Impurities test for Cefalexin (EP-8.0 method):

SAMPLE PREPARATION:

Test solution: Dissolve 50 mg of the substance to be examined in Mobile phase A and dilute to 50.0 ml with mobile phase A

Reference solution (a): Dissolve 10 mg of D-phenylglycine in mobile phase A and dilute to 10.0 ml with mobile phase A

Reference solution (b): Dissolve 10 mg of 7-Aminodesacetoxycephalosporanic acid in phosphate buffer solution pH 7.0 and dilute with mobile phase A

Reference solution (c) Dilute 1.0 ml of reference solution (a) and 1.0 ml of reference solution (b) to 100 ml with mobile phase A

Reference solution: Dissolve 10 mg of cefotaxime sodium in mobile phase A and dilute to 10.0 ml with mobile phase A. To 1.0 ml of this solution add 1.0 ml of the test solution and dilute to 100ml with mobile phase A.

CHROMATOGRAPHIC CONDITIONS:

Instrument: UltiMate 3000 LC
Column: Hypersil Gold (4.6*100mm, 5um, p/n 25005-104630, Sr. No.:0918795JR4)

Mobile phase A: Dissolve 2.72 g of potassium dihydrogen phosphate in 800 ml of water and adjust the pH to 5 with 1 M KOH and make up to 1 L.

Mobile phase B: Methanol

Diluent: Mobile Phase A
Separation Mode: Gradient
Column temperature: 25˚C
Flow rate: 1.5 mL/min
Injection Volume: 20 μl
**Detector wavelength:** UV 220nm
Run Time: 35min

Time (Min)	Mobile Phase A (Per cent V/V)	Mobile phase B (Per cent V/V)
0- 1	98	2
1- 20	98 → 70	2 → 30
20.1-35	98	2

System Suitability Results:

| Sr. No.	Parameters	USP Criteria	Obtained Results
1	• Resolution between imp A and Imp B	• NLT 2.0	• 5.42
2	• Resolution between Cefalexin and Cefotaxime	• NLT 1.5	• 6.45

CHROMATOGRAMS:

System Suitability:

No.	Peak Name	Retention Time min	Rel.Area %	Area mAU*min	Resolution (EP)	Asymmetry (EP)	Plates (EP)
1	Impurity A	1.202	51.50	2.706	0.00	1.24	5171
2	Impurity B	1.617	48.50	2.548	5.42	1.16	5563

ref Sol:

