CASE STUDY

USP Miconazole Nitrate Assay and Organic Impurities

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Introduction

Miconazole Nitrate belongs to the imidazole group of antifungal agents. The development of a quick and efficient analysis of Miconazole Nitrate and its related organic impurities is of interest for generic drug manufacturers. The USP monograph for Miconazole Nitrate Assay and Organic Impurities, which became official on May 1st, 2020, uses the Kinetex™ 2.6 µm, 100 x 4.6 mm Phenyl-Hexyl column. This method was revised by USP as part of the USP monograph modernization efforts and subject to the typical USP review and comment process before being becoming official. The monograph revision for Miconazole Nitrate replaced both the original titration method (Assay) and the original TLC procedure (Organic Impurities) with HPLC. It is worth noting that, while the official USP monograph does not specify a column by name (simply indicated as "L11, 2.6 µm, 100 x 4.6 mm"), the draft which was published in USP-PF 41(4) in 2015 does indicate that the Kinetex 2.6 µm, 100 x 4.6 mm Phenyl-Hexyl column was the column that was used in the elaboration of the monograph.

Since the USP does not publish chromatograms in the official monograph methods, we wanted to demonstrate in this case study that the Kinetex Phenyl-Hexyl column gave the desired results and to share the chromatographic results with those interested in this USP monograph method for Miconazole Nitrate.

System suitability per USP Monograph for the Miconazole Nitrate Assay is a resolution no less than (NLT) 1.5 between Miconazole Nitrate and Miconazole Related Compound F, a symmetry factor no more than (NMT) 2.0 for Miconazole Nitrate, and a percent relative standard deviation (%RSD) of NMT 0.73 % for Miconazole Nitrate. All system suitability requirements for Miconazole Nitrate Assay were met using the Kinetex Phenyl-Hexyl column (**Figure 1** and **2**).

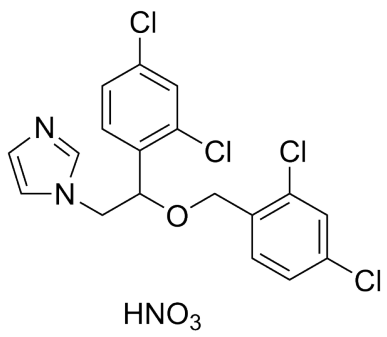
Key Concepts:

- Applicability of Core-shell Phenyl-Hexyl for USP Miconazole Nitrate Assay and Organic Impurities.
- Weak analyte absorbance at low UV and concentration differences between sample/solvent can lend to baseline shifts.

System suitability per USP Monograph for the Miconazole Nitrate Organic Impurities is a resolution NLT 1.5 between Miconazole Related Compound C and Miconazole Related Compound I, a resolution NLT 1.5 between Miconazole Related Compound I and Econazole Nitrate, a resolution NLT 1.5 between Miconazole Related Compound F and Miconazole Nitrate, as well as a %RSD NMT 3.0 % for Miconazole Nitrate. The separation of Miconazole Nitrate and its organic impurities was achieved with all requirements for System Suitability for Organic Impurities met using the Kinetex Phenyl-Hexyl column (**Figure 3**).

All solutions were prepared as indicated in the USP Monograph for Miconazole Nitrate. USP Miconazole Nitrate RS (Catalog No. 1443500), USP Econazole Nitrate RS (Catalog No. 1231808), USP Miconazole Related Compound C RS (Catalog No. 1443431), USP Miconazole Related Compound F RS (Catalog No. 1443464), and USP Miconazole Related Compound I RS (Catalog No. 1443497) were purchased from USP.

Figure 1. Miconazole Nitrate



Application Methods

LC-UV Conditions

Column: Kinetex™ 2.6 µm Phenyl-Hexyl
Dimensions: 100 x 4.6 mm
Part No.: 00D-4495-E0
Mobile Phase: A: Methanol/Water/1 M Triethylammonium Acetate (30:70:1, v/v/v)
B: Acetonitrile/Methanol/1 M Triethylammonium Acetate (25:75:0.2, v/v/v)

Gradient:

Time (min)	%B
0	30
5	30
10	56
27	56
30	75
35	75
36	30
40	30

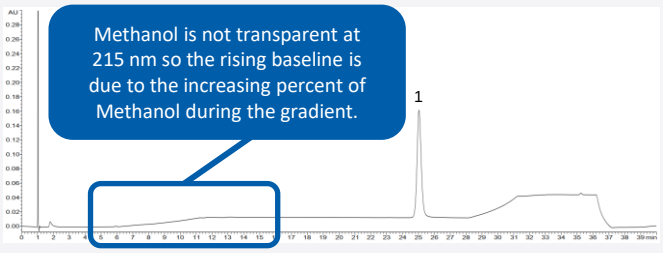
Flow Rate: 0.8 mL/min
Injection Volume: 10 µL
Temperature: 40 °C
Detector: UV @ 215
System: Waters® ACQUITY® I-Class UPLC

Table 1. Preparation of Solutions

Solution	Composition
Diluent – Assay	Methanol/Water (70:30, v/v)
Standard Solution – Assay	0.1 mg/mL USP Miconazole Nitrate RS in Diluent
System Suitability Solution – Assay	0.1 mg/mL of USP Miconazole Nitrate RS and 6 µg/mL of USP Miconazole Related Compound F RS in Diluent
Standard Solution – Organic Impurities	1.2 µg/mL each of USP Miconazole Nitrate RS, USP Econazole Nitrate RS, USP Miconazole Related Compound C RS, USP Miconazole Related Compound F RS, and USP Miconazole Related Compound I RS in Diluent

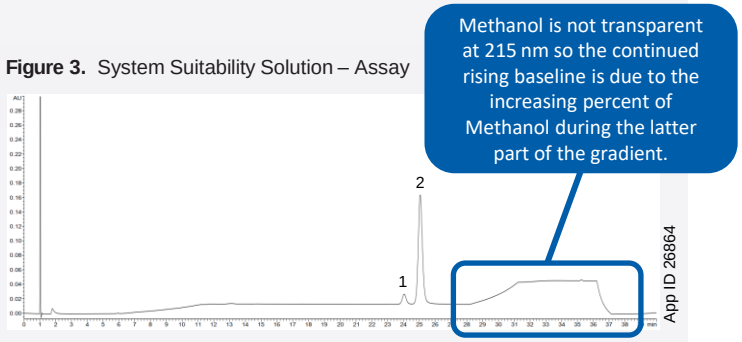
Results and Discussion

Figure 2. Standard Solution - Assay



Peak No.	Analyte	Symmetry Factor	Area %RSD
1	Miconazole Nitrate	1.11	0.421
N=6 Injections			

Figure 3. System Suitability Solution – Assay

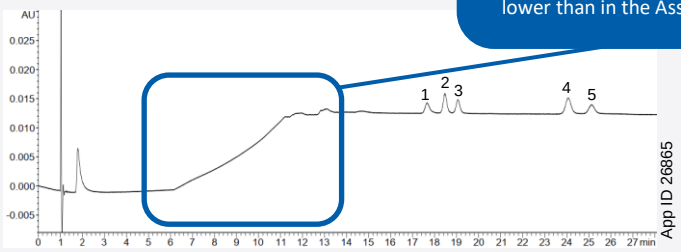


Peak No.	Analyte	Resolution
1	Miconazole Related Compound F	2.1
2	Miconazole Nitrate	
N=6 Injections		

In both **Figure 2** and **3**, there was a rise in the baseline after 10 minutes that continued and then increased in magnitude after 27 minutes. The baseline returned after 36 minutes when the mobile phase composition is returned to the initial conditions. This rise was also seen in the solvent blank (not shown), so it was not due to the composition of the prepared solutions for this application. The rising baseline is due to the increasing % of Methanol in the mobile phase during the gradient. Methanol has some absorbance (is not as UV transparent as Acetonitrile) at 215 nm, so as the % Methanol increases the UV absorbance also increases, resulting in the rising baseline.

The baseline rise due to the increase in Methanol is much more noticeable when the analyte concentrations are much lower than in the Assay.

Figure 4. Standard Solution – Organic Impurities



Peak No.	Analyte	Resolution		Area %RSD
1	Miconazole Related Compound C	2.417	-	-
2	Miconazole Related Compound I		1.867	-
3	Econazole Nitrate	-		-
4	Miconazole Related Compound F	2.2	-	-
5	Miconazole Nitrate		-	2.592
N=6 Injections				

Figure 4 also shows the rise in the baseline due to the increasing % of Methanol in the mobile phase during the gradient, but to what seems a greater extent. This, however, is the same rise that was observed in **Figure 2** and **3** but is much more noticeable because the analyte concentrations are much lower compared to the Assay and there is a difference is scaling.

Key Learnings:

- This method was revised by USP as part of the USP monograph modernization efforts and subject to the typical USP review and comment process before being becoming official.
- The results obtained using the Kinetex™ 2.6 µm Phenyl-Hexyl 100 x 4.6 mm column demonstrate that the Kinetex 2.6 µm Phenyl-Hexyl column met all system suitability requirements as published in the USP monograph.

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