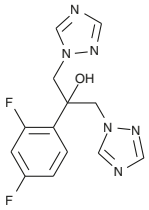


Fluconazole and Related Substances – USP

MACHEREY NAGEL application department · Dr. H. R. Wollseifen, T. Kretschmer, R. Nuessgen

Application benefits

- HPLC method with faster separations within allowable adjustments
- Shorter runtimes
- Lower solvent consumption
- Optimized system suitability

MN products

REF 760083.46
EC HPLC column (analytical), NUCLEODUR® C18 Gravity, 3 µm, 150 × 4.6 mm

REF 763136.46
EC HPLC column (analytical), NUCLEOSHELL® RP 18, 2.7 µm, 150 × 4.6 mm

REF 763134.46
EC HPLC column (analytical), NUCLEOSHELL® RP 18, 2.7 µm, 100 × 4.6 mm

REF 702107
Screw closure, N 9, PP, yellow, center hole, Silicone white/PTFE red, 1.0 mm

REF 702079
Screw neck vial, N 9, 11.6x32.0 mm, 1.5 mL, label, flat bottom, amber, silanized

MN application numbers

HPLC: 129330
HPLC: 129340
HPLC: 129350

Keywords

Fluconazole, USP, NUCLEOSHELL® RP18, NUCLEODUR® C18 Gravity, L1, United States Pharmacopeia

Introduction

The USP monograph describes the separation of Fluconazole from impurities. This work starts using a fully porous HPLC phase and shows the benefits using superficially porous particles. The method optimization was performed to achieve shorter run time and system suitability results within allowable adjustments.

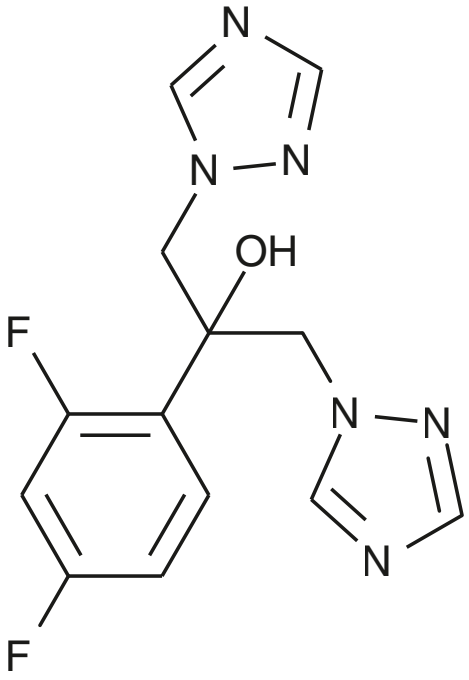


Figure 1: Structure of fluconazole.

USP Monograph: Fluconazole Method Details

Method Parameter	Description
Standard solution	10 µg/mL each of USP Fluconazole RS, USP Fluconazole Related Compound A RS, USP Fluconazole Related Compound B RS, and USP Fluconazole Related Compound C RS, dissolved in acetonitrile, and then diluted in Mobile phase.
Column size	150 × 4.6 mm
Stationary Phase	3.5-µm packing L1
Mobile Phase	Acetonitrile and water (20:80)
Flow Rate	0.5 mL/min
Temperature	40 °C
Detection	260 nm
Injection	20 µL
Run Time	1.5 times the retention time of Fluconazole (about 6 min)
Elution Order	1. Fluconazole related compound A 2. Fluconazole related compound B 3. Fluconazole related compound C 4. Fluconazole
Suitability requirements	
Resolution:	NLT 1.5 between fluconazole related compound B and fluconazole related compound C.
Relative standard deviation:	NMT 5.0 % for each peak.

* Fluconazole (USP-1271700), Fluconazole Related Compound A (USP-1271711), Fluconazole Related Compound B (USP-1271722), and Fluconazole Related Compound C (USP-1271733), were purchased from Labmix24 GmbH; Postal address: Industriestrasse 18A - 46499 Hamminkeln (Germany).

