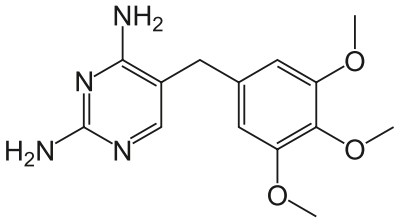

Trimethoprim and Related Substances – USP

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

Application benefits

- HPLC method with fast separation within allowable adjustments.
- Short runtimes
- Low solvent consumption
- Optimized system suitability

MN products

REF 763157.46
EC HPLC column (analytical), NUCLEOSHELL® RP 18, 5 µm, 250x4.6 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: 129400

Keywords

Trimethoprim, USP, NUCLEOSHELL® RP 18, L1, United States Pharmacopeia

Introduction

The USP monograph describes the separation of Trimethoprim from impurities. Using a superficially porous HPLC phase this separation can be achieved with short run times and therefore low solvent consumption while keeping the system suitability parameters within the allowed adjustment ranges.

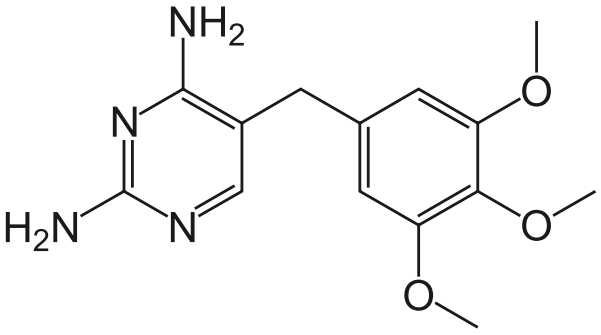

Figure 1: Structure of Trimethoprim.

USP Monograph: Trimethoprim Method Details

Method Parameter	Description
Resolution solution	Dissolve accurately weighed quantities of USP Trimethoprim RS* and Diaveridine; and dilute quantitatively, and stepwise if necessary, with Mobile phase to obtain a solution having known concentrations of about 10 µg per mL and 5 µg per mL, respectively.
Test solution	Transfer about 25.0 mg of Trimethoprim to a 25 mL volumetric flask, dissolve in and dilute with mobile phase to volume, and mix.
Column size	250 x 4.6 mm
Stationary phase	Base-deactivated packing L1
Mobile phase	Buffer solution: Prepare a 10 mM sodium perchlorate solution in water, adjust with phosphoric acid to a pH of 3.6, and mix. Mobile phase: Prepare a filtered and degassed mixture of Buffer solution and methanol (7:3).
Flow rate	1.3 mL/min
Detection	280 nm
Injection	20 µL
Run time	11 times the retention time of Trimethoprim
Elution order	1. Trimethoprim 2. Diaveridine
Suitability requirements	
Resolution:	NLT 2.5 between Trimethoprim and Diaveridine.
Relative standard deviation:	NMT 2.0% for each peak.

* Trimethoprim (USP-1692505 was purchased from Labmix24 GmbH; Postal address: Industriestrasse 18A - 46499 Hamminkeln (Germany).

Table 1: USP Monograph: Trimethoprim Method Details

Chromatographic methodology improvements

Figure 2:

