



## TN-1302

# USP Moxifloxacin Hydrochloride Assay and Organic Impurities On Luna<sup>®</sup> 5 μm Phenyl-Hexyl, 250 x 4.6 mm

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### Introduction

Moxifloxacin is an antibiotic used to treat several bacterial infections including pneumonia, conjunctivitis, and tuberculosis. It is in the fluoroquinolone family of medication and is taken by mouth, injection into vein, and as an eye drop. This technical note for the LC-UV assay and organic impurities for Moxifloxacin Hydrochloride drug substance is based on the USP monograph where a L11 (phenyl) column with 250 x 4.0 mm; 5 μm dimensions was used under isocratic conditions for both Assay and Organic impurity analysis. The Luna 5 μm Phenyl-Hexyl, 250 x 4.6 mm column was used for the analysis of Moxifloxacin Hydrochloride.

### Experimental Conditions

#### Materials and Methods

The standard solution, sensitivity solution, and system suitability solution were prepared in accordance with the USP monograph, using Moxifloxacin Hydrochloride reference standard (USP Catalog number 1448606) and Moxifloxacin Hydrochloride Related compound A (USP Catalog number 1448617). All solutions were protected from light. The Moxifloxacin Hydrochloride reference standard and the Moxifloxacin Related Compound A were obtained from USP (Rockville, Maryland, USA) and the solutions were prepared as indicated in the USP monograph. The original column dimension called for in the monograph is 250 x 4.0 mm, 5μm. We ran the experiment with Luna 5 μm Phenyl-Hexyl, 250 x 4.6 mm dimension. The flow rate was scaled to maintain the same linear velocity in accordance with the recommendations of USP General Chapter 621.

**Table 1. USP allowable adjustments and method comparison**

Parameter	Allowable Change	USP Method	Proposed Method
Column ID	Any (maintain constant linear velocity)	4.0 mm	4.6 mm (+15 %)
Length (L) / Particle Size (d <sub>p</sub> )	L / d <sub>p</sub> remains constant or between the -25 % to +50 % of prescribed ratio	50,000 (= 250 mm / 5 μm)	50,000
Flow Rate	± 50 %	0.9 mL/min	1.2 mL/min (same linear velocity maintained)
Temperature	± 10 %	45 °C	As Specified
Mobile Phase pH	± 0.2 unit	N/A	N/A
Injection Volume	Any reduction	25 μL	As specified
Wavelength	None	293 nm	As Specified
Mobile Phase A/B ratio	Minor ± 30 % relative, but ≤ ± 10 % absolute	Buffer:Methanol (72:28, v/v)	As Specified

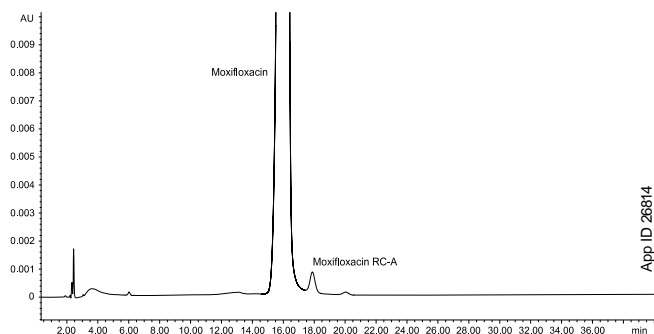


**Dr. Trivikram Reddy Gundala**

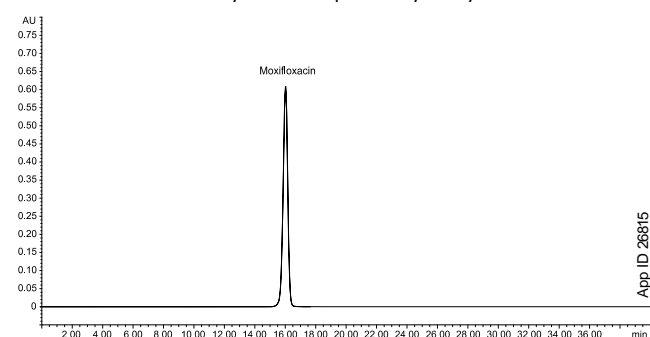
In addition to chromatography, he enjoys playing cricket and watching thriller movies. He loves to spend more time with his little one.



