Impurities test for Hydrochlorothiazide (EP- method):

SAMPLE PREPARATION:

Reference solution-a: Dissolve 3mg of Impurity-A and 3mg of Hydrochlorothiazide in 5ml of mixture of equal volumes of acetonitrile and methanol if necessary dilute to 20ml with phosphate buffer solution pH 3.2. Dilute 5ml of this solution to 100ml with solvent mixture.

Test Solution-a: Dissolve 30mg of Hydrochlorothiazide in 5ml of mixture of equal volumes of acetonitrile and methanol if necessary dilute to 20ml with phosphate buffer solution pH 3.2.

Reference solution-b: Dilute 1ml of test solution (a) to 100ml with the solvent mixture. Dilute 1 ml of this solution to 10ml with solvent mixture.

Solvent Mixture: Dilute 50ml of mixture of equal volumes of acetonitrile and methanol to 200ml of phosphate buffer solution pH 3.2.

CHROMATOGRAPHIC CONDITIONS:

Instrument: UltiMate 3000 LC
Column: Hypersil Gold (4.6*100mm, 3 um, p/n 25003-104630, lot no.:12337)
Mobile phase A: Buffer: Methanol: Tetrahydrofuran (940:60: 10)
Mobile phase B: Buffer: Methanol: Tetrahydrofuran (500:500: 50)
Diluent: Solvent mixture and phosphate buffer pH 3.2
Phosphate Buffer: 35.8 g/l of disodium hydrogen phosphate in water, adjust with phosphoric acid to a pH of 3.2.Dilute 100 mL of this solution to 2000 mL with water.
Separation Mode: Gradient

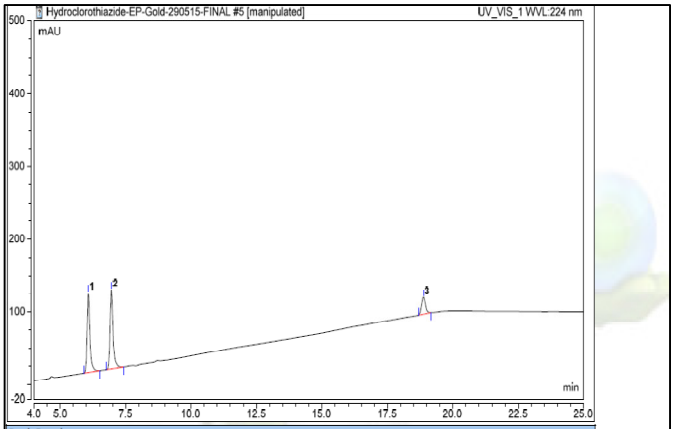
Time (min)	Mobile phase A (% v/v)	Mobile phase B (% v/v)
0	100	0
17	55	45
30	55	45
30.1	100	0
45	100	0

Column temperature: 25˚C
Flow rate: 0.8 mL/min
Injection Volume: 10 µl
Detector wavelength: UV 224nm
Run Time: 45 min

System Suitability Results:

Sr. No.	Parameters	USP Criteria	Obtained Results
1	Resolution between Hydrochlorothiazide & impurity-A in reference solution (a)	NLT 2.5	5.74
2	RRT of Impurity A, B and C with reference to Hydrochlorothiazide	Impurity-A= about 0.9 min Impurity-B= about 0.7 min Impurity-C= about 2.8 min	Imp-A=0.88 Imp-B= 0.67 Imp-C =2.94

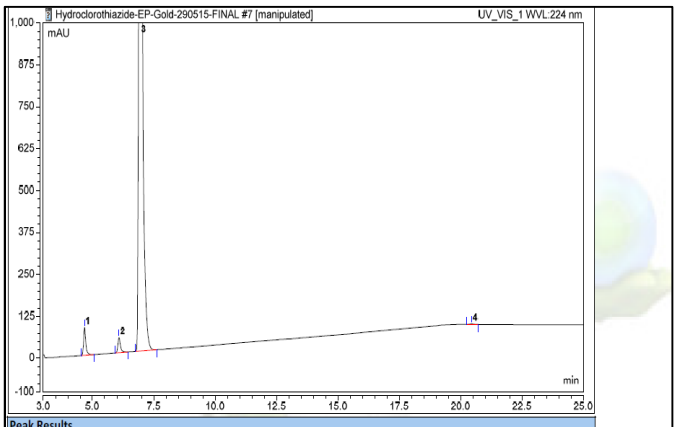
CHROMATOGRAMS:



Peak Results

No.	Peak Name	Retention Time (min)	Area (mAU*min)	Rel.Area (%)	Resolution (EP)	Asymmetry (EP)	Plates (EP)	Rel. Ret.Time (Hydrochlorothiazide)
1	Impurity-A	6.075	13.784	42.49	4.70	1.44	17636	0.87
2	Hydrochlorothiazide	6.955	14.522	44.77	0.00	1.43	20802	1.00
3	Unknown Impurity	18.875	4.132	12.74	52.03	1.15	80253	2.71

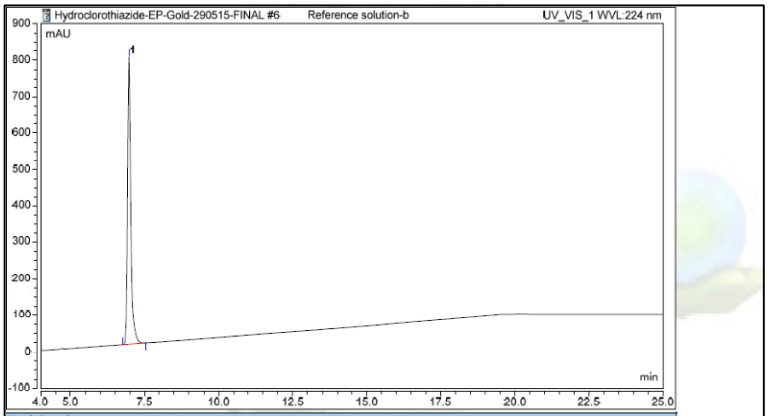
System Suitability:



Peak Results

No.	Peak Name	Retention Time (min)	Area (mAU*min)	Rel.Area (%)	Resolution (EP)	Asymmetry (EP)	Plates (EP)	Rel. Ret.Time (Hydrochlorothiazide)
1	Impurity-B	4.683	8.584	1.85	11.96	1.47	15715	0.67
2	Impurity-A	6.085	5.407	1.17	4.29	1.45	19798	0.88
3	Hydrochlorothiazide	6.948	448.628	96.91	0.00	1.61	14543	1.00
4	Impurity-C	20.433	0.293	0.06	50.74	1.16	73005	2.94

Impurity Mix:



Peak Results

No.	Peak Name	Retention Time (min)	Area (mAU*min)	Rel.Area (%)	Resolution (EP)	Asymmetry (EP)	Plates (EP)	Rel. Ret.Time (Hydrochlorothiazide)
1	Hydrochlorothiazide	6.977	99.777	100.00	0.00	1.45	22973	1.00

Reference Solution-b: