

Impurities test for Mefenamic Acid (EP- method):

SAMPLE PREPARATION:

Test solution: Dissolve 25 mg of Mefenamic acid in mobile phase and dilute to 25 ml with mobile phase.

Reference Solution A: Dissolve 1mL of test solution to 100 mL with mobile phase, dilute 1mL of this solution to 10mL with mobile phase.

Reference Solution B: Dissolve 50 mg of Impurity C and D in mobile phase and dilute to 10 mL with mobile phase, dilute 1 mL of this solution to 100 mL with mobile phase, dilute 1 mL of this solution to 100 mL with mobile phase A.

Reference Solution C: Dissolve 10 mg of Impurity A in mobile phase and dilute to 10 mL with mobile phase, dilute 1 mL of this solution to 100 mL with mobile phase, dilute again this 1mL solution to 100 mL with mobile phase.

Reference Solution D: Dissolve 20 mg of Impurity D in mobile phase and dilute to 1000 mL with mobile phase, dilute 1 mL of this solution to 100 mL with mobile phase.

CHROMATOGRAPHIC CONDITIONS:

Instrument: UltiMate 3000 LC

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

Mobile phase A: 14 volumes of Tetrahydrofuran, 40 volumes of 5.75 g/L solution of ammonium dihydrogen phosphate pH adjusted to 5.0 with dilute ammonia and 46 volumes of acetonitrile.

Separation Mode: Isocratic

Column temperature: 25°C

Flow rate: 1.0 mL/min

Injection Volume: 10 µL

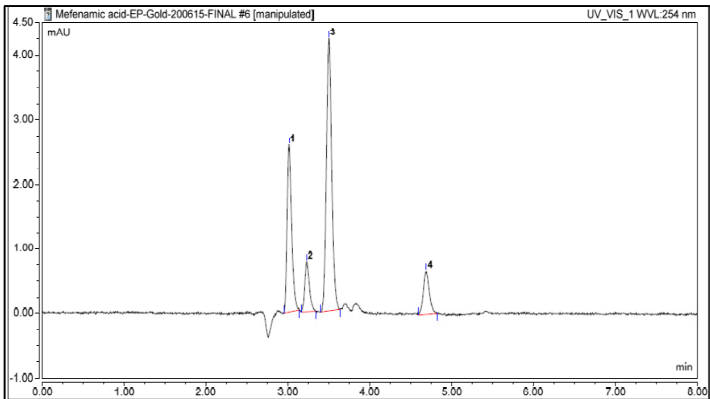
Detector wavelength: UV 254nm

Run Time: 35 min

System Suitability Results:

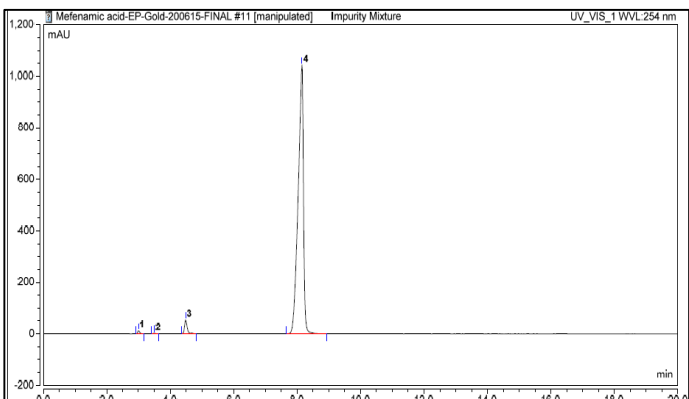
Sr. No.	Parameters	EP Criteria	Obtained Results
1	Resolution between Impurity C and Impurity D in reference solution (b)	Minimum 3.0	4.5
2	Retention Time of Mefenamic Acid	Around 8 minute	8.155 minute
3	RRT of Impurity A, C and D with reference to Mefenamic Acid	Impurity-A= about 0.5 Impurity-C= about 0.3 Impurity-D= about 0.35	Imp-A=0.55 Imp-C= 0.37 Imp-D=0.43

CHROMATOGRAMS:



Peak Results							
No.	Peak Name	Retention Time	Area	Rel.Area	Resolution (EP)	Asymmetry (EP)	Plates (EP)
		min	mAU*min	%			
1	Impurity-C	3.012	0.168	28.33	4.49	1.38	14332
2	Unknown Impurity-1	3.227	0.051	8.58	2.53	1.23	17097
3	Impurity-D	3.498	0.318	53.62	0.00	1.16	14367
4	Unknown Impurity-2	4.687	0.056	9.46	9.67	1.05	20894

Reference Solution B (System suitability):



Peak Results							
No.	Peak Name	Retention Time	Area	Rel.Area	Resolution (EP)	Asymmetry (EP)	Plates (EP)
		min	mAU*min	%			
1	Impurity-C	3.003	0.895	0.43	4.17	1.40	12267
2	Impurity-D	3.495	0.197	0.10	7.50	1.20	11934
3	Impurity-A	4.487	5.258	2.54	16.99	1.31	17145
4	Mefenamic Acid	8.155	200.794	96.93	n.a.	0.71	12155

Impurity Mix: