Impurities test for Ampicillin Sodium (EP-8.0 method):

SAMPLE PREPARATION:

Reference solution (a): Dissolve 27.0 mg of Ampicillin in Mobile phase A and dilute to 50ml with mobile phase A.

Reference solution (b): Dissolve 2mg of cefradine CRS in mobile phase A, , dilute to 50 ml with mobile phase A, To 5ml of this solution add 5ml of reference solution (a).

Reference solution (c): Dilute 1.0 ml of reference solution (a) to 20 ml with mobile phase A.

Reference solution (b): To 0.2gm of Ampicillin add 1 ml of water. Heat the solution to 60˚C for 1 hour. Dilute 0.5 ml of this solution to 50 ml with mobile phase A.

CHROMATOGRAPHIC CONDITIONS:

Instrument: UltiMate 3000 LC
Column: Acclaim120-C18 (4.6*250mm, 5um, p/n 059149, lot no.: 018-01-152)

Mobile phase:
Mobile phase A: Mix 0.5 ml of dilute acetic acid, 50ml of 0.2M potassium Dihydrogen phosphate and 50 ml of acetonitrile, then dilute to 1000ml with water.

Mobile phase B: Mix 0.5 ml of dilute acetic acid, 50ml of 0.2M potassium Dihydrogen phosphate and 400 ml of acetonitrile, then dilute to 1000ml with water.

Separation Mode: Solution (b) and solution (c) with Isocratic elution at Initial mobile phase composition for 20 minutes.

Solution (d), Impurity Mix solution and individual impurities as per the gradient described below:

Time (min)	Mobile phase A (% v/v)	Mobile phase B (% v/v)
0	90*	10*
11	90	10
41	0	100
56	0	100
57	90	10
70	90	10

Note: As described in Monograph the initial mobile phase composition of A and B can be adjusted to achieve the resolution, so the composition of A and B was finalized as 90 and 10 respectively.

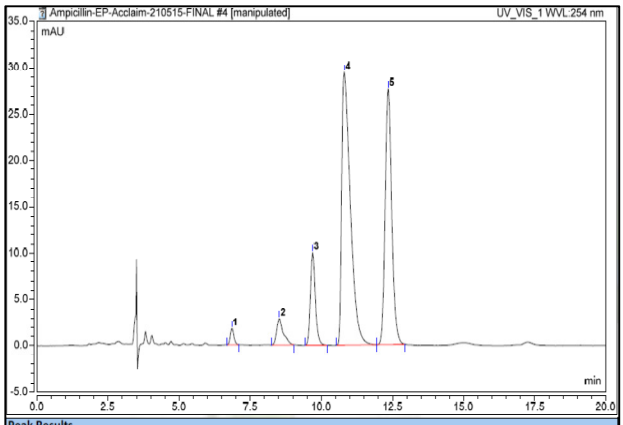
Column temperature: 25˚C
Flow rate: 1.0 mL/min
Injection Volume: 50 µl
Detector wavelength: UV 254 nm
Run Time: 70min

System Suitability Results:

Sr. No.	Parameters	EP Criteria	Obtained Results
1	Resolution b/w Ampicillin and Cefradine	Minimum 3.0	3.23
2	RRT with reference to ampicillin and ampicillin dimer in reference solution (d)	About 2.8	2.84

CHROMATOGRAMS:

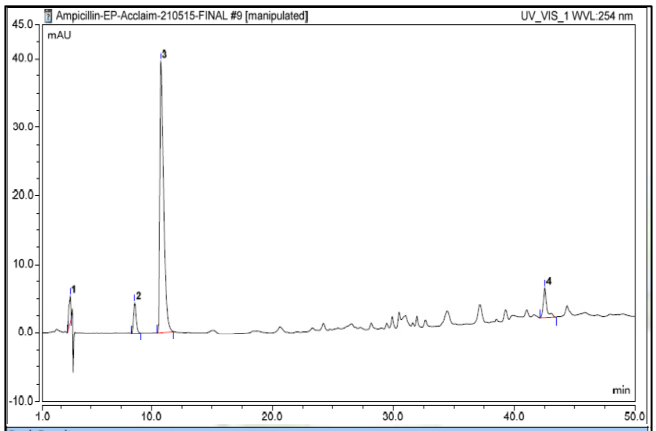
System Suitability:



Peak Results

No.	Peak Name	Retention Time min	Area mAU*min	Rel.Area %	Resolution (EP)	Plates (EP)	Rel. Ret.Time (Ampicillin)
1	Unknown Impurity-1	6.855	0.263	1.26	5.68	14712	0.63
2	Unknown Impurity-2	8.520	0.771	3.69	3.51	8862	0.79
3	Unknown Impurity-3	9.692	2.019	9.67	2.61	15918	0.90
4	Ampicillin	10.803	10.399	49.82	3.23	6229	1.00
5	Cefradine	12.350	7.423	35.56	n.a.	14283	1.14

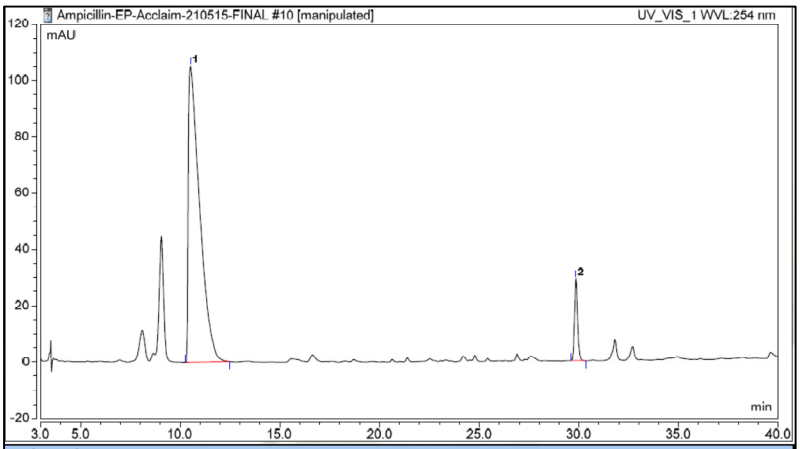
Impurity Mix:



Peak Results

No.	Peak Name	Retention Time min	Area mAU*min	Rel.Area %	Resolution (EP)	Plates (EP)	Rel. Ret.Time (Ampicillin)
1	Impurity-L	3.280	0.800	4.54	14.12	1149	0.30
2	Impurity-D	8.613	1.077	6.11	4.65	8661	0.80
3	Ampicillin	10.768	14.315	81.26	65.94	5955	1.00
4	Impurity-C	42.520	1.425	8.09	n.a.	174194	3.95

Reference solution (d):



Peak Results

No.	Peak Name	Retention Time min	Area mAU*min	Rel.Area %	Resolution (EP)	Plates (EP)	Rel. Ret.Time. (Ampicillin)
1	Ampicillin	10.508	69.316	93.10	29.78	1660	1.00
2	Ampicillin Dimer	29.852	5.133	6.90	n.a.	194036	2.84