



TN-1301

Ph. Eur. Monograph 2254: Moxifloxacin Hydrochloride Related Substances and Assay on Luna® 5 µm Phenyl-Hexyl

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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, and as an eye drop. In this technical note we are showing the effective separation of Moxifloxacin from its impurities and related substances according to Ph. Eur. Monograph 2254. The fully porous Luna 5 µm Phenyl-Hexyl 250 x 4.6 mm column under isocratic conditions met the system suitability requirement for both Related Substances and Assay.

All reference solutions were prepared as indicated in Ph.Eur. monograph 2254 for Moxifloxacin Hydrochloride. All Solutions were protected from light. The following Certified Reference Standards (CRS) were purchased from the European Directorate for the Quality of Medicines & HealthCare (EDQM) –Council of Europe; Postal address: Allee Kastner CS 30026F - 67081 Strasbourg (France): Moxifloxacin Peak Identification A CRS (Y0002099) and Moxifloxacin Peak identification B CRS (Y0002081).

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: Mixed 28 volumes of methanol and 72 volumes of a solution containing 0.5 g/L of Tetrabutylammonium hydrogen sulfate, 1.0 g/L of Potassium dihydrogen phosphate and 3.4 g/L phosphoric acid.

Diluent: 0.5 g of Tetrabutylammonium hydrogen sulfate and 1.0 g of Potassium dihydrogen phosphate in 500 mL of water. Add 2.0 mL of Phosphoric acid and 0.050 g of anhydrous sodium sulfite and dilute to 1000 mL with water.

Flow Rate: 1.3 mL/min (Isocratic, 40 min)

Injection Volume: 10 µL

Temperature: 45 °C

Detector: UV @ 293 nm

System: Waters® Arc™ HPLC

Table 1. Preparation of Test and Reference Solutions

Solution	Composition
Test Solution (a)	Dissolve 50 mg of substance to be examined (for this study we used Moxifloxacin HCl standard) and dilute to 50 mL with diluent.
Reference Solution (a)	Dilute 1 mL of the Test Solution (a) to 100 mL with diluent. Dilute 1 mL of this solution to 10 mL with diluent.
Reference Solution (b)	Dissolve 50 mg of Moxifloxacin HCl standard and dilute to 50 mL with diluent. Dilute 1 mL of the solution to 10 mL with diluent.
Reference Solution (c)	Dissolve 5 mg of Moxifloxacin Peak Identification A CRS (containing Impurity A, B, and E) in 5 mL of diluent.
Reference Solution (d)	Dissolve 5 mg of Moxifloxacin Peak Identification B CRS (containing Impurity F) in 5 mL of diluent.

Results and Discussion

Moxifloxacin reference solutions (a), (b), (c), and (d) 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 determination of related substances using reference solution (c) as system suitability solution and **Table 3** shows the summary of results in Assay using reference solution (b). The column and mobile phase are the same for both Assay and Related substances. Reference solution (b) was used for Assay and reference solutions (a), (c), and (d) were used for Related substances.



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.
Reference Solution (c) for Related Substance on Luna® 5 µm Phenyl-Hexyl

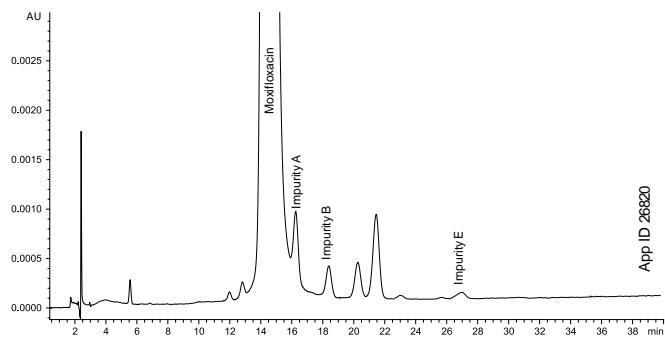


Figure 2.
Reference Solution (d) for Related Substances on Luna 5 µm Phenyl-Hexyl

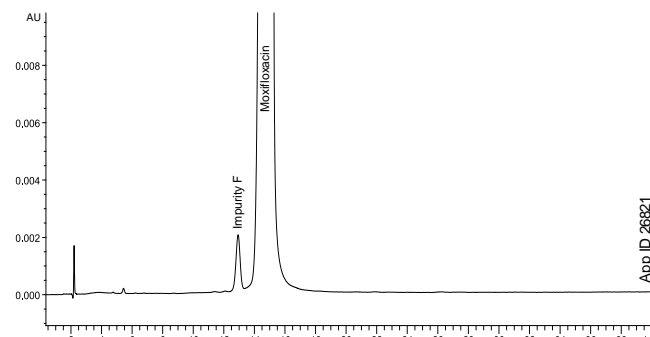


Figure 3.
Reference Solution (a) for Related Substances on Luna 5 µm Phenyl-Hexyl