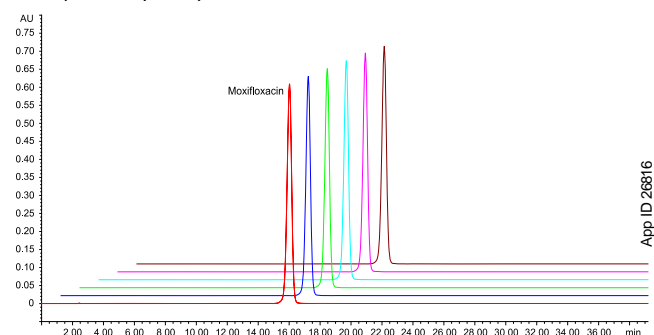
**Figure 1.**  
System Suitability Solution for Assay and Organic Impurities on Luna 5 µm Phenyl-Hexyl



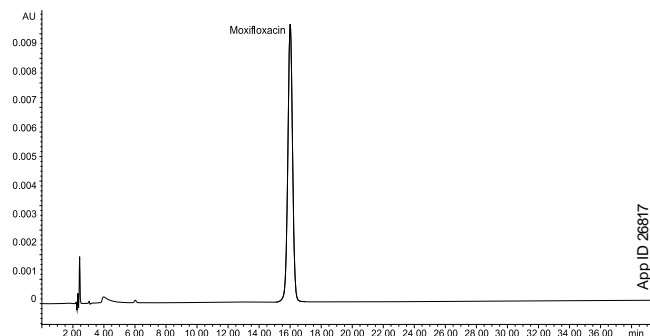
**Figure 2.**  
Standard Solution for Assay on Luna 5 µm Phenyl-Hexyl



**Figure 3.**  
Overlay Chromatogram of Six Injections of Standard Solution for Assay on Luna 5 µm Phenyl-Hexyl



**Figure 4.**  
Standard Solution for Organic Impurities on Luna 5 µm Phenyl-Hexyl



## LC-UV Conditions

**Column:** Luna® Phenyl-Hexyl 5 µm

**Dimension:** 250 x 4.6 mm

**Part No.:** [00G-4257-E0](#)

**Guard Column:** SecurityGuard™ Cartridge, Phenyl (Part No.: [AJ0-4351](#))  
SecurityGuard Holder (Part No.: [KJ0-4282](#))

**Mobile Phase:** Buffer:Methanol (72:28, v/v)

Buffer: 1.0 g of Tetrabutylammonium hydrogen sulfate and 2.0 g of Monobasic potassium phosphate in water, with 4.0 mL of Phosphoric acid diluted to 2000 mL.

Mobile Phase: 1440 mL of Buffer and 560 mL of Methanol was mixed and sonicated for 10 minutes.

**Diluent:** 20 mg of anhydrous sodium sulfite in 1000 mL Buffer

**Flow Rate:** 1.2 mL/min (Isocratic)

**Injection Volume:** 25 µL

**Temperature:** 45 °C

**Detector:** UV @ 293 nm

**System:** Waters® Arc™ HPLC

**Sample:** 1. *System Suitability Solution*: 0.1 mg/mL

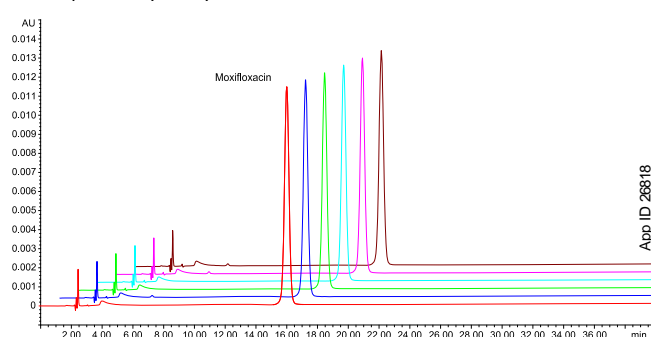
Moxifloxacin HCl and 1 µg/mL Moxifloxacin Related Compound A.

