Impurities test for Caffeine (EP-8.0 method):

SAMPLE PREPARATION:

Test Solution: Dissolve 0.1gm of Caffeine in Mobile phase, dilute to 50 ml, and dilute 1ml of this solution to 10ml with mobile phase.

Reference solution (a): Dilute 2ml of test solution to 100ml with mobile phase, dilute 1ml of this solution to 10ml with mobile phase.

Reference solution (b): Dissolve 5mg of caffeine for system suitability CRS (containing impurity A, C, D and F) in mobile phase and dilute to 5ml, Dilute 2ml of this solution to 10ml with mobile phase.

CHROMATOGRAPHIC CONDITIONS:

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

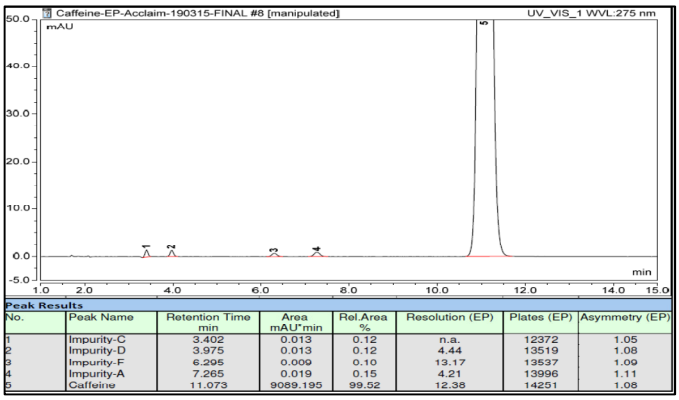
Mobile phase: Acetonitrile: Tetrahydrofuran: Buffer (25:20:955)
Buffer: 0.82 g/L of anhydrous sodium acetate, adjust pH to 4.5 with Glacial acetic acid.
Separation Mode: Isocratic
Column temperature: 25˚C
Flow rate: 1.0 mL/min
Injection Volume: 10 µl
Detector wavelength: UV 275nm
Run Time: 15min

System Suitability Results:

Sr. No.	Parameters	USP Criteria	Obtained Results
1	• Resolution between the impurities C & D. • Resolution between the impurities F & A	• NLT 2.5 • NLT 2.5	• 4.4 • 4.2
2	Tailing Factor for caffeine	NMT 2.0	1.1

CHROMATOGRAMS:

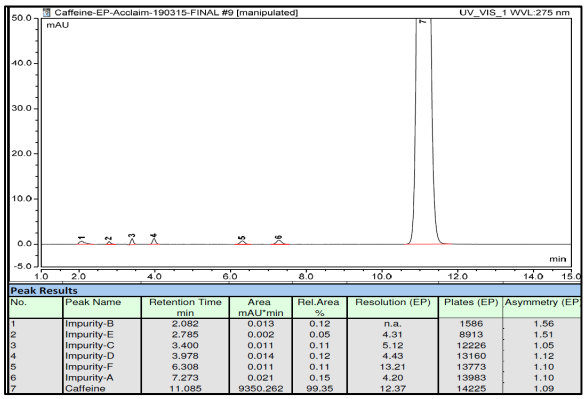
System Suitability:



Peak Results

No.	Peak Name	Retention Time min	Area mAU*min	Rel.Area %	Resolution (EP)	Plates (EP)	Asymmetry (EP)
1	Impurity-C	3.402	0.013	0.12	n.a.	12372	1.05
2	Impurity-D	3.975	0.013	0.12	4.44	13519	1.08
3	Impurity-F	6.295	0.009	0.10	13.17	13537	1.09
4	Impurity-A	7.265	0.019	0.15	4.21	13996	1.11
5	Caffeine	11.073	9089.195	99.52	12.38	14251	1.08

Impurity Mix:



Peak Results

No.	Peak Name	Retention Time min	Area mAU*min	Rel.Area %	Resolution (EP)	Plates (EP)	Asymmetry (EP)
1	Impurity-B	2.082	0.013	0.12	n.a.	1586	1.56
2	Impurity-E	2.785	0.002	0.05	4.31	8913	1.51
3	Impurity-C	3.400	0.011	0.11	5.12	12226	1.05
4	Impurity-D	3.978	0.014	0.12	4.43	13160	1.12
5	Impurity-F	6.308	0.011	0.11	13.21	13773	1.10
6	Impurity-A	7.273	0.021	0.15	4.20	13983	1.10
7	Caffeine	11.085	9350.262	99.35	12.37	14225	1.09