Impurities test for Cefradine (EP- method):

SAMPLE PREPARATION:

Reference solution-a: Dissolve 3.0 mg of Impurity-B in Mobile phase A and dilute to 50 ml with mobile phase A.

Reference solution-b: Dissolve 3.0 mg of Cefradine and Cefalexin monohydrate in Mobile phase A and dilute to 25 ml with mobile phase A.

Reference solution-c: Dilute 1ml of test solution to 100 ml with mobile phase A

Test Solution: Dissolve 0.3 g of Cefradine in mobile phase A and dilute to 50 ml with mobile phase A.

Reference solution-e: Dissolve content of vial of cefradine impurity mixture (Impurity A and G) in 1 ml mobile phase.

CHROMATOGRAPHIC CONDITIONS:

Instrument: UltiMate 3000 LC
Column: Hypersil Gold (4.6*150mm, 5 um, p/n 25005-154630, lot no.:12750)
Mobile phase A: 2.72 g/L solution of potassium Dihydrogen phosphate adjusted to pH 3 with OPA.
Mobile phase B: Methanol
Separation Mode: Gradient

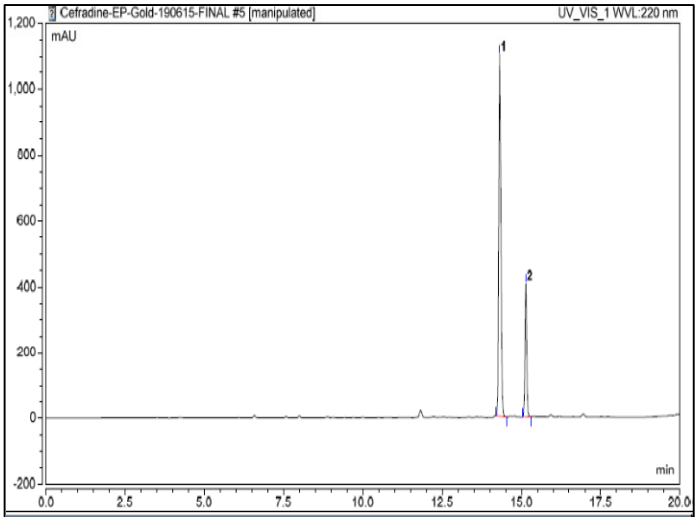
Time (min)	Mobile phase A (% v/v)	Mobile phase B (% v/v)
0	99.5	0.5
2.5	97	3
11	75	25
13	60	40
16	60	40
19	20	80
19.1	99.5	0.5
25	99.5	0.5

Column temperature: 30˚C
Flow rate: 1.0 mL/min
Injection Volume: 25 µl
Detector wavelength: UV 220nm
Run Time: 25min

System Suitability Results:

Sr. No.	Parameters	EP Criteria	Obtained Results
1	Resolution between Cefalexin and Cefradine	Minimum 4.0	7.32
2	Retention Time of Cefradine	About 15 minutes	15.057 min
3	RRT with reference to Cefradine	Imp-A= about 0.27, Imp-B= about 0.32 min, Imp-C= about 0.53 min, Imp-D=about 0.63 min, Imp-E= about 0.80 min, Imp-F= about 0.92 min, Cefalexin= about 0.95, 4',5' dihydrocefradine= about 1.06 min, Imp-G= about 1.32 min	Imp-A= 0.23 min, Imp-B= 0.28 min Imp-C= 0.53 min, Imp-D= 0.59 min Imp-E= 0.78 min, Imp-F= 0.98 min Cefalexin= 0.95 min 4',5' dihydrocefradine= 1.06 min Imp-G= 1.18 min