No.	Peak Name	Retention Time min	Rel.Area %	Area mAU*min	Resolution (EP)	Asymmetry (EP)	Plates (EP)
1	Cefalexin	11.050	45.19	3.419	0.00	1.07	37807
2	Cepatoxime	12.663	54.81	4.146	6.78	1.13	61161
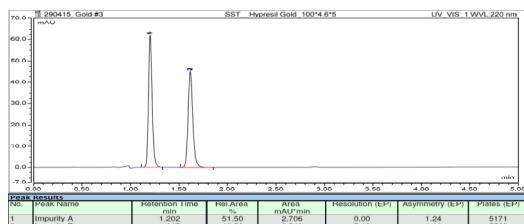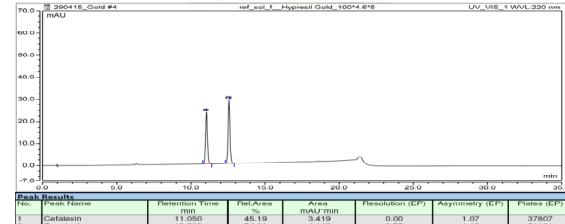
All Mix
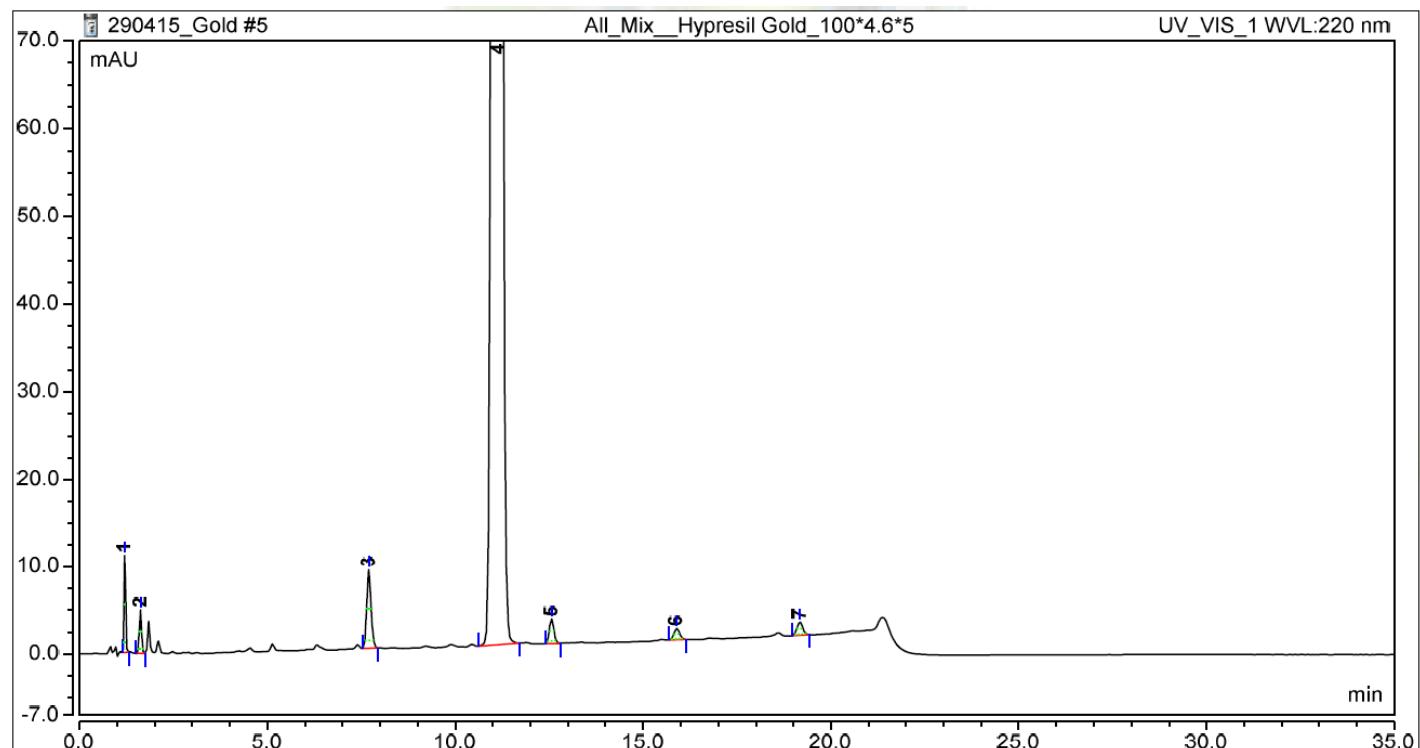
Peak Results

No.	Peak Name	Retention Time min	Rel.Area %	Area mAU*min	Resolution (EP)	Asymmetry (EP)	Plates (EP)
1	Impurity A	1.200	0.18	0.476	60.76	1.23	5207
2	Impurity B	1.618	0.11	0.288	54.66	1.12	5431
3	Impurity E	7.698	0.45	1.192	14.74	1.11	21831
4	Cefalexin	11.158	98.93	263.220	0.00	0.76	28982
5	Cepatoxime	12.562	0.15	0.392	5.84	1.09	52171
6	Impurity D	15.895	0.09	0.230	17.47	1.04	51054
7	Impurity C	19.173	0.10	0.272	29.41	1.06	72716