36 [p280] 2017.

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