SIMULTANEOUS ESTIMATION OF LATEST ANALYTICAL
METHOD IMPROVEMENT AND VALIDATION OF DABRAFENIB
AND TRAMETINIB BY MEANS OF HIGH-PERFORMANCE LIQUID
CHROMATOGRAPHY

Santhosh Illendula* and G. Mariya Therisa

Department of Pharmaceutical Analysis, Nalanda College of Pharmacy, Cherlapally(v),
Nalgonda(Dt), Telangana (St), India, 508001.

ABSTRACT

A correct, particular, easy, inexperienced and reproducible, isocratic Reversed section-high overall performance Liquid Chromatography (RP-HPLC) technique became developed and established for the simultaneous estimation of Dabrafenib and Trametinib in bulk and mixed pharmaceutical pill dosage paperwork. Dabrafenib and Trametinib were separated through the usage of a Symmetry ODS C18 (4.6mm×150mm) 5µm Particle length; Waters Alliance e2695 HPLC device with 2998 PDA detector and the cellular phase contained a combination of Methanol: zero.1% Orthophosphoric acid (64:36% v/v). The float price has become set to 1ml/min with the responses measured at 224nm. The retention time of Dabrafenib and Trametinib changed into discovered to be 2.808min and three.880min respectively with resolution of 5. sixty-eight. Linearity emerge as installation for Dabrafenib and Trametinib in the shape of 20-a hundred µg/ml for Dabrafenib and 60-one hundred forty µ g/ml for Trametinib with correlation coefficient 0.999. The percentage healing emerges as decided to be is a hundred.30% for Dabrafenib and a hundred.21% for Trametinib respectively. Validation parameters alongside specificity, linearity, precision, accuracy and robustness, restriction of detection (LOD) and restrict of quantitation (LOQ) were evaluated for the technique everyday with the worldwide conference on Harmonization (ICH) Q2 R1 guidelines. The superior method has end up effectively completed for the quantification of bulk and active pharmaceutical thing present and in blended pill dosage form.
KEYWORDS: Dabrafenib and Trametinib, RP-HPLC, Validation, Accuracy, Precision.

INTRODUCTION

Analytical Method Improvement

Steps of approach development: Documentation starts off evolved at the very beginning of the improvement method, a device for whole documentation of the development studies need to be set up.

1. All regarded information about the analyte and its form is gathered i.E., bodily and chemical homes, toxicity, purity, hygroscopic nature, solubility and balance.

Approach Requirements: The goals or requirements of the analytical method that want to be developed are taken into consideration and the analytical figures of benefit are defined. The specified detection limits, selectivity, linearity, variety, accuracy and precision are described.

Literature are searching for and in advance approach: Books, periodicals, chemical producers and regulatory enterprise compendia which include USP / NF, affiliation of official Analytical Chemists (AOAC) and American Society for testing and substances (ASTM) guides are reviewed.

Instrumental setup and initial studies

- The specified instrumentation is setup, installation, operational and preferred overall performance qualification of instrumentation the usage of laboratory fashionable running methods (SOP’s) are verified.
- Evaluation is executed the use of analytical conditions described inside the present day-day literature.

Optimization: during optimization one parameter is changed at a time, and set of conditions are isolated, in choice to the use of a trial and mistakes method. paintings has been completed from an organized methodical plan and every step is documented (in a lab pocket e-book) in case of lifeless ends.

Analytical Technique Validation

Specificity: In exercise this can be finished thru using using spiking the drug substance or product (placebo system, excipients degradation product, approach impurity) with suitable diploma and demonstrating the assay surrender end result is unaffected with the aid of
manner of using the presence of those extraneous substances.

**Precision:** The precision of an analytical approach is determined with the useful resource of using manner of assaying a enough wide form of aliquots of a homogenous sample if you want to calculate statistically legitimate estimates of deviation or relative elegant deviation.

**Accuracy:** within the case of a drug in the formulated product. Accuracy can be determined via software of analytical synthetic combos of the drug components to which the seemed quantity of analyte have been added indoors shape of the technique.

**Linearity:** ICH encouraged that, for the set-up order of linearity, at least 5 concentrations. it is also advocated that the subsequent minimum high-quality range need to be taken into consideration. The correlation coefficient of > 0.999 is normally considered as proof of desirable in shape of the facts to the regression line the analyte at to purpose diploma.

**Robustness (ICH 1994):** The robustness of the techniques changed into determined thru manner of appearing the assay of the triplicate with the useful aid of deliberately alternating parameters and that the outcomes aren't stimulated with the beneficial resource of diverse adjustments inside the beneath parameters.

- ✓ Change in column temperature: + 20C
- ✓ Alternate in go with the flow charge: + zero.2ml/min.
- ✓ Alternate in natural segment : + 10%
- ✓ Alternate in pH : + 0.2

**DRUG PROFILE**

**Drug:** Dabrafenib

**Shape**

![Chemical structure](image)

**Chemical call/ Nomenclature / IUPAC name:** N-three-[5-(2-aminopyrimidin-4-yl)-2- tert-butyl-1,3-thiazol-4-yl]-2-fluorophenyl-2,6-difluorobenzene-1-sulfonamide.
Molecular method: C23H20F3N5O2S2.
Molecular Weight: 519.562 gm/mole.
Description (physical kingdom): Dabrafenib is a stable Powder.
Bioavailability: 5%.
Half of-existence: mean absolute bioavailability of oral Dabrafenib is ninety : 8 hours.
Absorption: imply absolute bioavailability of oral Dabrafenib is ninety five%.
Quantity of Distribution: apparent quantity of distribution (Vd/F) = 70.three L.
Protein binding: 99.7% sure to human plasma protein.
Metabolism: Dabrafenib is metabolized within the liver.

Excretion: Fecal excretion is the foremost route of elimination accounting for 71% of radioactive dose on the equal time as urinary excretion accounted for 23% of preferred radioactivity as metabolites best.

Destructive Effects/Facet Effects: Fever, Joint ache, Papilloma (warts/growths), Hair loss, Hand-foot syndrome (Palmar-plantar erythrodyesthesia), expanded Alkaline phosphatase, Rash and lower back pain.

Mechanism of motion: Dabrafenib is an orally bioavailable inhibitor of B-raf (BRAF) protein with antineoplastic hobby. Dabrafenib selectively binds to and inhibits the hobby of B-raf, which can also inhibit the proliferation of tumor cells which include a mutated BRAF gene.

Interactions Drug interactions
Abatacept: The metabolism of Dabrafenib can be superior on the same time as blended with Abatacept.

Abiraterone: The metabolism of Dabrafenib can be reduced on the equal time as blended with Abiraterone.

Drug Profile For Trametinib
Name of The Drug: Trametinib
Chemical shape

![Chemical structure of Trametinib](image)

**IUPAC name:** N-(3-three-cyclopropyl-5-[(2-fluoro-4-iodophenyl) amino]-6,8-dimethyl-2,4,7-trioxo-1H,2H,3H,4H,6H,7H-pyrido[4,3-d] pyrimidin-1-yl phenyl) acetamide

**Molecular formula:** C$_{26}$H$_{23}$FIN$_{5}$O$_{4}$

**Molecular weight:** 615.3948 g/mole

**Description (bodily nation):** Trametinib is a crystalline sturdy powder.

**Description (physical kingdom):** Trametinib is a crystalline strong powder.

**Bioavailability:** A unmarried 2 mg oral dose has a bioavailability of seventy %.

**Absorption:** while a dose of 2mg/day is given, the height plasma consciousness (C$_{\text{max}}$) is 22.2ng/mL.

**Quantity of Distribution:** obvious amount of distribution (V$_{d}$/F) = 214 L.

**Protein binding:** ninety seven.four% first rate to human plasma proteins.

**Metabolism:** Trametinib is metabolized predominantly thru deacetylation on my own or with mono-oxygenation or in mixture with glucuronidation biotransformation pathways in vitro.

**Route of Removal:** 80% of the dose is excreted within the feces. <20% of the dose is excreted within the urine with <zero.1% of the excreted dose within the shape of the figure compound.

**Half of Life:** elimination half of of-life = 3.9-4.eight days.

**Mechanism of action:** Trametinib is a mainly selective reversible allosteric inhibitor of MEK1 and MEK2 interest. it's far an ATP non-competitive inhibitor that binds MEK adjoining to the ATP binding net web page in not unusual with awesome MEK allosteric inhibitors.

**Drug Interactions:** Abacavir: Abacavir can also lower the excretion fee of Trametinib that could result in a higher serum diploma.
**Drug-meals Interactions:** Take reduce loose meals. Take at least one hour earlier than or hours after a meal.

**EVALUATION OF LITERATURE**

**C M Nijenhuis, et al. (2016):** Dabrafenib (Tafinlar®) and trametinib (Mekinist®) are registered for the treatment of sufferers with BRAF V600 mutation splendid unresectable or metastatic most cancers. Human plasma samples had been amassed on an outpatient base and stored at nominally -20°C. Analytes and internal requirements (robust isotope categorized compounds) had been extracted with TBME. The dry extract become then reconstituted with a hundred μL acetonitrile and five μL of the final extract grow to be injected and separated on a C18 column with gradient elution, and analysed with triple quadrupole mass spectrometry in incredible-ion mode. The examined assay degrees from 50 to 5000ng/mL for dabrafenib and zero.5-50ng/mL for trametinib have been linear, and correlation coefficient (r(2)) of 0.996 or better. in any respect concentrations of each analytes the biases had been inside ±15% of the nominal concentrations and precisions have been ≤15%.

**NVS Naidu, et al., (2018):** Trametinib showed maximum absorption at 245nm. This approach finished through using isocratic elution with ratio ammonium acetate buffer: methanol: Acetonitrile: tetrahydrofuran cellular segment at forty: 30: 20: 10 V/V. The evolved HPLC method have end up installed for Linearity, Accuracy, Inter day Precision, Specificity & Selectivity, Robustness, solution stability, restriction of detection & limit of quantification. balance Indicating methods had been advanced for Trametinib in Pharmaceutical Dosage shape underneath hydrolytic stress state of affairs (5N HCl, 5N NaOH); Oxidation situation (five% H2O2) and dry warmth state of affairs, Thermal scenario, UV mild.

**PLAN OF WORK**

**Step 1 – Literature survey:** collect the following statistics approximately Physico chemical property of medication from surely taken into consideration one in every of a type e-books, net and masses of others. Make compilation and format the plan for growth the approach.

**Knowledge of the samples of medication**

- Molecular weight variety
- Nature of sample components and structure of sample components
- Variety of compounds gift
- Sample matrix and pKa values of pattern additives
- Awareness range and Solubility
- Other pertinent records

**Step 2 – Method Improvement**

I. Choice of initial situations.
II. Selection of cell section solvent power
III. Selection of desk positive phase (HPLC column) and selection of wavelength
IV. Selectivity optimization
V. Gadget parameter optimization and approach Optimization.

**Step 3 - Approach Validation**

- System Suitability
- Specificity
- Linearity
- Accuracy
- Precision
- Repeatability
- Intermediate precision
- Reproducibility
- Restriction of Detection (LOD) and restrict of Quantification (LOQ)
- Robustness and Ruggedness

**EXPERIMENTAL METHODS HPLC METHOD IMPROVEMENT**

**Preparation of standard solution:** As it should be weigh and transfer 10 mg of Dabrafenib and Trametinib working favored proper right into a 10ml of smooth dry volumetric flasks upload approximately 7ml of Methanol and sonicate to dissolve and removal of air in fact and make extent on pinnacle of things with the identical Methanol. in addition pipette 0.6ml of Dabrafenib and 1ml of Trametinib from the above inventory solutions proper right into a 10ml volumetric flask and dilute up to the mark with Methanol.

**Procedure:** Inject the samples via using changing the chromatographic situations and report the chromatograms, word the situations of proper peak elution for appearing validation parameters as in keeping with ICH guidelines.
Mobile Phase Optimization: to begin with the mobile section attempted turn out to be Methanol: Water and ACN: Water with numerous proportions. finally, the mobile phase turn out to be optimized to Methanol: zero.1% Orthophosphoric acid in percent sixty 4:36 v/v respectively.

Optimization of Column: The method have grow to be finished with diverse C18columns like Symmetry, X terra and ODS column. Symmetry ODS C18 (four.6mm×150mm) 5µm Particle size have end up determined to be outstanding as it gave fantastic top form and resolution at 1ml/min go together with the flow.

Method Validation
Preparation of mobile phase: correctly measured 640ml of Acetonitrile (sixty four%) of and 360ml of HPLC Water (36%) were mixed and degassed in a digital fairly sonicater for 15 minutes and then filtered through 0.45 µ smooth out underneath vacuum filtration.

Diluent Preparation: The mobile segment turn out to be used because the diluent.

Validation Parameters System Suitability
Procedure: The contemporary-day solution turn out to be injected for five instances and measured the area for all 5 injections in HPLC. The %RSD for the region of 5 reflect injections changed into decided to be within the tremendous limits.

Specificity Study of Drug
Preparation of Standard Solution and Preparation of Sample Solution
Procedure: Inject the three reflect injections of massive and pattern answers and calculate the assay through manner of the use of components:

\[
\%\text{ASSAY} = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Weight of tablet}} \times \frac{\text{Dilution of sample}}{\text{Dilution of standard}} \times \frac{\text{Purity}}{100 \% \text{Label claim}}
\]

Preparation of Drug Solutions For Linearity
Appropriately weigh and switch 10 mg of Dabrafenib and Trametinib strolling huge into a 10ml of clean dry volumetric flasks upload about 7ml of Diluents and sonicate to dissolve it in reality and make amount on pinnacle of things with the same solvent. (inventory solution) Similarly prepared the final interest stages from 20ppm – 100ppm of Dabrafenib and 60ppm
– 140ppm of Trametinib respectively.

**Procedure:** Inject every degree into the chromatographic gadget and degree the height vicinity. Plot a graph of top location rather than interest (on X-axis hobby and on Y-axis height area) and calculate the correlation coefficient.

**Precision: Repeatability**

**Preparation of Standard Solutions for Dabrafenib and Trametinib for Precision**

**Intermediate Precision:** To examine the intermediate precision (moreover known as Ruggedness) of the method, Precision modified into finished on one-of-a-type days through manner of maintaining equal situations.

**Method: DAY 1 and DAY 2:** The well-knownfamous answer have emerge as injected for six times and measured the vicinity for all Six injections in HPLC. The %RSD for the place of Six replicate injections come to be decided to be within the pleasant limits.

**Accuracy**

**Method:** Inject the 3 mirror injections of man or woman concentrations (50%, a hundred%, 100 and fifty%) have been made below the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the quantity decided and quantity introduced for Dabrafenib and Trametinib and calculate the individual healing and propose restoration values.

**Robustness:** The evaluation changed into achieved in particular situations to discover the kind of take a look at effects. The following situations are checked for version of outcomes.

**For preparation of Standard solution**

**Effect of version of flow conditions:** The pattern became analyzed at 0.nine ml/min and 1.1 ml/min instead of 1ml/min, very last conditions are same. 20µl of the above pattern became injected and chromatograms were recorded.

**Effect of variation of cell segment herbal composition:** The sample became analyzed by means of the use of model of cell phase i.e. Methanol: zero.1% Orthophosphoric acid (64:36% v/v) become taken in the ratio and sixty nine:31, 59:forty one in region of sixty four:36 closing conditions are same. 20µl of the above sample became injected and chromatograms were recorded.
RESULTS AND DISCUSSION

Trail 1
Mobile phase: Acetonitrile: Methanol (40% -60% v/v)
Column: Zorbax C18 (4.6mm×250mm) 5µm particle size
Flow rate: 0.8 ml/min
Wavelength: 224 nm
Column temp: Ambient
Sample Temp: Ambient
Injection Volume: 10 µl
Run time: 9 minutes

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Peak Name</th>
<th>R_t</th>
<th>Area</th>
<th>Height</th>
<th>USP Resolution</th>
<th>USP Tailing</th>
<th>USP plate count</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dabrafenib</td>
<td>2.485</td>
<td>536521</td>
<td>59898</td>
<td>1.13</td>
<td></td>
<td>4265</td>
</tr>
<tr>
<td>2</td>
<td>Trametinib</td>
<td>4.030</td>
<td>8658452</td>
<td>87984</td>
<td>2.35</td>
<td>1.26</td>
<td>3412</td>
</tr>
</tbody>
</table>

Observation: This trial show very less plate count, and show improper baseline in the chromatogram, so more trials were required for obtaining good peaks.

Trail 2
Mobile phase: Acetonitrile: 0.1% Orthophosphoric acid (70:30% v/v)
Column: Develosil C18 (4.6mm×250mm, 5µm).
Flow rate: 1.0 ml/min
Wavelength: 224 nm
Column temp: Ambient
Sample Temp : Ambient
Injection Volume: 0.9µl/min
Run time : 10 minutes
<table>
<thead>
<tr>
<th>S. No</th>
<th>Peak Name</th>
<th>Rt</th>
<th>Area</th>
<th>Height</th>
<th>USP Resolution</th>
<th>USP Tailing</th>
<th>USP plate count</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Trametinib</td>
<td>1.573</td>
<td>1852456</td>
<td>52365</td>
<td>1.26</td>
<td>2653</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Dabrafenib</td>
<td>4.611</td>
<td>125875</td>
<td>2546</td>
<td>4.35</td>
<td>4536</td>
<td></td>
</tr>
</tbody>
</table>

**Observation:** This trial shows improper baseline and shows less plate count in the chromatogram, so more trials were required for obtaining peaks.

**Trail 3**
Mobile phase: Methanol: 0.1% Orthophosphoric acid (50:50% v/v)
Column: Symmetry ODS C18 (4.6mm×150mm) 5µm Particle Size
Flow rate: 1.0 ml/min.
Wavelength: 224 nm.
Column temp: 36°C.
Sample Temp: Ambient
Injection Volume: 1.0µl/min.
Run time: 10 minutes.
Santhosh et. al.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Peak Name</th>
<th>$R_t$</th>
<th>Area</th>
<th>Height</th>
<th>USP Resolution</th>
<th>USP Tailing</th>
<th>USP plate count</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Trametinib</td>
<td>6.025</td>
<td>4265985</td>
<td>325685</td>
<td>0.96</td>
<td>1.08</td>
<td>1365.6</td>
</tr>
<tr>
<td>2</td>
<td>Dabrafenib</td>
<td>7.977</td>
<td>53625</td>
<td>45685</td>
<td>2.51</td>
<td>1.08</td>
<td>3856.5</td>
</tr>
</tbody>
</table>

**Observation:** This trial shows improper baseline and peak in the chromatogram, so more trials were required for obtaining peaks.

**Trial 4: (Optimized Condition)**

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

![](chart.png)

<table>
<thead>
<tr>
<th>S. No</th>
<th>Peak name</th>
<th>$R_t$</th>
<th>Area</th>
<th>Height</th>
<th>USP Resolution</th>
<th>USP Tailing</th>
<th>USP plate count</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dabrafenib</td>
<td>2.808</td>
<td>65258</td>
<td>4326</td>
<td>1.08</td>
<td>1.42</td>
<td>5685.4</td>
</tr>
<tr>
<td>2</td>
<td>Trametinib</td>
<td>3.880</td>
<td>8659854</td>
<td>659823</td>
<td>5.68</td>
<td>1.42</td>
<td>6895.7</td>
</tr>
</tbody>
</table>

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

Figure: Chromatogram for system suitability.

Table:-: Results of system suitability parameters for Dabrafenib and Trametinib.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Name</th>
<th>Retention time(min)</th>
<th>Area (µV sec)</th>
<th>Height (µV)</th>
<th>USP resolution</th>
<th>USP tailing</th>
<th>USP plate count</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dabrafenib</td>
<td>2.816</td>
<td>65358</td>
<td>4536</td>
<td>1.08</td>
<td>5689.6</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Trametinib</td>
<td>3.893</td>
<td>8658746</td>
<td>658985</td>
<td>5.69</td>
<td>1.42</td>
<td>6892.4</td>
</tr>
</tbody>
</table>

Fig:-: Chromatogram showing blank (mobile phase preparation).

METHOD VALIDATION PARAMETERS

Assay (Standard)

Fig:-: Chromatogram showing assay of standard injection-1.
Fig:- Chromatogram showing assay of standard injection-2.

Fig: Chromatogram showing assay of standard injection-3 Table:- Showing assay standard Results.

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

Assay
(Sample)

Fig: Chromatogram showing assay of sample injection -1.
Fig: Chromatogram showing assay of sample injection -2.

Fig: Chromatogram showing assay of sample injection -3 Table-: Showing assay sample results.

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

The % purity of Dabrafenib and Trametinib in pharmaceutical dosage form was found to be 99.68% and 99.46% respectively.

**PRECISION**
Table:- Results of method precision for Dabrafenib.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Name</th>
<th>Rt</th>
<th>Area</th>
<th>Height</th>
<th>USP plate count</th>
<th>USP Tailing</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dabrafenib</td>
<td>2.808</td>
<td>65898</td>
<td>4365</td>
<td>5682.2</td>
<td>1.08</td>
</tr>
<tr>
<td>2</td>
<td>Dabrafenib</td>
<td>2.808</td>
<td>65487</td>
<td>4375</td>
<td>5628.6</td>
<td>1.09</td>
</tr>
<tr>
<td>3</td>
<td>Dabrafenib</td>
<td>2.808</td>
<td>65324</td>
<td>4395</td>
<td>5649.7</td>
<td>1.08</td>
</tr>
<tr>
<td>4</td>
<td>Dabrafenib</td>
<td>2.808</td>
<td>65982</td>
<td>4328</td>
<td>5638.4</td>
<td>1.09</td>
</tr>
<tr>
<td>5</td>
<td>Dabrafenib</td>
<td>2.808</td>
<td>65248</td>
<td>4371</td>
<td>5698.3</td>
<td>1.08</td>
</tr>
<tr>
<td>6</td>
<td>Dabrafenib</td>
<td>2.808</td>
<td>65734</td>
<td>4391</td>
<td>5682.7</td>
<td>1.09</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>65612.17</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Std. Dev</td>
<td></td>
<td></td>
<td>304.8425</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% RSD</td>
<td></td>
<td></td>
<td>0.464613</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table: Results of method precision for Trametinib.

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

Accuracy: Accuracy Standard

Figure: Chromatogram for Accuracy std. Injection-1.

Figure: Chromatogram for Accuracy std. Injection-2.

Figure: Chromagram for Accuracy std. Injection-3.
Table:- Results of Accuracy standard values.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Name</th>
<th>Rt</th>
<th>Area</th>
<th>Height</th>
<th>USP Resolution</th>
<th>USP Tailing</th>
<th>USP plate count</th>
<th>Injection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dabrafenib</td>
<td>2.860</td>
<td>65359</td>
<td>4358</td>
<td>1.09</td>
<td>5698.5</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Trametinib</td>
<td>3.949</td>
<td>8659825</td>
<td>659862</td>
<td>5.68</td>
<td>1.42</td>
<td>6859.4</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>Dabrafenib</td>
<td>2.860</td>
<td>65874</td>
<td>4395</td>
<td>1.08</td>
<td>5672.4</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Trametinib</td>
<td>3.949</td>
<td>8659875</td>
<td>653485</td>
<td>5.68</td>
<td>1.43</td>
<td>6824.2</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>Dabrafenib</td>
<td>2.860</td>
<td>65398</td>
<td>4382</td>
<td>1.08</td>
<td>5683.1</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Trametinib</td>
<td>3.949</td>
<td>8674587</td>
<td>6587458</td>
<td>5.69</td>
<td>1.42</td>
<td>6875.6</td>
<td>3</td>
</tr>
</tbody>
</table>

Table:- Accuracy (recovery) data for Dabrafenib.

<table>
<thead>
<tr>
<th>% Concentration (at specification Level)</th>
<th>Area</th>
<th>Amount Added (mg)</th>
<th>Amount Found (mg)</th>
<th>% Recovery</th>
<th>Mean Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>50%</td>
<td>35921.67</td>
<td>30</td>
<td>30.134</td>
<td>100.446%</td>
<td>100.30%</td>
</tr>
<tr>
<td>100%</td>
<td>70894.33</td>
<td>60</td>
<td>60.205</td>
<td>100.341%</td>
<td></td>
</tr>
<tr>
<td>150%</td>
<td>105654.7</td>
<td>90</td>
<td>90.093</td>
<td>100.103%</td>
<td></td>
</tr>
</tbody>
</table>

Table:- Accuracy (recovery) data for Trametinib.

<table>
<thead>
<tr>
<th>% Concentration (at specification Level)</th>
<th>Area</th>
<th>Amount Added (mg)</th>
<th>Amount Found (mg)</th>
<th>% Recovery</th>
<th>Mean Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>50%</td>
<td>4276302</td>
<td>50</td>
<td>50.208</td>
<td>100.416%</td>
<td>100.21%</td>
</tr>
<tr>
<td>100%</td>
<td>8484717</td>
<td>100</td>
<td>100.148</td>
<td>100.148%</td>
<td></td>
</tr>
<tr>
<td>150%</td>
<td>10160609</td>
<td>150</td>
<td>150.091</td>
<td>100.060%</td>
<td></td>
</tr>
</tbody>
</table>

ACCURACY 50%

Figure: Chromatogram for sample concentration-50% Injection-1.
Figure: Chromatogram for sample concentration-50% Injection-2.

Figure: Chromatogram for Accuracy std. Injection-1 Figure: Chromatogram for sample concentration-50% Injection-3.

Table:-: Results of Accuracy sample 50% values.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Name</th>
<th>Rt</th>
<th>Area</th>
<th>Height</th>
<th>USP Resolution</th>
<th>USP Tailing</th>
<th>USP plate count</th>
<th>Injection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dabrafenib</td>
<td>2.816</td>
<td>35929</td>
<td>3896</td>
<td>0.98</td>
<td>4896.2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Trametinib</td>
<td>3.893</td>
<td>4274645</td>
<td>578452</td>
<td>5.08</td>
<td>1.28</td>
<td>6895.1</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>Dabrafenib</td>
<td>2.816</td>
<td>35989</td>
<td>3958</td>
<td>5.08</td>
<td>1.28</td>
<td>6826.7</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>Trametinib</td>
<td>3.893</td>
<td>4275698</td>
<td>586592</td>
<td>5.09</td>
<td>1.29</td>
<td>6826.7</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>Dabrafenib</td>
<td>2.816</td>
<td>35847</td>
<td>3874</td>
<td>0.99</td>
<td>4879.4</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Trametinib</td>
<td>3.893</td>
<td>4278563</td>
<td>586874</td>
<td>5.08</td>
<td>1.28</td>
<td>6895.3</td>
<td>3</td>
</tr>
</tbody>
</table>

Accuracy 100%

Figure: Chromatogram for sample concentration-100% Injection-1.
Figure: Chromatogram for sample concentration-100% Injection-2.

Figure:-: Chromatogram for sample concentration-100% Injection-3.

Table:-: Results of Accuracy sample 100% values.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Name</th>
<th>Rt</th>
<th>Area</th>
<th>Height</th>
<th>USP Resolution</th>
<th>USP Tailing</th>
<th>USP plate count</th>
<th>Injection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dabrafenib</td>
<td>2.860</td>
<td>70989</td>
<td>4485</td>
<td>1.09</td>
<td>5698.8</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Trametinib</td>
<td>3.949</td>
<td>8488468</td>
<td>659822</td>
<td>5.70</td>
<td>1.43</td>
<td>6985.4</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>Dabrafenib</td>
<td>2.860</td>
<td>70896</td>
<td>4398</td>
<td>1.10</td>
<td>5786.9</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Trametinib</td>
<td>3.949</td>
<td>8478696</td>
<td>658952</td>
<td>5.71</td>
<td>1.44</td>
<td>6975.4</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>Dabrafenib</td>
<td>2.860</td>
<td>70798</td>
<td>4458</td>
<td>1.09</td>
<td>5864.7</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Trametinib</td>
<td>3.949</td>
<td>8486987</td>
<td>658754</td>
<td>5.70</td>
<td>1.43</td>
<td>6898.9</td>
<td>3</td>
</tr>
</tbody>
</table>

Accuracy 150%

Figure: Chromatogram for sample concentration-150% Injection-1.
Figure: Chromatogram for sample concentration-150% Injection-2.

Figure: Chromatogram for sample concentration-150% Injection-3.

Table: Results of Accuracy sample 150% values.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Name</th>
<th>Rt</th>
<th>Area</th>
<th>Height</th>
<th>USP Resolution</th>
<th>USP Tailing</th>
<th>USP plate count</th>
<th>Injection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dabrafenib</td>
<td>2.824</td>
<td>105753</td>
<td>6528</td>
<td>1.24</td>
<td>6587.4</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Trametinib</td>
<td>3.914</td>
<td>12695265</td>
<td>752454</td>
<td>6.82</td>
<td>1.68</td>
<td>8695.3</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>Dabrafenib</td>
<td>2.824</td>
<td>105584</td>
<td>6584</td>
<td>1.25</td>
<td>6582.2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Trametinib</td>
<td>3.914</td>
<td>12689898</td>
<td>752658</td>
<td>6.83</td>
<td>1.69</td>
<td>8759.6</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>Dabrafenib</td>
<td>2.824</td>
<td>105627</td>
<td>6539</td>
<td>1.24</td>
<td>6538.6</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Trametinib</td>
<td>3.914</td>
<td>12694574</td>
<td>753689</td>
<td>6.82</td>
<td>1.68</td>
<td>8698.5</td>
<td>3</td>
</tr>
</tbody>
</table>

Linearity
Figure 6.3.4: Calibration graph for Dabrafenib Linearity Results: (for Dabrafenib).

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Linearity Level</th>
<th>Concentration (ppm)</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>I</td>
<td>20</td>
<td>24759</td>
</tr>
<tr>
<td>2</td>
<td>II</td>
<td>40</td>
<td>47859</td>
</tr>
<tr>
<td>3</td>
<td>III</td>
<td>60</td>
<td>70898</td>
</tr>
<tr>
<td>4</td>
<td>IV</td>
<td>80</td>
<td>93985</td>
</tr>
</tbody>
</table>

Figure 6.3.4: Calibration graph for Trametinib Linearity Results: (for Trametinib).

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Linearity Level</th>
<th>Concentration (ppm)</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>I</td>
<td>60</td>
<td>4928578</td>
</tr>
<tr>
<td>2</td>
<td>II</td>
<td>80</td>
<td>6687842</td>
</tr>
<tr>
<td>3</td>
<td>III</td>
<td>100</td>
<td>8389878</td>
</tr>
<tr>
<td>4</td>
<td>IV</td>
<td>120</td>
<td>10085847</td>
</tr>
<tr>
<td>5</td>
<td>V</td>
<td>140</td>
<td>11769854</td>
</tr>
</tbody>
</table>

Correlation Coefficient

Limit of Detection

Dabrafenib: Result: $= 0.97\mu g/ml$.

Trametinib: Result: $= 2.06\mu g/ml.$
Quantitation Limit
Dabrafenib: Result: =2.91µg/ml
Trametinib: Result: =6.18µg/ml

ROBUSTNESS
Variation in flow

Figure:- Chromatogram showing less flow of 0.9ml/min.

Figure:- Chromatogram showing more flow of 1.1ml/min.

Table:- System suitability results for Dabrafenib.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Flow Rate (ml/min)</th>
<th>System Suitability Results</th>
<th>USP Plate Count</th>
<th>USP Tailing</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.9</td>
<td></td>
<td>5784.6</td>
<td>1.06</td>
</tr>
<tr>
<td>2</td>
<td>1.0</td>
<td></td>
<td>5685.4</td>
<td>1.08</td>
</tr>
<tr>
<td>3</td>
<td>1.1</td>
<td></td>
<td>5869.5</td>
<td>1.09</td>
</tr>
</tbody>
</table>

Table:- System suitability results for Trametinib.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Flow Rate (ml/min)</th>
<th>System Suitability Results</th>
<th>USP Plate Count</th>
<th>USP Tailing</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.9</td>
<td></td>
<td>6698.3</td>
<td>1.46</td>
</tr>
<tr>
<td>2</td>
<td>1.0</td>
<td></td>
<td>6895.7</td>
<td>1.42</td>
</tr>
<tr>
<td>3</td>
<td>1.1</td>
<td></td>
<td>6983.6</td>
<td>1.49</td>
</tr>
</tbody>
</table>
Variation of mobile phase organic composition

Figure:- Chromatogram showing more organic composition.

Table:- System suitability results for Dabrafenib.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Change in Organic Composition in the Mobile Phase</th>
<th>System Suitability Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>USP Plate Count</td>
</tr>
<tr>
<td>1</td>
<td>10% less</td>
<td>5895.3</td>
</tr>
<tr>
<td>2</td>
<td>*Actual</td>
<td>5685.4</td>
</tr>
<tr>
<td>3</td>
<td>10% more</td>
<td>5964.2</td>
</tr>
</tbody>
</table>

Table:- System suitability results for Trametinib.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Change in Organic Composition in the Mobile Phase</th>
<th>System Suitability Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>USP Plate Count</td>
</tr>
<tr>
<td>1</td>
<td>10% less</td>
<td>6785.2</td>
</tr>
<tr>
<td>2</td>
<td>*Actual</td>
<td>6895.7</td>
</tr>
<tr>
<td>3</td>
<td>10% more</td>
<td>6982.4</td>
</tr>
</tbody>
</table>

CONCLUSION

The test is targeted to increase and validate HPLC strategies for estimation of Dabrafenib and Trametinib in bulk and pill dosage shape.
For habitual analytical purpose it's far applicable to installation strategies capable of reading large quantity of samples in a quick time period with well robustness, accuracy and precision with none in advance separation steps. HPLC technique generates big quantity of incredible records, which characteristic incredibly powerful and to be had analytical device.

The method expertise right reproducibility and suitable healing. From the specificity research, it has emerge as observed that the evolved techniques had been particular for Dabrafenib and Trametinib.

REFERENCES
5. P.D. Sethi. HPLC: Quantitative analysis pharmaceutical formulations, CBS publishers and groups, New Delhi (India), 2001; 3-137.
9. Approach validation suggestions international convention on Harmonization; GENEVA; 1996.


