**5. METHOD DEVELOPMENT**

**5.1 METHOD DEVELOPMENT TRAILS**

**TRAIL 1:**

Mobile Phase:OPA: Methanol (60:40) ;PH-2.3

Column :WATERS, C18, 15cmx4.6mm, 5µm

Flow Rate : 1.0ml/Min

Temperature : 25˚C

Volume : 10µl

Run time : 15min

Detector : PDA



**Fig 6: Typical chromatogram of Trail 1**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Retention Time | Area | USP Resolution | USP Tailing | USP Plate Count |
| 4.254 | 41411 |  | 0.94 | 8218 |

Observation: Only One peak observed

Reason: may be column not suitable

Corrective Action: change the column

**TRAIL 2:**

Mobile Phase:OPA: Methanol (60:40) ;PH-2.3

Column :WATERS, C18, 25cmx4.6mm, 5µm

Flow Rate : 1.0ml/Min

Temperature : 25˚C

Volume : 10µl

Run time : 15min

Detector : PDA



**Fig 7: Typical chromatogram of Trail 2**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Retention Time | Area | USP Resolution | USP Tailing | USP Plate Count |
| 2.922 | 20866 | 7.60 | 0.95 | 7212 |
| 1.915 | 14806 |  | 1.13 | 6526 |

Observation: Two peaks eluted but peak shape is not good.

Reason: may be column not suitable

Corrective Action: change the column

**TRAIL 3:**

Mobile Phase: KH2PO4: Methanol (60:40) ;PH-4.3

Column :SUPELCO, C18, 15cmx4.6mm, 5µm

Flow Rate : 1.0ml/Min

Temperature : 25˚C

Volume : 10µl

Run time : 15min

Detector : PDA



**Fig 8: Typical chromatogram of Trail 3**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Retention Time | Area | USP Resolution | USP Tailing | USP Plate Count |
| 2.894 | 38581 | 5.95 | 1.10 | 4462 |
| 1.903 | 19248 |  | 1.69 | 4677 |

**Observation:** Two peaks eluted but peak shape is not good.

Reason: may be mobile phase composition is not suitable

Corrective Action: change the mobile phase composition

**TRAIL 4:**

Mobile Phase: KH2PO4: Methanol (60:40) ;PH-4.3

Column :SUPELCO, C18, 25cmx4.6mm, 5µm

Flow Rate : 1.0ml/Min

Temperature : 25˚C

Volume : 10µl

Run time : 15min

Detector : PDA



**Fig 9: Typical chromatogram of Trail 4**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Retention Time | Area | USP Resolution | USP Tailing | USP Plate Count |
| 4.502 | 12871 | 4.97 | 1.29 | 17030 |
| 3.833 | 14774 |  | 2.40 | 23838 |

**Observation:** Two peaks eluted but peak shape is not good.

Reason: may be mobile phase composition is not suitable

Corrective Action: change the mobile phase composition.

**Trail: 5**

Mobile Phase: KH2PO4: Methanol (60:40); PH-4.3

Column :THERMO HYPERSIL, C18, 25cmx4.6mm, 5µm

Flow Rate : 1.0ml/Min

Temperature : 25˚C

Volume : 10µl

Run time : 15min

Detector : PDA



**Fig 9: Typical chromatogram of Trail 5**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Retention Time | Area | USP Resolution | USP Tailing | USP Plate Count |
| 4.419 | 815888 | 4.14 | 1.45 | 12853 |
| 3.700 | 973730 |  | 1.23 | 6879 |

**Observation:** Two peaks eluted but peak shape is not good.

Reason: may be mobile phase composition is not suitable

Corrective Action: change the mobile phase composition.

**Trail: 6**

Mobile Phase: K2HPO4: Methanol (70:30); PH-4.5

Column :THERMO HYPERSIL, C18, 25cmx4.6mm, 5µm

Flow Rate : 1.0ml/Min

Temperature : 25˚C

Volume : 10µl

Run time : 8min

Detector : 231nm



**Fig 9: Typical chromatogram of Trail 6**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Name | Retention Time | Area | USP Resolution | USP Tailing | USP Plate Count |
| 2 | Rosuvstatin | 4.023 | 545523 | 6.56 | 1.32 | 12306 |
| 1 | Fenofibrate | 3.026 | 983696 |  | 1.07 | 6992 |

Observation: Two peaks eluted and all the system suitability parameters are within the limit.