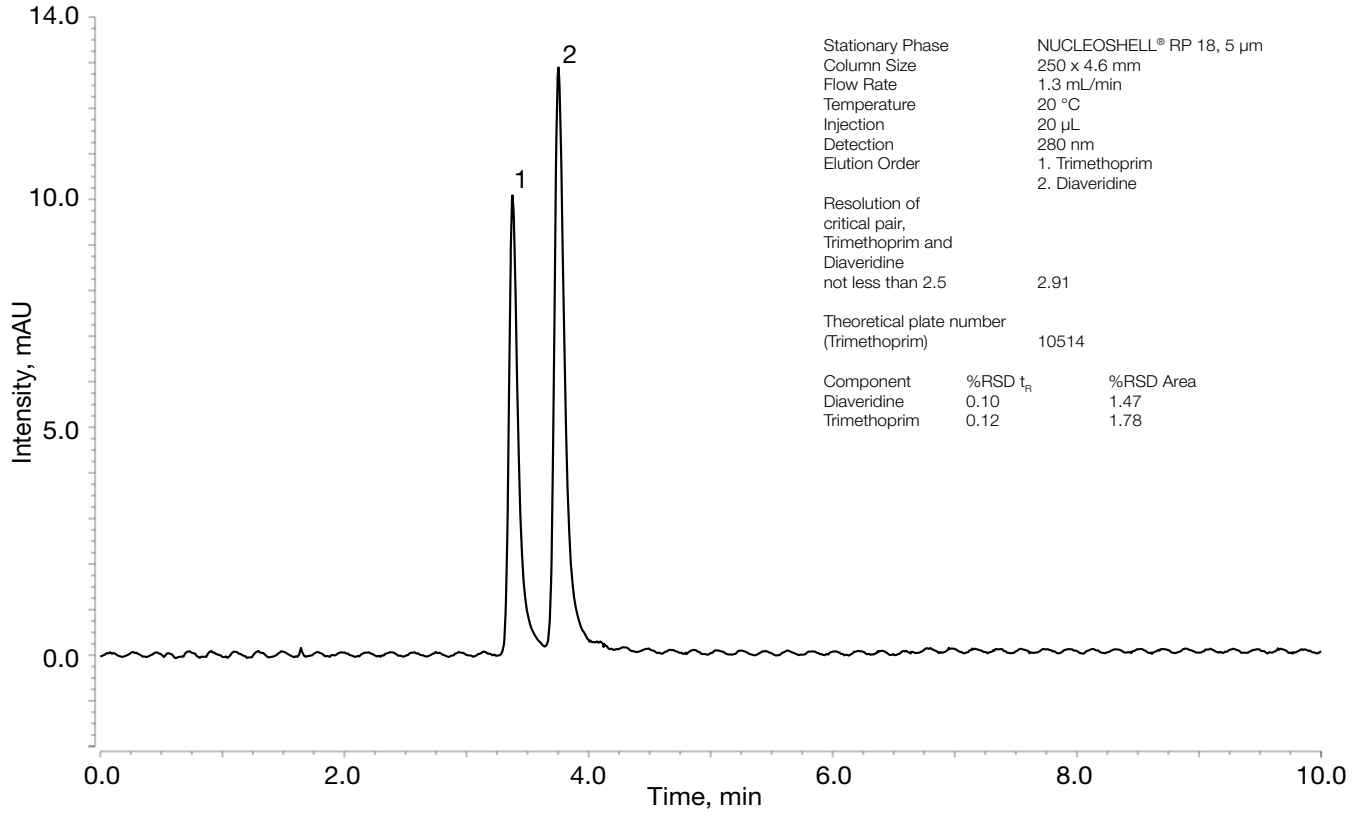

Figure 2: EC HPLC column (analytical), NUCLEOSHELL® RP 18, 5 µm, 250x4.6 mm.

Results

Method Parameter	Allowed Adjustments (isocratic elution)	Method 1 (figure 2)
Mobile phase pH	± 0.2 units	As specified
Concentration of salts in buffer	± 10%	As specified
Composition of the mobile phase	± 30% relative; cannot exceed ± 10% absolute change; cannot be reduced to zero	As specified
Stationary phase	No change of C18 allowed	NUCLEOSHELL® RP 18
Ratio column length/particle size	Column length to particle size diameter ratio can be adjusted between – 25% and +50%	250 mm / 5 µm
Column internal diameter	Can be adjusted so long as linear velocity is maintained	4.6 mm as specified
Flow rate	± 50% after adjustment due to a change in column dimensions	1.3 mL as specified
Column temperature	± 10 °C	20 °C
Injection volume	Can be adjusted as much as needed; must be consistent with linearity, precision, and detection requirements	20 µL as specified
Detection [nm]	No change permitted	280 nm as specified
Retention time Trimethoprim [min]		3.748
Theoretical plate number (Trimethoprim)	Within –25% to 50%, relative to the prescribed column*	10514
Suitability requirements		
Resolution:	NLT 2.5 between Trimethoprim and Diaveridine	2.71
%RSD t_r:	NMT 2.0% for each peak.	0.10 – 0.12 (see Figure 2)
%RSD Area:	NMT 2.0% for each peak.	1.47 – 1.78 (see Figure 2)

* column used in method 1.

Conclusion

The superficially porous NUCLEOSHELL® RP 18, 5 µm, 250x4.6 mm HPLC column from MACHEREY NAGEL fulfills all requirements of the USP monograph (Trimethoprim and Diaveridine).

By using superficially porous particles, the separation between Trimethoprim from impurities can be achieved in less than 4 minutes leading to a low solvent consumption while keeping all method parameters well within the allowed adjustment range of the USP monograph.

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