Table 1a: USP Monograph: Fluconazole Method Details

Chromatographic methodology improvements

Figure 2: a

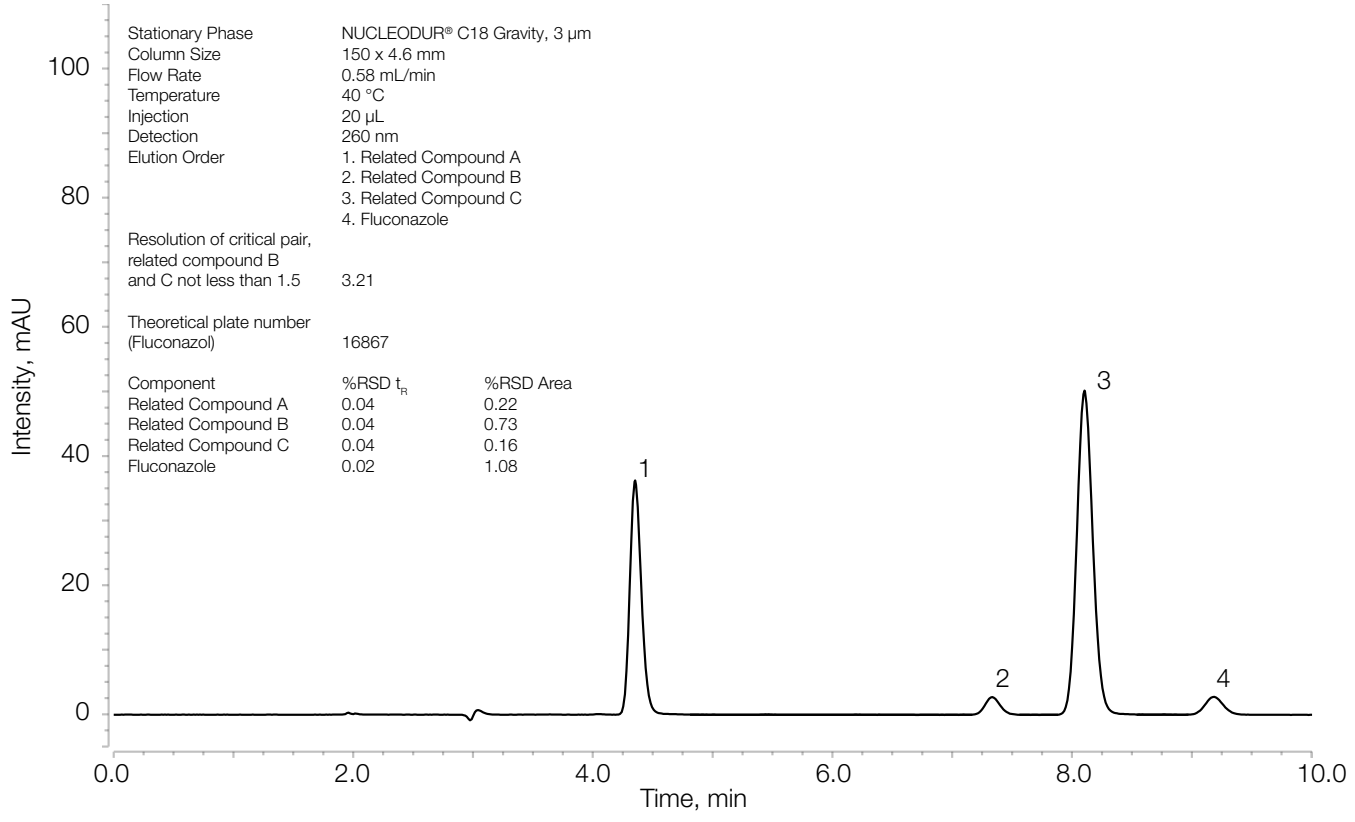
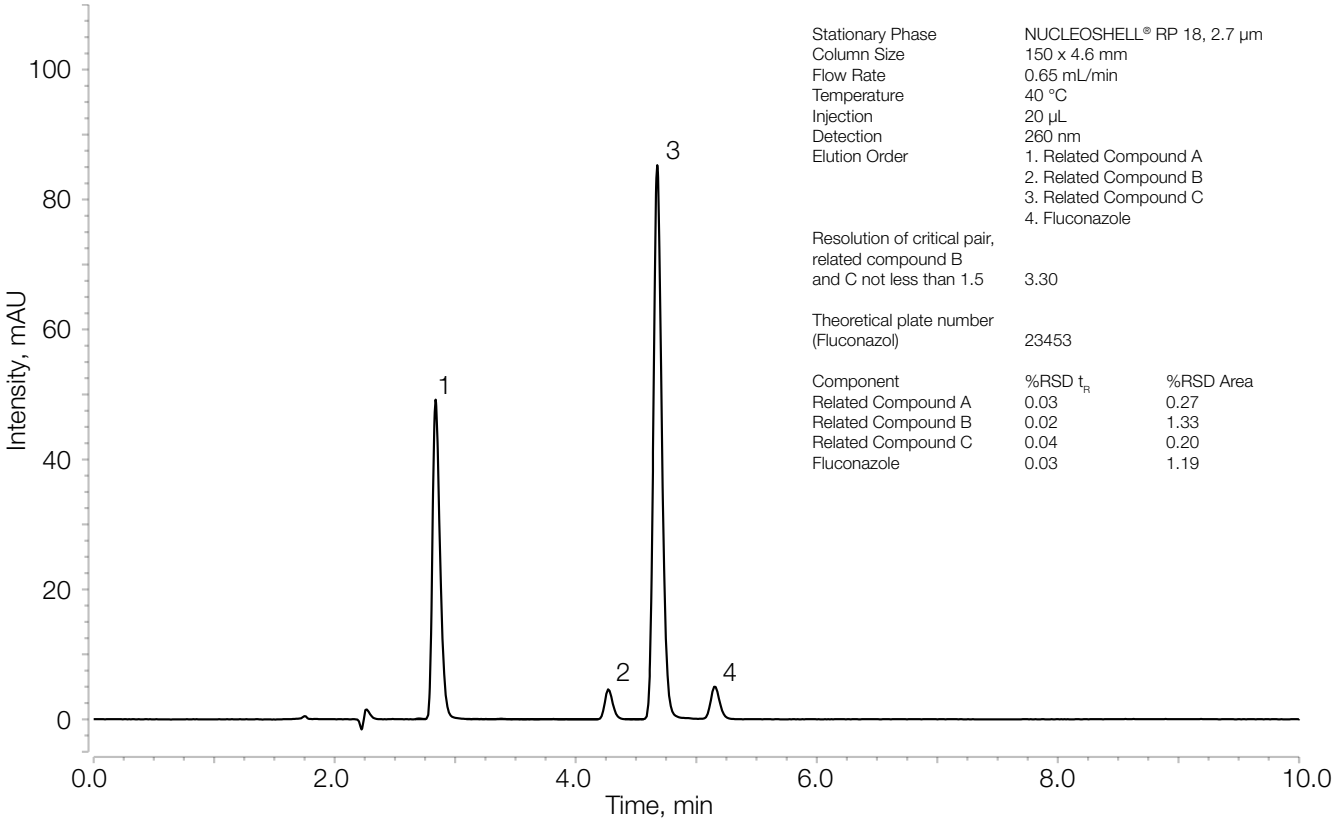


