Impurities test for Amoxicillin (EP-8.0 method):

SAMPLE PREPARATION:

Test Solution (a): Dissolve 30 mg of Metronidazole in Mobile phase A and dilute to 50ml with mobile phase A.

Test Solution (b): Dissolve 30 mg of Metronidazole in Mobile phase A, and dilute to 20ml with mobile phase A.

Reference solution (a): Dissolve 30 mg of Metronidazole in Mobile phase A and dilute to 50ml with mobile phase A.

Reference solution (b): Dissolve 4mg of cefadroxil CRS in mobile phase A, , dilute to 50 ml with mobile phase A, To 5ml of this solution add 5ml of reference solution (a) and dilute to 100 ml with mobile phase A.

Reference solution (c): Dilute 2ml of reference solution (a) t0 20 ml with mobile phase A; dilute 5ml of this solution to 20 ml with mobile phase A.

CHROMATOGRAPHIC CONDITIONS:

Instrument: UltiMate 3000 LC
Column: Hypersil Gold (4.6*250mm, 5um, p/n 25005-254630, lot no.: 13285)

Mobile phase:
Mobile phase A: Mix 1 volumes of Acetonitrile and 99 volumes of 25 % v/v solution of 0.2M potassium Dihydrogen phosphate adjusted to pH 5.0 with dilute sodium hydroxide.

Mobile phase B: Mix 20 volumes of Acetonitrile and 80 volumes of 25 % v/v solution of 0.2M potassium Dihydrogen phosphate adjusted to pH 5.0 with dilute sodium hydroxide.

Separation Mode: Gradient

Time (min)	Mobile phase A (% v/v)	Mobile phase B (% v/v)
0	92	8
10	92	8
35	0	100
50	0	100
52	92	8
65	92	8

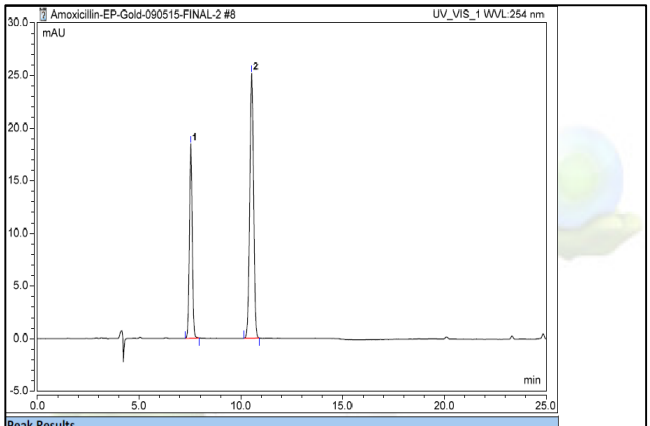
Column temperature: 25˚C
Flow rate: 1.0 mL/min
Injection Volume: 50 µl
Detector wavelength: UV 254 nm
Run Time: 65min

System Suitability Results:

Sr. No.	Parameters	USP Criteria	Obtained Results
1	Resolution b/w Amoxicillin and Cefadroxil peak in reference solution b	NLT 2.0	10.01
2	RRT of Impurity C and Impurity J with respect to Amoxicillin peak	• Imp C- 3.4 • Imp-J- 4.1	• 3.2 • 3.8

CHROMATOGRAMS:

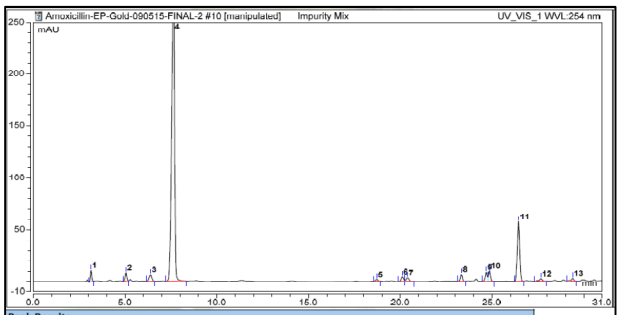
System Suitability:



Peak Results

No.	Peak Name	Retention Time min	Area mAU*min	Rel.Area %	Resolution (EP)	Asymmetry (EP)	Plates (EP)	Rel. Ret.Time. (Amoxicillin)
1	Amoxicillin	7.540	2.964	34.97	10.01	1.02	14326	1.0
2	Cefadroxil	10.530	5.513	65.03	n.a.	0.98	14730	1.4

Impurity Mix:



Peak Results

No.	Peak Name	Retention Time min	Area mAU*min	Rel.Area %	Resolution (EP)	Asymmetry (EP)	Plates (EP)	Rel. Ret.Time. (Amoxicillin)
1	Impurity-I	3.142	0.976	1.40	12.72	1.05	8935	0.4
2	Impurity-D	5.047	0.855	1.23	5.94	1.04	14535	0.7
3	Impurity-A	6.390	1.108	1.59	4.44	0.94	7964	0.8
4	Amoxicillin	7.640	52.369	75.15	43.98	0.78	12109	1.0
5	Impurity-G	18.718	0.269	0.39	6.05	0.94	108364	2.5
6	Unknown Impurity-1	20.113	0.690	0.99	1.09	n.a.	117066	2.6
7	Unknown Impurity-2	20.393	0.652	0.94	12.45	n.a.	93479	2.7
8	Impurity-E	23.330	0.808	1.16	6.54	0.98	239201	3.1
9	Impurity-C	24.683	1.128	1.62	0.81	n.a.	194435	3.2
10	Unknown Impurity-3	24.860	1.285	1.84	7.08	n.a.	220443	3.3
11	Impurity-F	26.442	8.610	12.36	5.18	1.00	200369	3.5
12	Impurity-H	27.663	0.497	0.71	7.96	0.78	218422	3.6
13	Impurity-J	29.393	0.438	0.63	n.a.	0.75	348637	3.8