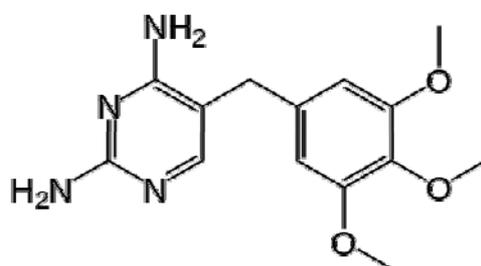


Trimethoprim



Trimethoprim

Trimethoprim is a bacteriostatic antibiotic used mainly in the prophylaxis and treatment of urinary tract infections. Trimethoprim binds to dihydrofolate reductase and inhibits the reduction of dihydrofolic acid (DHF) to tetrahydrofolic acid (THF). THF is an essential precursor in the thymidine synthesis pathway and interference with this pathway inhibits bacterial DNA synthesis.

Trimethoprim's affinity for bacterial dihydrofolate reductase is several thousand times greater than its affinity for human dihydrofolate reductase. Human dihydrofolate reductase is a target for numerous anti cancer drugs.

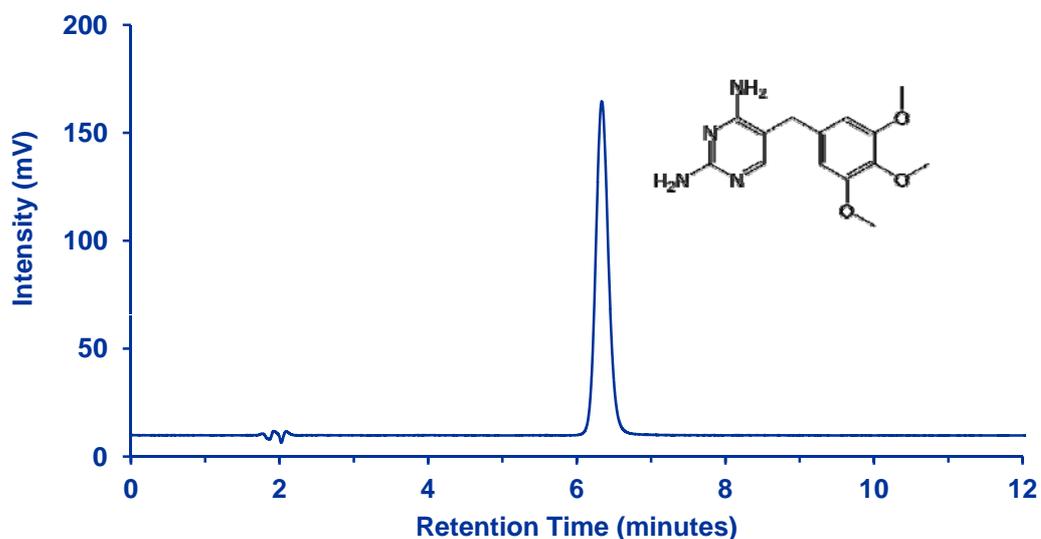
The current USP assay method for trimethoprim is potentiometric titration with 0.1 N perchloric acid.

Trimethoprim

SeQuant[®] ZIC[®]-cHILIC

Chromatographic Conditions

Column:	SeQuant [®] ZIC [®] -cHILIC (3 μm, 100 Å) PEEK 150 × 4.6 mm	1.50661.0001
Injection:	10 μL	
Detection:	Hitachi Chromaster-5430 Diode Array Detector, UV=280 nm	
Cell:	13 μL	
Flow Rate:	1.0 mL/min	
Mobile Phase:	Buffer: Dissolve 1.261 g of ammonium formate in 1000 ml water (20 mM). Mix Acetonitrile and Buffer 90:10 (v/v)	
Temperature:	25 °C	
Diluent:	Mobile phase	
Sample:	Trimethoprim RS 100 ppm	
Pressure Drop:	105 Bar (1500 psi)	



Chromatographic Data

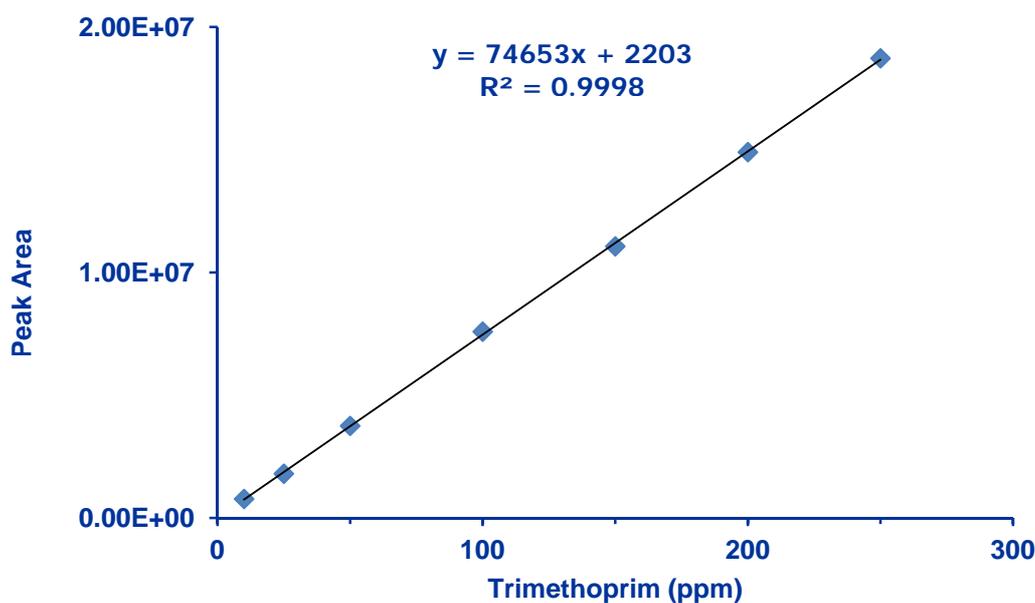
No.	Compound	Retention Time (min)	Retention factor K ^o	Asymmetry
1	t ₀	1.50	-	-
2	Trimethoprim RS	6.40	3.27	1.10

Linearity and repeatability for Trimethoprim

SeQuant® ZIC® -cHILIC

Calibration Data – Trimethoprim

Day	Replicate	Mean Response (Arbitrary Area Units)		
		Set 1	Set 2	Set 3
1	1	7664046	7497835	7652008
	2	7534304	7526457	7613707
	3	7473688	7437704	7478460
	4	7538221	7523075	7511738
	5	7511779	7510099	7500177
	Mean	7544408	7499034	7551218
	Response (100ppm)	101.0	100.4	101.1
	Std.dev	71623	36108	76683
	RSD	0.9	0.5	1.0



The linearity, accuracy and precision of the proposed assay method for Trimethoprim using the SeQuant® ZIC®- cHILIC column was well within the criteria normal for a USP monograph method.

LOD= 5 ppm

LOQ= 13 ppm