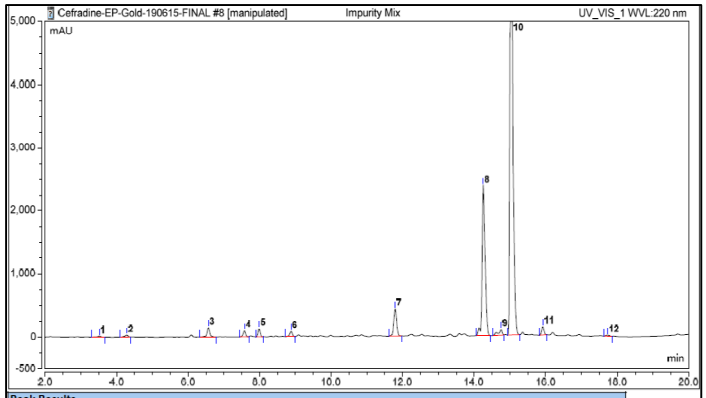
CHROMATOGRAMS:



Peak Results

No.	Peak Name	Retention Time min	Area mAU*min	Rel.Area %	Resolution (EP)	Asymmetry (EP)	Plates (EP)
1	Cefalexine	14.312	87.231	77.00	7.32	1.25	207780
2	Cefradine	15.137	26.062	23.00	0.00	1.10	363107

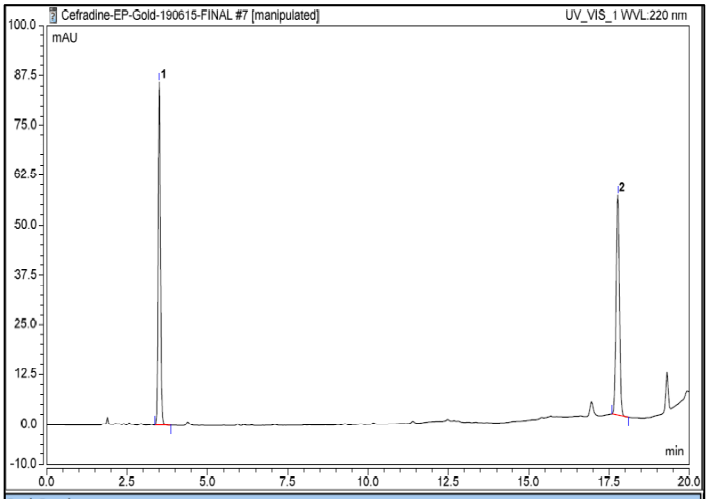
Reference solution –b (System Suitability)



Peak Results

No.	Peak Name	Retention Time min	Area mAU*min	Rel.Area %	Resolution (EP)	Asymmetry (EP)	Plates (EP)	Rel. Ret.Time. (Cefradine)
1	Impurity-A	3.518	2.119	0.23	71.86	0.72	8906	0.23
2	Impurity-B	4.282	3.461	0.38	68.98	0.77	14889	0.28
3	Unknown Impurity-1	6.567	12.965	1.43	57.65	0.94	46028	0.44
4	Unknown Impurity-2	7.572	6.920	0.76	51.83	0.96	67342	0.50
5	Impurity-C	7.985	8.733	0.96	50.21	1.02	84968	0.53
6	Impurity-D	8.883	6.985	0.77	40.96	0.82	75422	0.59
7	Impurity-E	11.790	38.529	4.24	21.01	1.03	115219	0.78
8	Cefalexine	14.253	229.984	25.31	5.00	1.48	145296	0.95
9	Impurity-F	14.753	10.997	1.21	2.10	0.65	254237	0.98
10	Cefradine	15.057	577.056	63.49	0.00	1.15	121372	1.00
11	4' , 5' dihydrocefradine	15.918	9.242	1.02	6.04	1.02	316096	1.06
12	Impurity-G	17.730	1.848	0.20	15.44	1.02	165478	1.18

Impurity Mix



Peak Results

No.	Peak Name	Retention Time min	Area mAU*min	Rel.Area %	Resolution (EP)	Asymmetry (EP)	Plates (EP)
1	Impurity-A	3.502	6.919	51.31	n.a.	1.08	12408
2	Impurity-G	17.773	6.567	48.69	n.a.	1.02	141935

Reference solution –e