

## **Impurities test for Ampicillin (USP-38 method):**

### **SAMPLE PREPARATION:**

**Standard stock solution:** 0.5mg/ml of Ampicillin in Acetonitrile, water and Solution D (4:91:5), prepared as follows. Dissolve first in a mixture of acetonitrile, water and solution-D (4:30:5), sonicate and dilute with water.

**Standard solution:** 0.05mg/ml of Ampicillin in Solution D and water (1:19) from the standard stock solution.

**Sensitivity solution:** 0.05µg/ml of Ampicillin in Solution D and water (1:19) from the standard solution.

**System suitability solution:** 0.5mg/ml of Ampicillin and 0.1 mg/ml of Amoxicillin in Acetonitrile, water and Solution D (4:91:5), prepared as follows. Dissolve first in a mixture of acetonitrile, water and solution-D (4:30:5), sonicate and dilute with water.

### **CHROMATOGRAPHIC CONDITIONS:**

**Instrument:** UltiMate 3000 LC

**Column:** Hypersil Gold (4.6\*150mm, 5µm, p/n: 25005-154630, lot no.: 12750)

**Solution A:** 6.54g/l of monobasic potassium phosphate and 0.34g/l of dibasic potassium phosphate adjusted with 1N sodium hydroxide or 1N phosphoric acid to a pH of 5.5 before final dilution.

**Solution B:** Acetonitrile and Solution A (2:23).

**Solution C:** Acetonitrile and solution A (3:7).

**Solution D:** 46.3 g/L of monobasic potassium phosphate and 27.8 g/L of dibasic potassium phosphate adjusted with 1N sodium hydroxide or 1N OPA to a pH of 6.5.

**Mobile phase:** As per Gradient below:

**Separation Mode:** Gradient

Time (min)	Solution B (% v/v)	Solution C (% v/v)
0	100	0
6	100	0
15	0	100
16	0	100
18	100	0
20	100	0

**Column temperature:** 25°C

**Flow rate:** 1.5 mL/min

**Injection Volume:** 20 µl

**Detector wavelength:** UV230 nm

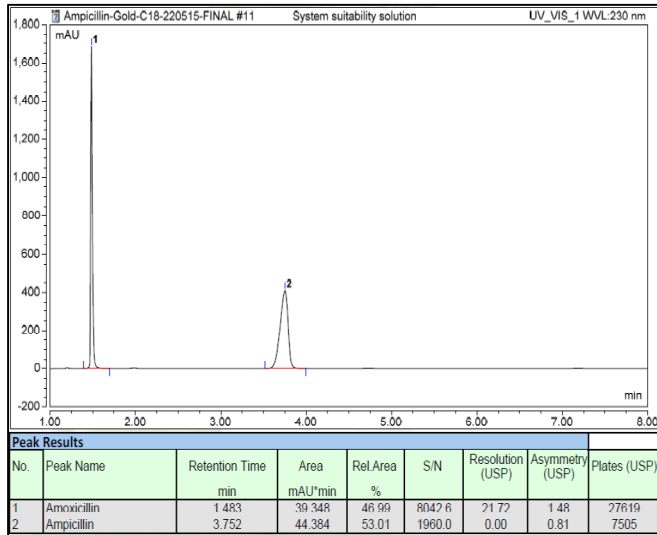
**Run Time:** 20min

**System Suitability Results:**

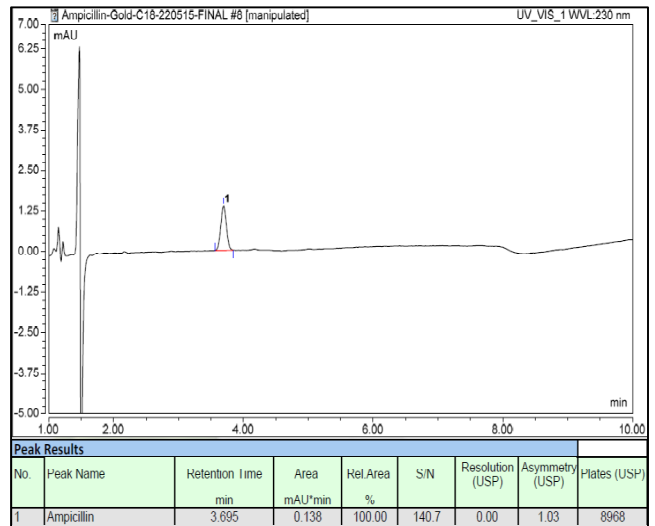
Sr. No.	Parameters	USP Criteria	Obtained Results
1	Signal to Noise ratio for Sensitivity solution	NLT 3	140.7
2	Resolution between Amoxicillin and Ampicillin peak in system suitability solution	NLT 10	21.72
3	Tailing factor of Ampicillin peak in System suitability solution	NMT 1.4	0.81
4	Relative standard deviation for standard solution	NMT 10.0%	0.04%

**CHROMATOGRAMS:**

**System Suitability:**



**Sensitivity solution:**



**Impurity Mix:**

