Assay Analysis of Tetracycline Hydrochloride (USP-38 method):

SAMPLE PREPARATION:

Diluent: Mix 680ml of 0.1M ammonium oxalate and 270 ml of Dimethylformamide.

Standard solution: 0.5mg/ml of Tetracycline HCl in Diluent.

Resolution solution: 100µg/ml of Tetracycline HCl and 25µg/ml of USP 4-Epianhydrotetracycline HCl in Diluent.

CHROMATOGRAPHIC CONDITIONS:

Instrument: UltiMate 3000 LC
Column: Acclaim 120-C8 (4.6*250mm, 5um, p/n: 059141, lot no.: 018-01-158)

Mobile phase: Dimethylformamide, 0.1M ammonium oxalate and 0.2M dibasic ammonium phosphate (27:68:5), adjust if necessary with 3N ammonium hydroxide or 3N phosphoric acid to a pH of 7.6 to 7.7

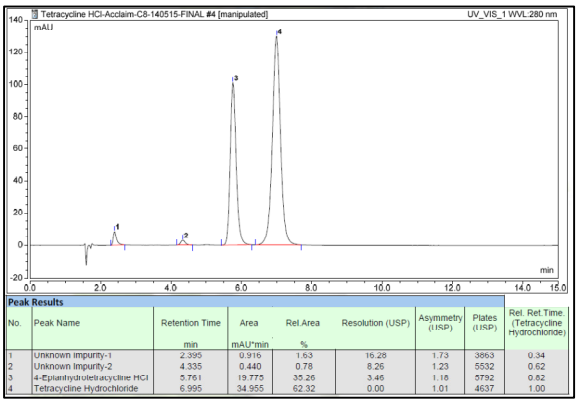
Separation Mode: Isocratic
Column temperature: 25˚C
Flow rate: 2.0 mL/min
Injection Volume: 20 µl
Detector wavelength: UV280 nm
Run Time: 25min

System Suitability Results:

Sr. No.	Parameters	USP Criteria	Obtained Results
1	Resolution between 4-Epianhydrotetracycline HCl and Tetracycline	NLT 1.2	3.46
2	RRT of 4-Epianhydrotetracycline HCl with respect to Tetracycline HCl peak	About 0.9	0.82
3	The %RSD for replicate injection of standard solution	NMT 2.0%	0.4%

CHROMATOGRAMS:

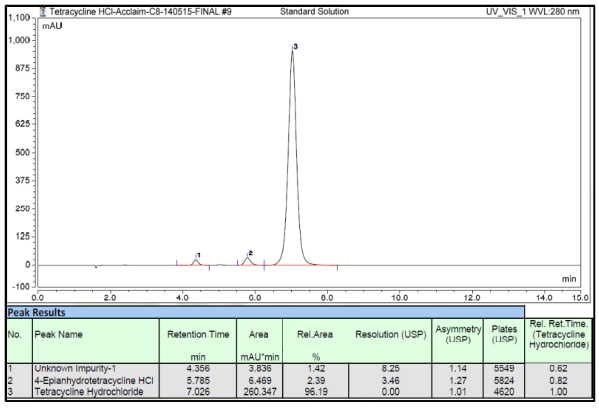
System Suitability:



Peak Results

No.	Peak Name	Retention Time (min)	Area (mAU*min)	Rel.Area (%)	Resolution (USP)	Asymmetry (USP)	Plates (USP)	Rel. Ret.Time (Tetracycline Hydrochloride)
1	Unknown Impurity-1	2.395	0.916	1.63	16.28	1.73	3863	0.34
2	Unknown Impurity-2	4.335	0.440	0.78	8.26	1.23	5532	0.62
3	4-Epianhydrotetracycline HCl	5.761	19.775	35.26	3.46	1.16	5792	0.82
4	Tetracycline Hydrochloride	6.995	34.955	62.32	0.00	1.01	4637	1.00

Standard Solution:



Peak Results

No.	Peak Name	Retention Time (min)	Area (mAU*min)	Rel.Area (%)	Resolution (USP)	Asymmetry (USP)	Plates (USP)	Rel. Ret.Time (Tetracycline Hydrochloride)
1	Unknown Impurity-1	4.356	3.836	1.42	8.25	1.14	5549	0.62
2	4-Epianhydrotetracycline HCl	5.785	6.469	2.39	3.46	1.27	5824	0.82
3	Tetracycline Hydrochloride	7.026	260.347	96.19	0.00	1.01	4620	1.00