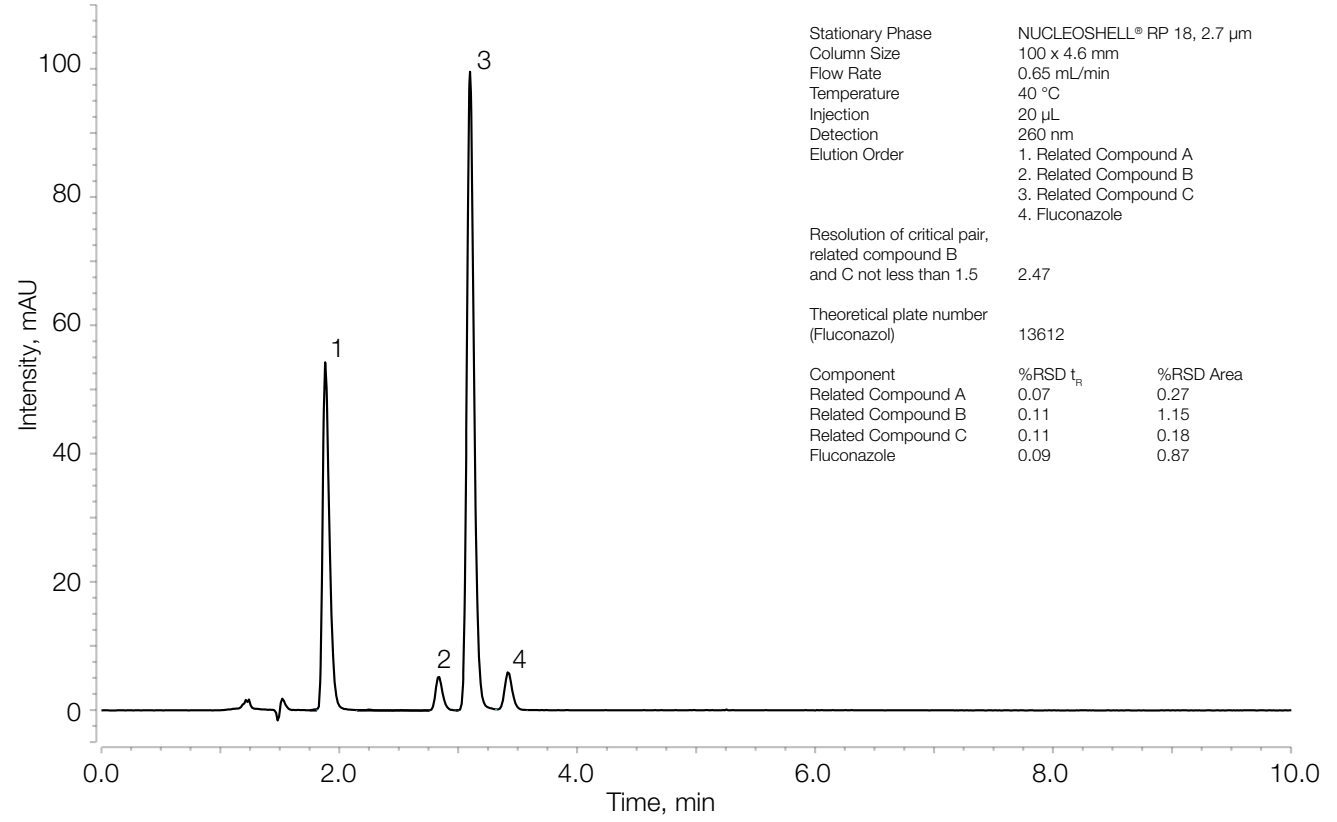
Figure 2: b



Stationary Phase	NUCLEOSHELL® RP 18, 2.7 µm
Column Size	150 x 4.6 mm
Flow Rate	0.65 mL/min
Temperature	40 °C
Injection	20 µL
Detection	260 nm
Elution Order	1. Related Compound A 2. Related Compound B 3. Related Compound C 4. Fluconazole
Resolution of critical pair, related compound B and C not less than 1.5	3.30
Theoretical plate number (Fluconazol)	23453

Component	%RSD t_R	%RSD Area
Related Compound A	0.03	0.27
Related Compound B	0.02	1.33
Related Compound C	0.04	0.20
Fluconazole	0.03	1.19

Figure 2: c



Stationary Phase	NUCLEOSHELL® RP 18, 2.7 µm
Column Size	100 x 4.6 mm
Flow Rate	0.65 mL/min
Temperature	40 °C
Injection	20 µL
Detection	260 nm
Elution Order	1. Related Compound A 2. Related Compound B 3. Related Compound C 4. Fluconazole
Resolution of critical pair, related compound B and C not less than 1.5	2.47
Theoretical plate number (Fluconazol)	13612

Component	%RSD t_R	%RSD Area
Related Compound A	0.07	0.27
Related Compound B	0.11	1.15
Related Compound C	0.11	0.18
Fluconazole	0.09	0.87

Figure 2: a: EC HPLC column (analytical), NUCLEODUR® C18 Gravity, 3 µm, 150 × 4.6 mm, b: EC HPLC column (analytical), NUCLEOSHELL® RP 18, 2.7 µm, 150 × 4.6 mm, c: EC HPLC column (analytical), NUCLEOSHELL® RP 18, 2.7 µm, 100 × 4.6 mm.

