***:***

diluent and mix well. **[Label as sample preparation-1 and 2].**

***Order of injection:***

|  |  |  |
| --- | --- | --- |
| *Name of the preparations* | *No. of injections* | *Purpose* |
| Blank | 1 | Blank |
| System suitability solution | 1 | System suitability |
| Sample preparation-1 | 1 | Sample analysis |
| Sample preparation-2 | 1 |

peak is about 29.39 min. The relative retention time are given below.



***Reference Chromatograms:***

***Consider stage IV in the below chromatogram as stage III, stage-IV dimer as*** Insitu dimer, stage-III as KSM-II, KSM-II as KSM-II/II,



***Reporting:***

Report the results on the basis of % area average of two injections.

***Gradient Program:***

***Seal Wash:***

***Standard stock solution preparation:***

Weigh and transfer about 5.0 ± 0.5 mg each of Stage III (Insitu) impurity III, standard into a 50

***Evaluation of system suitability:***

***Reference Chromatograms:***

***Consider stage IV in the below chromatogram as stage III, stage-IV insitu as stage-III insitu***

***Order of injection:***

|  |  |  |
| --- | --- | --- |
| *Name of the preparations* | *No. of injections* | *Purpose* |
| Blank | 1 | Blank |
| Standard solution | 3 | System suitability |
| Sample preparation-1 | 1 | Sample analysis |
| Sample preparation-2 | 1 | Sample analysis |

***Calculations:***

1. **Assay (By HPLC, %w/w) (On dried basis) :**

***Preparation of diluted orthophosphoric acid solution (10% v/v):***

Add about 10 mL of orthophosphoric acid into a 100 mL volumetric flask. Make up to the volume with water. Mix well.

***Buffer solution Preparation:***

Transfer 2.0 mL of triethylamine into a 1000 mL of water, and mix well. Adjust the pH

8 ± 0.05 with diluted orthophosphoric acid solution. Filter it through 0.45 µ filter paper [Make: Millipore] and degas.

***Mobile phase preparation:***

Transfer about 350 mL of buffer and 650 mL of Acetonitrile into a suitable container. Mix well. Sonicate to degas.

***Seal Wash:***

Transfer about 900 mL of water and 100 mL of Acetonitrile into a suitable container. Mix well. Sonicate to degas.

***Reference Chromatograms:***

***Consider stage IV in the below chromatogram as stage III***