Impurities test for Trimethoprim (USP-38 method):

SAMPLE PREPARATION:

Test Solution: Transfer about 25 mg of Trimethoprim to 25 ml volumetric flask, dissolve and dilute with mobile phase to volume.

Resolution solution: Dissolve accurately weighed quantity of Trimethoprim and Diaveridine and dilute quantitatively with mobile phase to obtain solution of 10 µg per ml and 5µg/ml respectively.

CHROMATOGRAPHIC CONDITIONS:

Instrument: UltiMate 3000 LC
Column: Acclaim 120 C18 (4.6*250mm, 5um, p/n: 059149, lot no.: 018-01-152)

Buffer solution: 10mM Sodium perchlorate solution in water adjust pH to 3.6 with phosphoric acid

Mobile phase: Prepare a mixture of Buffer solution and Methanol (7:3).

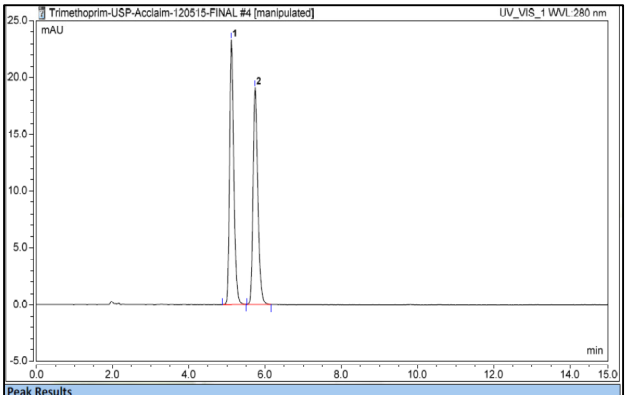
Separation Mode: Isocratic
Column temperature: 25˚C
Flow rate: 1.3 mL/min
Injection Volume: 20 µl
Detector wavelength: UV280 nm
Run Time: 55min

System Suitability Results:

Sr. No.	Parameters	USP Criteria	Obtained Results
1	Resolution between Trimethoprim and Diaveridine	NLT 2.5	2.97

CHROMATOGRAMS:

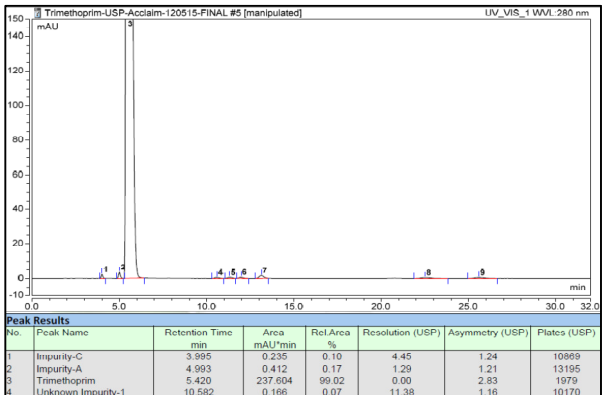
System Suitability:



Peak Results

No.	Peak Name	Retention Time min	Area mAU*min	Rel.Area %	Resolution (USP)	Asymmetry (USP)	Plates (USP)
1	Trimethoprim	5.115	2.986	51.83	2.97	1.29	10808
2	Diaveridine	5.738	2.775	48.17	n.a.	1.28	10615

Impurity Mix:



Peak Results

No.	Peak Name	Retention Time min	Area mAU*min	Rel.Area %	Resolution (USP)	Asymmetry (USP)	Plates (USP)
1	Impurity-C	3.995	0.235	0.10	4.45	1.24	10869
2	Impurity-A	4.993	0.412	0.17	1.29	1.21	13195
3	Trimethoprim	5.420	237.604	99.02	0.00	2.83	1979
4	Unknown Impurity-1	10.582	0.166	0.07	11.38	1.16	10170
5	Impurity-G	11.313	0.192	0.08	12.51	1.17	9903
6	Impurity-D	11.967	0.178	0.07	14.36	1.22	12698
7	Impurity-B	13.132	0.525	0.22	16.07	1.12	12353
8	Impurity-F	22.515	0.310	0.13	16.81	1.29	3388
9	Impurity-J	25.595	0.338	0.14	22.50	1.23	6146