Impurities test for Caffeine (USP 38 method):

SAMPLE PREPARATION:

System Suitability Solution: 0.02 mg/ml of Theophylline in Mobile phase.

Standard solution: Transfer 5.0mg of Caffeine standard to 25 ml volumetric flask, add 5.0 ml of System suitability solution and 10 ml of Mobile phase, make up volume with mobile phase.

Sample solution: 0.2 mg/ml of Caffeine in Mobile phase.

CHROMATOGRAPHIC CONDITIONS:

Instrument: UltiMate 3000 LC
Column: Acclaim 120 C18 (4.6*150mm, 5um, p/n 059148, lot no.:018-01-171)

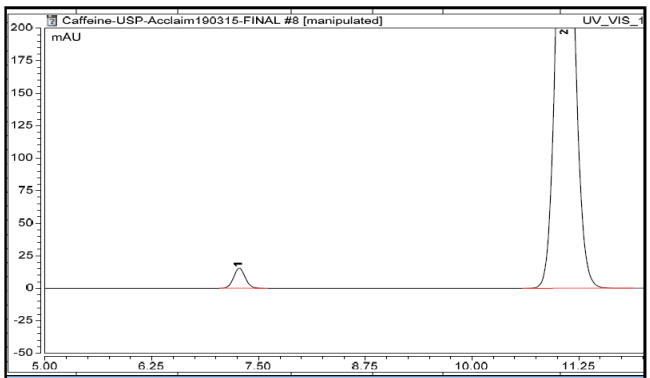
Mobile phase: Acetonitrile: Tetrahydrofuran:Buffer (25:20:955) adjust pH to 4.5 with Glacial acetic acid.
Buffer: 0.82gm/lt of anhydrous sodium acetate.
Separation Mode: Isocratic
Column temperature: 25˚C
Flow rate: 1.0 mL/min
Injection Volume: 10 µl
Detector wavelength: UV 275nm
Run Time: 15 minutes

System Suitability Results:

Sr. No.	Parameters	USP Criteria	Obtained Results
1	Resolution b/w Theophylline and Caffeine Peak	NLT 6.0	12.3
2	Tailing Factor for Theophylline and Caffeine peaks	NMT 2.0	For Both peaks 1.1

CHROMATOGRAMS:

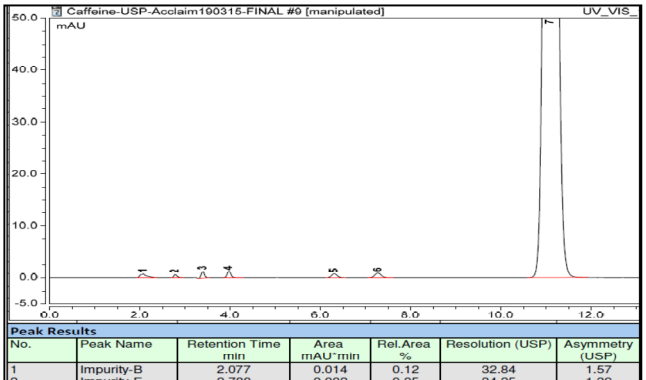
System Suitability:



Peak Results

No.	Peak Name	Retention Time min	Area mAU*min	Rel.Area %	Resolution (USP)	Asymmetry (USP)
1	Theophylline	7.273	5.834	2.38	12.30	1.07
2	Caffeine	11.088	9838.687	97.62	0.00	1.08

Impurity Mix:



Peak Results

No.	Peak Name	Retention Time min	Area mAU*min	Rel.Area %	Resolution (USP)	Asymmetry (USP)
1	Impurity-B	2.077	0.014	0.12	32.84	1.57
2	Impurity-E	2.780	0.002	0.05	34.25	1.39
3	Impurity-C	3.393	0.011	0.11	30.92	1.10
4	Impurity-D	3.972	0.014	0.12	27.74	1.12
5	Impurity-F	6.305	0.010	0.10	16.36	1.09
6	Impurity-A	7.277	0.021	0.15	12.27	1.06
7	Caffeine	11.088	9321.921	99.34	0.00	1.08