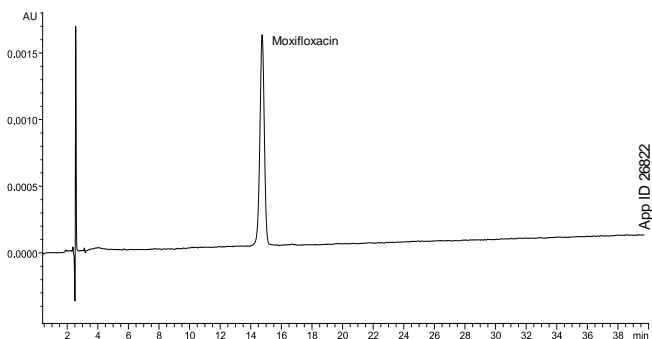


Figure 4.
Reference Solution (b) for Assay on Luna 5 µm Phenyl-Hexyl

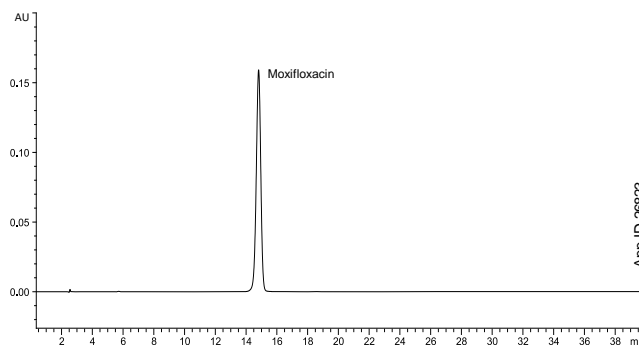


Figure 5.
Overlay Chromatogram of Six Injections of Reference Solution (b) for Assay on Luna 5 µm Phenyl-Hexyl

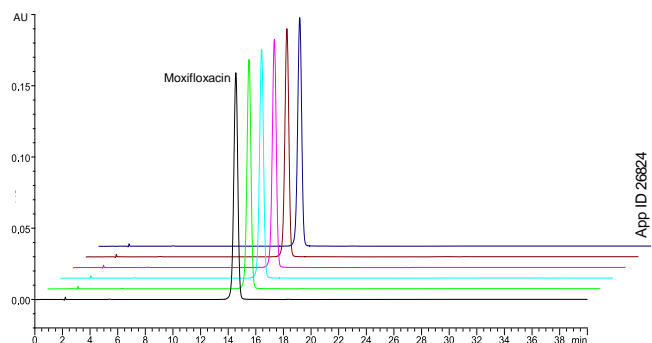


Table 2 . Summary of Results: Reference Solution (c) for Related Substances

Analyte	Retention Time, min	P/V Ratio (Minimum 1.5)
Moxifloxacin Hydrochloride	14.61	-
Moxifloxacin Impurity A	16.20	2.34

Table 3 . Summary of Results: Reference Solution (b) for Assay

Injection Number	Moxifloxacin Peak Area	Retention Time, min
1	3312263	14.58
2	3302233	14.57
3	3292187	14.56
4	3291311	14.56
5	3284965	14.56
6	3263960	14.55
Mean	3291153	14.56
STD Dev	16414.891	0.01
%RSD	0.50	0.07

Conclusions

Moxifloxacin hydrochloride Ph. Eur. Monograph 2254 method verification was successfully completed on Luna 5 µm Phenyl-Hexyl, 250 x 4.6 mm column. The system suitability requirements for the Related substances: Peak-to-Valley ratio (p/v) minimum 1.5 between the Moxifloxacin and Moxifloxacin Impurity A from reference solution (c) was easily achieved. The system suitability requirements for the Assay: RSD NMT 0.73% for Moxifloxacin peak from reference solution (b) was achieved. The Luna 5 µm Phenyl-Hexyl, 250 x 4.6 mm column was demonstrated to be a suitable column for the analysis of Moxifloxacin hydrochloride as per the Ph. Eur. Liquid chromatography 2.2.29 general chapter guidelines.

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)	—	00B-4274-E0	—	00D-4274-E0	00F-4274-E0	00G-4274-E0	AJ0-4348
C5	—	00B-4043-E0	—	00D-4043-E0	00F-4043-E0	00G-4043-E0	AJ0-4293
C8(2)	00A-4249-E0	00B-4249-E0	00C-4249-E0	00D-4249-E0	00F-4249-E0	00G-4249-E0	AJ0-4290
C18(2)	00A-4252-E0	00B-4252-E0	00C-4252-E0	00D-4252-E0	00F-4252-E0	00G-4252-E0	AJ0-4287
CN	00A-4255-E0	00B-4255-E0	00C-4255-E0	00D-4255-E0	00F-4255-E0	00G-4255-E0	AJ0-4305
Phenyl-Hexyl	00A-4257-E0	00B-4257-E0	—	00D-4257-E0	00F-4257-E0	00G-4257-E0	AJ0-4351
NH ₂	—	00B-4378-E0	—	00D-4378-E0	00F-4378-E0	00G-4378-E0	AJ0-4302
SCX	—	00B-4398-E0	—	00D-4398-E0	00F-4398-E0	00G-4398-E0	AJ0-4308
HILIC	—	—	—	00D-4450-E0	00F-4450-E0	00G-4450-E0	AJ0-8329
PFP(2)	—	00B-4448-E0	—	—	—	—	AJ0-8327

for ID: 3.2-8.0 mm

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

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