Impurities test for Betamethasone (EP- method)

SAMPLE PREPARATION:

Test solution: Dissolve 25mg of Betamethasone in a mixture of equal volumes of Acetonitrile and Methanol and dilute to 10 ml with same solution.

Reference solution-a: Dissolve 2 mg of Betamethasone and 2 mg of Methylprednisolone in mobile phase A then dilute to 100 ml with mobile phase A.

Reference solution-b: Dilute 1ml of test solution to 100ml with Mobile phase A.

CHROMATOGRAPHIC CONDITIONS:

Instrument: UltiMate 3000 LC
Column: Syncronis C18 (4.6*250mm, 5 um, p/n 97105-254630, lot no.:11811)
Mobile phase A: Water: Acetonitrile (250:750)
Mobile phase B: Acetonitrile
Separation Mode: Gradient

Time (min)	Mobile phase A (% v/v)	Mobile phase B (% v/v)
0	100	0
15	100	0
40	0	100
41	100	0
46	100	0

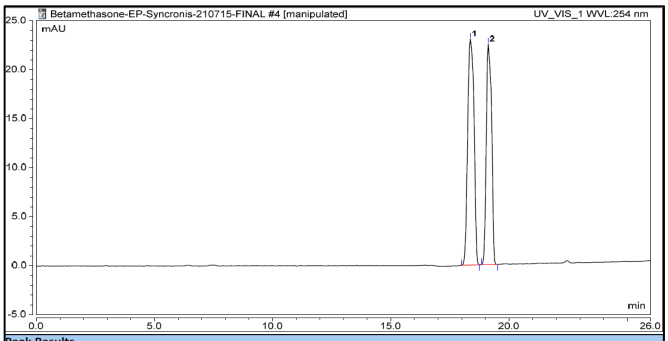
Column temperature: 45˚C
Flow rate: 2.5 mL/min
Injection Volume: 20 µl
Detector wavelength: UV 254nm
Run Time: 46 min

System Suitability Results:

Sr. No.	Parameters	EP Criteria	Obtained Results
1	Resolution between Methylprednisolone and Betamethasone peak	Minimum 1.5	1.55*

*** The obtained results are keeping all the chromatographic parameters same as per requirement of USP monograph, however as per monograph, the required resolution can further be increased by changing the acetonitrile concentration in mobile phase A.**

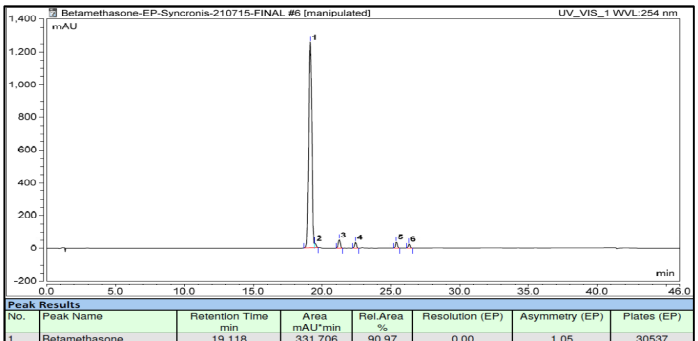
CHROMATOGRAMS:



Peak Results

No.	Peak Name	Retention Time min	Area mAU*min	Rel.Area %	Resolution (EP)	Asymmetry (EP)	Plates (EP)
1	Methylprednisolone	18.365	7.151	54.63	1.55	1.07	19527
2	Betamethasone	19.123	5.938	45.37	0.00	1.17	28219

System Suitability:



Peak Results

No.	Peak Name	Retention Time min	Area mAU*min	Rel.Area %	Resolution (EP)	Asymmetry (EP)	Plates (EP)
1	Betamethasone	19.118	331.706	90.97	0.00	1.05	30537
2	Impurity-A	19.465	5.073	1.39	n.a.	n.a.	n.a.
3	Impurity-E	21.232	9.827	2.69	5.64	1.05	72950
4	Impurity-C	22.410	6.575	1.80	9.12	1.05	98101
5	Impurity-B	25.377	6.929	1.90	17.15	1.13	119079
6	Impurity-D	26.310	4.539	1.24	20.22	1.07	145771

Impurity Mix: