Use of New YMC Meteoric Core Core-shell and YMC-Triart Hybrid Material for an Improved USP Chlorhexidine Gluconate Assay Method

Jeffrey Kakaley[1], Takashi Sato[2], Ernest J. Sobkow[1]

1. YMC America, Inc., Allentown, PA USA 2. YMC Co., Ltd., Kyoto, Japan

Introduction

USP methods are used widely in the pharmaceutical industry and are often not optimized for use with modern instrumentation and packing materials. As it becomes increasingly important to boost throughput in the modern laboratory, new instrumentation and smaller particle size packing materials are allowing scientists to achieve these goals while also realizing cost savings in terms of man-hours and solvent usage. In this poster we investigate the use of 3μm YMC-Triart C18 and 2.7μm YMC-Meteoric Core C18 stationary phases for improving a USP assay method for chlorhexidine gluconate.

Experimental

Method Parameters

Mobile Phases

Mobile Phase A: 0.1M NaH_2PO_4, 0.5% Triethylamine, pH=3.0
Mobile Phase B: 100% Acetonitrile

All columns were equilibrated with a minimum 10 column volumes of mobile phase prior to 1st injection.

Original USP Instrument Parameters

Column:	250x4.6mm, 5μm L1 packing
Flowrate:	1.5mL/min
Column Temp:	40° C
Sample Temp:	4° C
Detection λ:	215 nm
Injection Vol:	50μL

Original USP Gradient

Time (min)	%A	%B
0	100	0
9	100	0
10	45	55
15	45	55
16	100	0
21	100	0

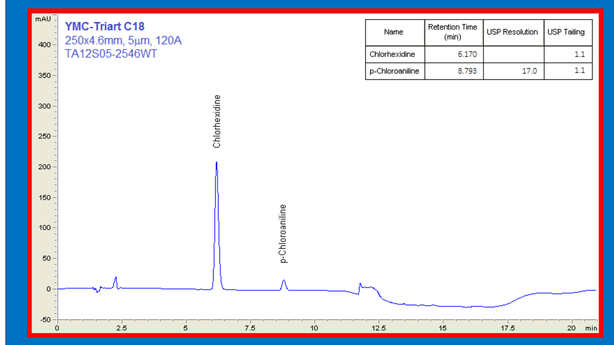
Columns Used

YMC-Triart C18, 250x4.6mm, 5μm, 120Å, P/N: TA12S05-2546WT
YMC-Triart C18, 100x2.0mm, 3μm, 120Å, P/N: TA12S03-1002WT
YMC Meteoric Core C18, 100x2.1mm, 2.7μm, 80Å, P/N: CAS08SQ7-10Q1PT

Samples

Diluent Blank: 0.1M NaH_2PO_4, adjusted to pH=3.0 with o-phosphoric acid
Test Sample: 50μg/mL Chlorhexidine Acetate with 1μg/mL p-Chloroaniline in diluent blank solution.

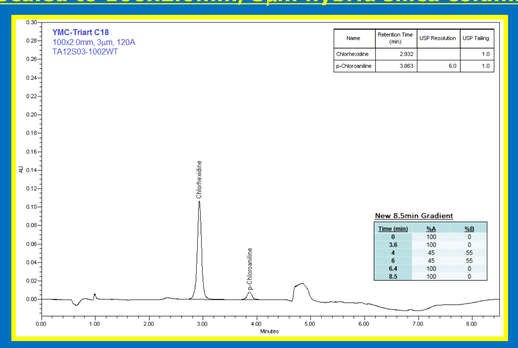
Original USP Conditions



Name	Retention Time (min)	USP Resolution	USP Tailing
Chlorhexidine	6.170		1.1
p-Chloroaniline	8.793	17.0	1.1

Scaled Parameters
Flowrate = 0.3mL/min
Inj. Volume = 4μL
Gradient = 8.5min

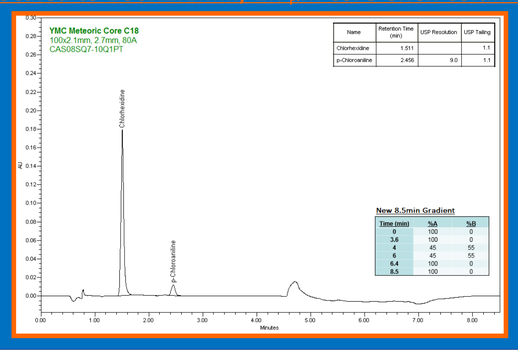
Scaled to 100x2.0mm, 3μm hybrid silica column



Name	Retention Time (min)	USP Resolution	USP Tailing
Chlorhexidine	2.932		1.0
p-Chloroaniline	3.863	6.0	1.0

Time (min)	%A	%B
0	100	0
3.6	100	0
4	45	55
6	45	55
6.4	100	0
8.5	100	0

Scaled to 100x2.1mm, 2.7μm core-shell column

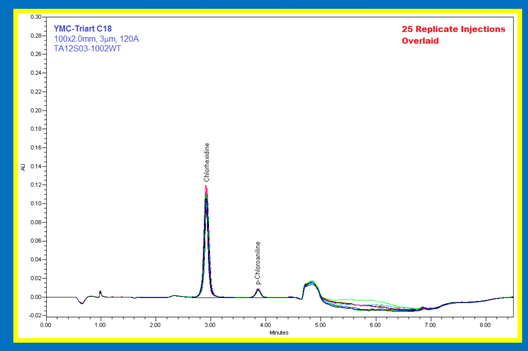


Name	Retention Time (min)	USP Resolution	USP Tailing
Chlorhexidine	1.511		1.1
p-Chloroaniline	2.456	9.0	1.1

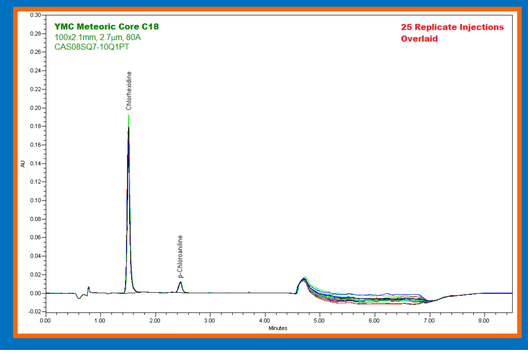
Time (min)	%A	%B
0	100	0
3.6	100	0
4	45	55
6	45	55
6.4	100	0
8.5	100	0

Lower Flow rate!
Faster Run time!
Smaller Injection!
Less Solvent!
Lower Cost!
Greater Total Savings!

25 Repeat Injections



25 Repeat Injections



Results and Discussion

The original USP assay method for chlorhexidine gluconate was first evaluated on a 5μm, 250x4.6mm YMC-Triart C18 column to insure appropriate selectivity and separation could be obtained on the hybrid-silica stationary phase. Once these parameters were confirmed, the method was next scaled down for use with the 100x2.0mm 3μm porous (Triart C18) and 2.7μm core-shell (Meteoric Core) columns. Flow rate and injection volume were reduced to 0.3mL/min and 4μL respectively, due to the decrease of column I.D. and the gradient was adjusted accordingly to take into account these parameter changes as well as the decreased column length. The scaled down method exhibited results for resolution (on both columns) that would easily pass the USP system suitability specification (NLT 3.0). 25 consecutive injections were then made on each column to determine how reproducible the scaled down method was. All retention times exhibited %RSD values of ≤0.3% for chlorhexidine and p-chloroaniline on both columns.

Conclusions

- YMC-Triart C18 and YMC Meteoric Core stationary phases exhibit the selectivity necessary to run the USP assay method for chlorhexidine gluconate.
- The resolution gained from smaller particle sizes (3mm Triart C18) and core-shell material (2.7mm Meteoric Core) allow for the use of shorter length and smaller I.D. columns.
- These columns allow for faster run times at a lower flow rate, enabling cost savings in solvent usage (12X less solvent) as well as time (1/3 total run time) savings for employees.
- YMC-Triart C18 3μm and YMC Meteoric Core 2.7μm core-shell materials are good choices for scaling down the USP chlorhexidine gluconate assay method.