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**Research**

**Simultaneous Estimation Of New Analytical Method Development And Validation Of Dabrafenib And Trametinib By High Performance Liquid Chromatography**

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|  |  |
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|  | **Abstract**  |
| Published on: 20 Oct 2023 |  An accurate, precise, simple, efficient and reproducible, isocratic Reversed Phase-High Performance Liquid Chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of Dabrafenib and Trametinib in bulk and combined pharmaceutical tablet dosage forms. Dabrafenib and Trametinib were separated by using a Symmetry ODS C18 (4.6mm×150mm) 5µm Particle Size; Waters Alliance e2695 HPLC system with 2998 PDA detector and the mobile phase contained a mixture of Methanol: 0.1% Orthophosphoric acid (64:36% v/v). The flow rate was set to 1ml/min with the responses measured at 224nm. The retention time of Dabrafenib and Trametinib was found to be 2.808min and 3.880min respectively with resolution of 5.68. Linearity was established for Dabrafenib and Trametinib in the range of 20-100µg/ml for Dabrafenib and 60-140µg/ml for Trametinib with correlation coefficient 0.999. The percentage recovery was found to be is 100.30% for Dabrafenib and 100.21% for Trametinib respectively. Validation parameters such as specificity, linearity, precision, accuracy and robustness, limit of detection (LOD) and limit of quantitation (LOQ) were evaluated for the method according to the International Conference on Harmonization (ICH) Q2 R1 guidelines. The developed method was successfully applied for the quantification of bulk and active pharmaceutical ingredient present and in combined tablet dosage form. |
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| **Keywords:** Dabrafenib and Trametinib, RP-HPLC, Validation, Accuracy, Precision |

**INTRODUCTION**

 Analytical chemistry**1** is the branch of chemistry involved in separating, identifying and determining the relative amounts of the components making up a sample of matter. It is mainly involved in the qualitative identification or detection of compounds and the quantitative measurement of the substances present in bulk and pharmaceutical preparation.

 Measurements of physical properties of analytes such as conductivity, electrode potential, light absorption or emission, mass to charge ratio, and fluorescence, began to be used for quantitative analysis of variety of inorganic and biochemical analytes. Highly efficient chromatographic and electrophoretic techniques began to replace distillation, extraction and precipitation for the separation of components of complex mixtures prior to their qualitative or quantitative determination. These newer methods for separating and determining chemical species are known collectively as instrumental methods of analysis. Most of the instrumental methods fit into one of the three following categories viz spectroscopy, electrochemistry and chromatography

Advantages of instrumental methods

* Small samples can be used
* High sensitivity is obtained
* Measurements obtained are reliable
* Determination is very fast
* Even complex samples can be handled easily

Limitations of instrumental methods

* An initial or continuous calibration is required
* Sensitivity and accuracy depends on the instrument
* Cost of equipment is large
* Concentration range is limited
* Specialized training is needed
* Sizable space is required

Principle types of chemical instrumentation:

**Spectrometric techniques**

1. Ultraviolet and visible Spectrophotometry
2. Fluorescence and phosphorescence Spectrophotometry.
3. Atomic Spectrometry (emission and absorption)
4. Infrared Spectrophotometry
5. Raman Spectroscopy
6. X-Ray Spectroscopy
7. Radiochemical Techniques including activation analysis
8. Nuclear Magnetic Resonance Spectroscopy
9. Electron Spin Resonance Spectroscopy

**Electrochemical techniques**

1. Potentiometry
2. Voltametry
3. Voltametric Techniques
4. Amperometric Techniques
5. Colorimetry
6. Electrogravimetry
7. Conductance Techniques

**Chromatographic techniques**

1. Gas Chromatography
2. High Performance Liquid Chromatography
3. High Performance Thin Layer Chromatography

**Miscellaneous techniques**

1. Thermal Analysis
2. Mass Spectrometry
3. Kinetic Techniques
4. Hyphenated techniques
5. GC-MS (Gas Chromatography – Mass Spectrometry)
6. GC-IR (Gas Chromatography – Infrared Spectroscopy)
7. MS-MS (Mass Spectrometry – Mass Spectrometry)

**1.1 INTRODUCTION TO HPLC**

HPLC**3** is a type of liquid chromatography that employs a liquid mobile phase and a very finely divided stationary phase. In order to obtain satisfactory flow rate liquid must be pressurized to a few thousands of pounds per square inch.

 The rate of distribution of drugs between Stationary and mobile phase is controlled by diffusion process. If diffusion is minimized faster and effective separation can be achieved. The techniques of high performance liquid chromatography are so called because of its improved performance when compared to classical column chromatography advances in column chromatography into high speed, efficient, accurate and highly resolved method of separation.

 For the recent study Clonazepam and Propranolol was selected for estimation of amount of analyte present in formulation and bulk drug. The HPLC method is selected in the field of analytical chemistry, since this method is specific, robust, linear, precise and accurate and the limit of detection is low and also it offers the following advantages

* Speed many analysis can be accomplished in 20min (or) less.
* Greater sensitivity (various detectors can be employed).
* Improved resolution (wide variety of stationary phases).
* Re usable columns (expensive columns but can be used for many analysis).
* Ideal for the substances of low viscosity.
* Easy sample recovery, handling and maintenance.
* Instrumentation leads itself to automation and quantification (less time and less labour).
* Precise and reproducible.
* Integrator itself does calculations.
* Suitable for preparative liquid chromatography on a much larger scale.

**MATERIALS AND METHODS**

Dabrafenib from Sura labs, Trametinib from Sura labs, Water and Methanol for HPLC from LICHROSOLV (MERCK). Acetonitrile for HPLC from Merck, Phosphate buffer from Sura labs.

**HPLC METHOD DEVELOPMENT**

**TRAILS**

**Preparation of standard solution**

Accurately weigh and transfer 10 mg of Dabrafenib and Trametinib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.6ml of Dabrafenib and 1ml of Trametinib from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

**Procedure**

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

**OPTIMIZED CHROMATOGRAPHIC CONDITIONS**

Instrument used : Waters Alliance 2695 HPLC with PDA Detector 996 model.

Temperature : 38ºC

Column : Symmetry ODS C18 (4.6mm×150mm) 5µm Particle Size

Mobile phase : Methanol: 0.1% Orthophosphoric acid (64:36% v/v)

Flow rate : 1ml/min

Wavelength : 224nm

Injection volume : 20µl

Run time : 7.0minutes

**VALIDATION**

**PREPARATION OF MOBILE PHASE**

**Preparation of mobile phase**

Accurately measured 640ml of Acetonitrile (64%) of and 360ml of HPLC Water (36%) were mixed and degassed in a digital ultra sonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.

**Diluent Preparation**

The Mobile phase was used as the diluent.

**RESULTS AND DISCUSSION**

**Optimized Chromatogram (Standard)**

Mobile phase : Methanol: 0.1% Orthophosphoric acid (64:36% v/v)

Column : Symmetry ODS C18 (4.6mm×150mm) 5µm Particle Size

Flow rate : 1 ml/min

Wavelength : 224 nm

Column temp : 38ºC

Sample Temp : Ambient

Injection Volume : 20 µl

Run time : 7 minutes



**Fig 1: Chromatogram for Trail 5**

**Table 1: Peak Results for Trail 5**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **S. No** | **Peak name** | **Rt** | **Area** | **Height** | **USP Resolution** | **USP Tailing** | **USP plate count** |
| 1 | Dabrafenib | 2.808 | 65258 | 4326 |  | 1.08 | 5685.4 |
| 2 | Trametinib | 3.880 | 8659854 | 659823 | 5.68 | 1.42 | 6895.7 |

From the above chromatogram it was observed that the Dabrafenib and Trametinib peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it’s optimized trial.

Retention time of Dabrafenib–2.808min

Retention time of Trametinib – 3.880 min

**Assay (Standard)**

**Table 2: Showing assay standard Results**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| S.No. | Name | Rt | Area | Height | USP Resolution | USP Tailing | USP plate count | Injection |
| 1 | Dabrafenib | 2.813 | 65684 | 4365 |  | 1.08 | 5632.4 | 1 |
| 2 | Trametinib | 3.886 | 8659824 | 659824 | 5.69 | 1.42 | 6859.2 | 1 |
| 3 | Dabrafenib | 2.813 | 65985 | 4329 |  | 1.09 | 5682.3 | 2 |
| 4 | Trametinib | 3.886 | 8645872 | 658266 | 5.68 | 1.43 | 6824.1 | 2 |
| 5 | Dabrafenib | 2.813 | 65784 | 4426 |  | 1.08 | 5692.8 | 3 |
| 6 | Trametinib | 3.886 | 8657847 | 6589412 | 5.69 | 1.43 | 6895.4 | 3 |

**Assay (Sample)**

**Table 3: Showing assay sample results**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| S.No. | Name | Rt | Area | Height | USP Resolution | USP Tailing | USP plate count | Injection |
| 1 | Dabrafenib | 2.799 | 66859 | 4458 |  | 1.09 | 5785.4 | 1 |
| 2 | Trametinib | 3.863 | 8756854 | 669585 | 5.69 | 1.43 | 6956.7 | 1 |
| 3 | Dabrafenib | 2.799 | 66258 | 4462 |  | 1.10 | 5789.5 | 2 |
| 4 | Trametinib | 3.861 | 8769582 | 663598 | 5.68 | 1.44 | 6945.2 | 2 |
| 5 | Dabrafenib | 2.799 | 66435 | 4438 |  | 1.09 | 5784.1 | 3 |
| 6 | Trametinib | 3.863 | 8754985 | 668548 | 5.69 | 1.44 | 6927.7 | 3 |

 Sample area Weight of standard Dilution of sample Purity Weight of tablet

 %ASSAY = \_\_\_\_\_\_\_\_\_\_\_ × \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ × \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_× \_\_\_\_\_\_\_× \_\_\_\_\_\_\_\_\_\_\_\_\_\_×100

 Standard area Dilution of standard Weight of sample 100 Label claim

The % purity of Tinidazole and Diloxanide in pharmaceutical dosage form was found to be 99.4 %.

**LINEARITY**

**CHROMATOGRAPHIC DATA FOR LINEARITY STUDY**

**Tinidazole**

**Fig 2: calibration graph for Dabrafenib**

**Linearity Results: (for** **Dabrafenib)**

|  |  |  |  |
| --- | --- | --- | --- |
| **S.No.** | **Linearity Level** | **Concentration (ppm)** | **Area** |
| 1 | I | 20 | 24759 |
| 2 | II | 40 | 47859 |
| 3 | III | 60 | 70898 |
| 4 | IV | 80 | 93985 |
| 5 | V | 100 | 116698 |
| Correlation Coefficient | 0.999 |

*Correlation coefficient should be not less than 0.999.*

**Linearity Results: (for Trametinib)**

**Fig 3: Calibration graph for Trametinib**

|  |  |  |  |
| --- | --- | --- | --- |
| **S.No.** | **Linearity Level** | **Concentration (ppm)** | **Area** |
| 1 | I | 60 | 4928578 |
| 2 | II | 80 | 6687842 |
| 3 | III | 100 | 8389878 |
| 4 | IV | 120 | 10085847 |
| 5 | V | 140 | 11769854 |
| Correlation Coefficient | 0.999 |

* *Correlation coefficient should be not less than 0.99.*

**REPEATABILITY**

**Table 4: Results of method precision for Dabrafenib**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| S.No. | Name | Rt | Area | Height | USP plate count | USP Tailing |
| 1 | Dabrafenib | 2.808 | 65898 | 4365 | 5682.2 | 1.08 |
| 2 | Dabrafenib | 2.808 | 65487 | 4375 | 5628.6 | 1.09 |
| 3 | Dabrafenib | 2.808 | 65324 | 4395 | 5649.7 | 1.08 |
| 4 | Dabrafenib | 2.808 | 65982 | 4328 | 5638.4 | 1.09 |
| 5 | Dabrafenib | 2.808 | 65248 | 4371 | 5698.3 | 1.08 |
| 6 | Dabrafenib | 2.808 | 65734 | 4391 | 5682.7 | 1.09 |
| Mean |  |  | 65612.17 |  |  |  |
| Std. Dev |  |  | 304.8425 |  |  |  |
| % RSD |  |  | 0.464613 |  |  |  |

**Table 5: Results of method precision for Trametinib**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| S.No. | Name | Rt | Area | Height | USP plate count | USP Tailing | USP Resolution |
| 1 | Trametinib | 3.880 | 8659824 | 658784 | 6859.4 | 1.42 | 5.68 |
| 2 | Trametinib | 3.880 | 8658547 | 657489 | 6824.6 | 1.43 | 5.69 |
| 3 | Trametinib | 3.880 | 8659824 | 652368 | 6829.3 | 1.42 | 5.68 |
| 4 | Trametinib | 3.880 | 8659875 | 658745 | 6892.7 | 1.43 | 5.69 |
| 5 | Trametinib | 3.880 | 8658745 | 658213 | 6875.2 | 1.42 | 5.68 |
| 6 | Trametinib | 3.880 | 8659862 | 652354 | 6859.8 | 1.42 | 5.69 |
| Mean |  |  | 8659446 |  |  |  |  |
| Std. Dev |  |  | 623.2924 |  |  |  |  |
| % RSD |  |  | 0.007198 |  |  |  |  |

* *%RSD for sample should be NMT 2.*
* *The %RSD for the standard solution is below 1, which is within the limits hence method is precise.*

**Intermediate precision**

**Table 6: Results of Intermediate precision for Dabrafenib:**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| S.No. | Name | Rt | Area | Height | USP plate count | USP Tailing |
| 1 | Dabrafenib | 2.808 | 66895 | 4468 | 5784.2 | 1.09 |
| 2 | Dabrafenib | 2.808 | 66986 | 4523 | 5835.1 | 1.09 |
| 3 | Dabrafenib | 2.808 | 66258 | 4475 | 5864.4 | 1.10 |
| 4 | Dabrafenib | 2.808 | 66457 | 4514 | 5864.6 | 1.09 |
| 5 | Dabrafenib | 2.808 | 66539 | 4489 | 5784.9 | 1.10 |
| 6 | Dabrafenib | 2.808 | 66298 | 4565 | 5748.5 | 1.10 |
| Mean |  |  | 66572.17 |  |  |  |
| Std. Dev |  |  | 304.536 |  |  |  |
| % RSD |  |  | 0.457452 |  |  |  |

**Table 7: Results of Intermediate precision for Trametinib**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| S.No. | Name | Rt | Area | Height | USP plate count | USP Tailing | USP Resolution |
| 1 | Trametinib | 3.882 | 8758568 | 669583 | 6982.4 | 1.43 |  |
| 2 | Trametinib | 3.882 | 8756982 | 665984 | 6935.3 | 1.44 | 5.69 |
| 3 | Trametinib | 3.882 | 8746925 | 665345 | 6984.7 | 1.44 |  |
| 4 | Trametinib | 3.882 | 8723654 | 665325 | 6952.8 | 1.43 | 5.70 |
| 5 | Trametinib | 3.882 | 8754982 | 669852 | 6898.9 | 1.44 |  |
| 6 | Trametinib | 3.882 | 8754698 | 665874 | 6976.5 | 1.43 | 5.69 |
| Mean |  |  | 8749302 |  |  |  |  |
| Std. Dev |  |  | 13188.56 |  |  |  |  |
| % RSD |  |  | 0.150738 |  |  |  |  |

* *%RSD of five different sample solutions should not more than 2.*
* *The %RSD obtained is within the limit, hence the method is rugged.*

**ACCURACY**

**Table 8: Accuracy (recovery) data for Dabrafenib**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **%Concentration****(at specification Level)** | **Area** | **Amount Added****(mg)** | **Amount Found****(mg)** | **% Recovery** | **Mean Recovery** |
| 50% | 35921.67 | 30 | 30.134 | 100.446% | 100.30% |
| 100% | 70894.33 | 60 | 60.205 | 100.341% |
| 150% | 105654.7 | 90 | 90.093 | 100.103% |

* *The % Recovery for each level should be between 98.0 to 102.0%.*

**Table 9: Accuracy (recovery) data for Trametinib**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **% Concentration****(at specification Level)** | **Area** | **Amount Added****(mg)** | **Amount Found****(mg)** | **% Recovery** | **Mean Recovery** |
| 50% | 4276302 | 50 | 50.208 | 100.416% | 100.21% |
| 100% | 8484717 | 100 | 100.148 | 100.148% |
| 150% | 10160609 | 150 | 150.091 | 100.060% |

* *The percentage recovery was found to be within the limit (97-103%).*

*The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.*

**Robustness**

**Table 10: System suitability results for** **Dabrafenib:**

|  |  |  |
| --- | --- | --- |
| **S.No** | **Flow Rate (ml/min)** | **System Suitability Results** |
| **USP Plate Count** | **USP Tailing** |
|  1 | 0.9 | 5784.6 | 1.06 |
| 2 | 1.0 | 5685.4 | 1.08 |
| 3 |  1.1 | 5869.5 | 1.09 |

***\**** *Results for actual flow (1.0 ml/min) have been considered from Assay standard.*

**Table 11: System suitability results for** **Trametinib:**

|  |  |  |
| --- | --- | --- |
| **S.No** | **Flow Rate (ml/min)** | **System Suitability Results** |
| **USP Plate Count** | **USP Tailing** |
| 1 | 0.9 | 6698.3 |  1.46 |
| 2 | 1.0 | 6895.7 | 1.42 |
| 3 |  1.1 |  6983.6 | 1.49 |

 **\*** Results for actual flow (1.0ml/min) have been considered from Assay standard.

**CONCLUSION**

The study is focused to develop and validate HPLC methods for estimation of Dabrafenib and Trametinib in bulk and tablet dosage form. For routine analytical purpose it is desirable to establish methods capable of analyzing huge number of samples in a short time period with good robustness, accuracy and precision without any prior separation steps. HPLC method generates large amount of quality data, which serve as highly powerful and convenient analytical tool. The method shows good reproducibility and good recovery. From the specificity studies, it was found that the developed methods were specific for Dabrafenib and Trametinib

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