2. *Moxifloxacin HCl Standard Solution for Assay*: 0.1 mg/mL

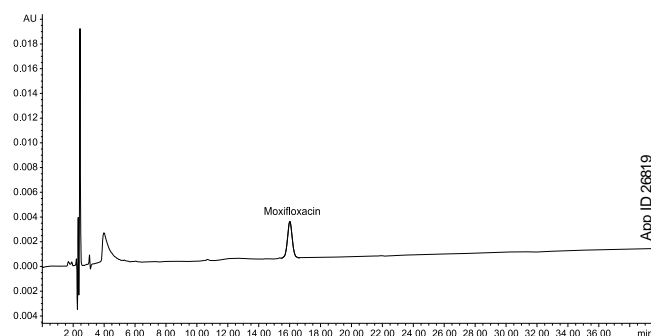
3. *Moxifloxacin HCl Standard Solution for Related Compounds*: 2 µg/mL

4. *Moxifloxacin HCl Sensitivity Solution*: 0.05 µg/mL

**Figure 5.**  
Overlay Chromatogram of Six Standard Solutions for Organic Impurities on Luna 5 µm Phenyl-Hexyl



**Figure 6.**  
Sensitivity Solution for Organic Impurities on Luna 5 µm Phenyl-Hexyl



## Results and Discussion

The resolution mixture, sensitivity solution, and standard solutions were run on a Luna® 5 µm Phenyl-Hexyl, 250 x 4.6 mm column and the results demonstrated that the system suitability requirements were satisfied. **Table 2** shows the summary of results for Assay and organic impurities using the system suitability solution. The column and mobile phase are the same for both assay and organic impurities. The standard and system suitability solutions were used for both assay and organic impurities. The system suitability requirements for the organic impurities: Resolution NLT 1.5 between the Moxifloxacin and Moxifloxacin Related Compound-A from system suitability solution, tailing factor NMT 2.0 and RSD NMT 2 % for Moxifloxacin peak from standard solution and Signal-to-Noise ratio NLT 10 for Moxifloxacin peak from sensitivity solution were easily achieved. The system suitability requirements for the Assay: Resolution NLT 1.5 between the Moxifloxacin and Moxifloxacin Related Compound-A from system suitability solution, tailing factor NMT 2.0 and RSD NMT 0.73 % for Moxifloxacin peak from standard solution were easily achieved.

**Table 2 . Summary of Results: System Suitability Solution for Assay and Organic Impurities**

Analyte	Retention Time, min	Resolution (NLT 1.5)
Moxifloxacin Hydrochloride	16.03	-
Moxifloxacin RC-A	17.87	3.14

**Table 3 . Summary of Results: Standard Solution for Assay**

Injection Number	Moxifloxacin Peak Area	Retention Time, min	Symmetry Factor
1	12974639	16.02	0.92
2	12957470	16.01	0.92
3	12936576	16.01	0.93
4	12897756	16.01	0.93
5	12908424	16.02	0.93
6	12843672	16.02	0.93
Mean	12919756	16.00	0.93
STD Dev	47156.317	0.005	0.005
%RSD	0.36	0.03	0.50

## Conclusions

Moxifloxacin HCl USP monograph method verification was successfully completed on the Luna 5 µm Phenyl-Hexyl, 250 x 4.6 mm column. All requirements for system suitability for both Assay and Organic Impurities were achieved here. The Luna 5 µm Phenyl-Hexyl, 250 x 4.6 mm column can be used for the analysis of Moxifloxacin HCl as per the USP general chapter 621 guidelines.