Results

Method Parameter	Allowed Adjustments (isocratic elution)*	Method 1 (figure 2a)	Method 2 (figure 2b)	Method 3 (figure 2c)
Mobile phase pH	± 0.2 units	As specified	As specified	As specified
Concentration of salts in buffer	± 10%	As specified	As specified	As specified
Composition of the mobile phase	± 30% relative; cannot exceed ± 10% absolute change; cannot be reduced to zero	As specified	As specified	As specified
Stationary phase	No change of C18 allowed	NUCLEODUR® C18 Gravity	NUCLEOSHELL® RP 18	NUCLEOSHELL® RP 18
Ratio column length/ particle size	Column length to particle size diameter ratio can be adjusted between – 25% and + 50%	150 mm / 3 µm (+ 16.7%*)	150 mm / 2.7 µm (+ 29.6%*)	100 mm / 2.7 µm (– 13.6%*)
Column internal diameter	Can be adjusted so long as linear velocity is maintained	4.6 mm as specified	4.6 mm as specified	4.6 mm as specified
Flow rate	± 50% after adjustment due to a change in column dimensions	0.58 mL/min (± 0% after adjustments)	0.65 mL/min (± 0% after adjustments)	0.65 mL/min (± 0% after adjustments)
Column temperature	± 10 °C	40 °C as specified	40 °C as specified	40 °C as specified
Injection volume	Can be adjusted as much as needed; must be consistent with linearity, precision, and detection requirements	20 µL as specified	20 µL as specified	20 µL as specified
Detection [nm]	No change permitted	260 nm as specified	260 nm as specified	260 nm as specified
Retention time Fluconazole [min]		8.101 min	5.152 min (– 36.4%**)	3.418 min (– 57.8%**)
Theoretical plate number (Fluconazol)	Within -25% to 50%, relative to the prescribed column***	16867	23453 (+ 39.1%**)	13612 (– 19.3%**)
Suitability requirements				
Resolution:	NLT 1.5 between flucon-azole related compound B and fluconazole related compound C	3.21	3.30	2.47
%RSD t_r:	NMT 5.0% for each peak.	0.02 – 0.04 (see Figure 2a)	0.02 – 0.04 (see Figure 2b)	0.07 – 0.11 (see Figure 2c)
%RSD Area:	NMT 5.0% for each peak.	0.16 – 1.08 (see Figure 2a)	0.20 – 1.33 (see Figure 2b)	0.18 – 1.15 (see Figure 2c)

* change in comparison to USP method ** change in comparison to method 1 *** column used in method 1.

Conclusion

The fully porous NUCLEODUR® C18 Gravity, 3 µm, 150 × 4.6 mm HPLC column from MACHEREY NAGEL fulfills all requirements of the USP monograph (Fluconazole and Related Substances). By using superficially porous NUCLEOSHELL® analytical columns the runtime of the method can be reduced by up to 57.8 % (with NUCLEOSHELL® RP 18, 2.7 µm, 100 × 4.6 mm) compared to fully porous NUCLEODUR® silica gel, while keeping all method parameters well within the allowed adjustment range of the USP monograph. The reduction in runtime leads to a lower solvent consumption optimizing the analysis of Fluconazol with regard to the guidelines of green chemistry. We were also able to improve the peak intensity with NUCLEOSHELL® columns compared to the original method with fully porous silica gel.

MACHEREY-NAGEL GmbH & Co. KG · Valencienner Str. 11 · 52355 Düren · Germany
DE +49 24 21 969-0 info@mn-net.com
CH +41 62 388 55 00 sales-ch@mn-net.com
FR +33 388 68 22 68 sales-fr@mn-net.com
US +1 888 321 62 24 sales-us@mn-net.com