**Table 4 . Summary of Results: Standard Solution for Organic Impurities**

Injection Number	Moxifloxacin Peak Area	Retention Time, min	Symmetry Factor
1	243464	16.00	0.99
2	242906	16.00	0.99
3	241179	16.00	0.99
4	240393	16.00	0.99
5	239347	16.01	0.99
6	239347	16.00	0.99
Mean	241170	16.00	0.99
STD Dev	1689.445	0.004	0
%RSD	0.70	0.03	0

**Table 5 . Summary of Results: System Suitability Solution for Assay and Organic Impurities**

Analyte	Retention Time, min	Signal-to-Noise Ratio (NLT 10)
Moxifloxacin Hydrochloride	16.02	88

## Luna® Ordering Information

5 µm Analytical Columns (mm)							SecurityGuard™ Cartridges (mm)
Phases	30 x 4.6	50 x 4.6	75 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	4 x 3.0* /10pk
Silica(2)	—	<a href="#">00B-4274-E0</a>	—	<a href="#">00D-4274-E0</a>	<a href="#">00F-4274-E0</a>	<a href="#">00G-4274-E0</a>	<a href="#">AJ0-4348</a>
C5	—	<a href="#">00B-4043-E0</a>	—	<a href="#">00D-4043-E0</a>	<a href="#">00F-4043-E0</a>	<a href="#">00G-4043-E0</a>	<a href="#">AJ0-4293</a>
C8(2)	<a href="#">00A-4249-E0</a>	<a href="#">00B-4249-E0</a>	<a href="#">00C-4249-E0</a>	<a href="#">00D-4249-E0</a>	<a href="#">00F-4249-E0</a>	<a href="#">00G-4249-E0</a>	<a href="#">AJ0-4290</a>
C18(2)	<a href="#">00A-4252-E0</a>	<a href="#">00B-4252-E0</a>	<a href="#">00C-4252-E0</a>	<a href="#">00D-4252-E0</a>	<a href="#">00F-4252-E0</a>	<a href="#">00G-4252-E0</a>	<a href="#">AJ0-4287</a>
CN	<a href="#">00A-4255-E0</a>	<a href="#">00B-4255-E0</a>	<a href="#">00C-4255-E0</a>	<a href="#">00D-4255-E0</a>	<a href="#">00F-4255-E0</a>	<a href="#">00G-4255-E0</a>	<a href="#">AJ0-4305</a>
Phenyl-Hexyl	<a href="#">00A-4257-E0</a>	<a href="#">00B-4257-E0</a>	—	<a href="#">00D-4257-E0</a>	<a href="#">00F-4257-E0</a>	<a href="#">00G-4257-E0</a>	<a href="#">AJ0-4351</a>
NH <sub>2</sub>	—	<a href="#">00B-4378-E0</a>	—	<a href="#">00D-4378-E0</a>	<a href="#">00F-4378-E0</a>	<a href="#">00G-4378-E0</a>	<a href="#">AJ0-4302</a>
SCX	—	<a href="#">00B-4398-E0</a>	—	<a href="#">00D-4398-E0</a>	<a href="#">00F-4398-E0</a>	<a href="#">00G-4398-E0</a>	<a href="#">AJ0-4308</a>
HILIC	—	—	—	<a href="#">00D-4450-E0</a>	<a href="#">00F-4450-E0</a>	<a href="#">00G-4450-E0</a>	<a href="#">AJ0-8329</a>
PFP(2)	—	<a href="#">00B-4448-E0</a>	—	—	—	—	<a href="#">AJ0-8327</a>

for ID: 3.2-8.0 mm

\*SecurityGuard™ Analytical Cartridges require holder, Part No.: [KJ0-4282](#)

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