Original Article

Development and Validation of Novel and Highly Sensitive Stability-Indicating Reverse Phase UPLC Method for Quantification of Dabrafenib and its ten Degradation Products

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ABSTRACT

Objectives: Dabrafenib is used as an active pharmaceutical ingredient acts as an inhibitor of the associated enzyme B-Raf, which plays a role in the regulation of cell growth. In the current study, we focused on developing a robust, highly sensitive and stability-indicating RP-UPLC method for estimation of DBR and its degradation products for the very first time. A stress study has been performed to demonstrate the stability-indicating capability of the method. **Materials and Methods:** Chromatographic separation was achieved using advanced UPLC technology with high resolution on Acquity BEH C-18 (100 mm × 2.1 mm, 1.8 μm) column using a mobile phase composed of orthophosphoric acid and methanol with very less consumption of solvents results in an eco-friendly method. Analysis was performed at 225 nm detector wavelength with 5 μL of injection volume and 0.3 mL min^{-1} of flow rate. **Results:** Forced degradation study was performed under hydrolytic (acid, alkali and neutral), oxidative, photolytic and thermal degradation as per ICH Q1 (R2) guidelines. The optimized method was observed to be linear in the concentration range of 12.5 to 125ng mL^{-1} with R^2 value of 1.0000. **Conclusion:** This is the first very sensitive stability-indicating UPLC method capable of separating dabrafenib and its ten degradation products at the nanogram (ng) level. The method was validated as stated by ICH guidelines.

Keywords: Dabrafenib, RP-UPLC, Validation, Stability-indicating, Forced degradation.

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INTRODUCTION

The development of oral targeted anticancer drugs has increased strongly in the past two decades and is expected to continue. Dabrafenib (Tafinlar), is BRAF (v-raf murine sarcoma viral oncogene homolog B1) inhibitor protein that is orally bio-available and is approved for the management of nonresectable stage III or stage IV metastatic melanoma that is responsible for mutation. BRAF, a serine/threonine-protein kinase is stimulated by somatic point mutations in human malignancy. This kinase is an important molecule of the RAS (rat sarcoma gene) that triggered the mitogen-activated protein (MAP) kinase/extracellular signal-regulated kinase (ERK) signaling pathway resulting in increased cell growth. Approximate 7% of all cancers,[1] including 60–70% of melanomas,[2] 12% of colorectal cancers[3] and 15% of papillary thyroid carcinomas contributed due to mutation in BRAF gene. To somewhat lesser extent patients with hairy cell leukemia,[4-5] papillary pharyngeal cancer[6-7] and 1.6–4.9% of lung cancer cells may be due to these BRAF mutations in the gene. After understanding the very crucial role of BRAF mutation in the case of

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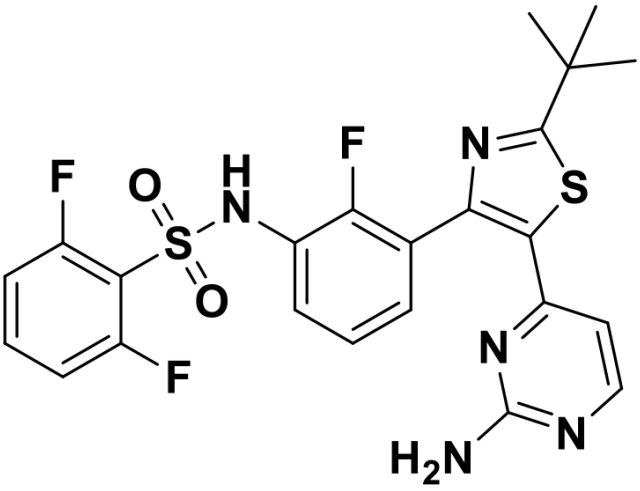

Figure 1: Chemical structure of Dabrafenib.

melanoma, more focus on the advanced development of an inhibitor of BRAF mutation to treat malignant melanoma. In this, Dabrafenib plays a vital role as it is a competitive inhibitor of BRAF kinase that is responsible for mutation in cell lines, Xenografts and kinase screening[8-9] as it is potent adenosine triphosphate (ATP). The structure of dabrafenib is given below in Figure 1.

For treatment of metastatic BRAFV600E-positive melanoma, dabrafenib is approved in Europe and USA. Dabrafenib drug has been approved by different regulatory agencies like USFDA (The US Food and Drug Administration) and EMA (European Medicines Agency) either alone or in combination with other anticancer drugs like trametinib for the treatment of metastatic melanomas.

Looking into the emergent applications of selective BRAF inhibitors, we decided to execute stress degradation studies of dabrafenib and to establish a highly sensitive and fast RP-UPLC method for the estimation of DBR and its ten degradation products.

An LC-MS method to measure dabrafenib and its six metabolites are described by Vikingsson *et al.* in human plasma.[10] Another LC-MS assay method of dabrafenib is reported on plasma samples of the mouse.[11] Few LC-MS recent methods on simultaneous quantification of dabrafenib along with other drugs like trametinib, vemurafenib, niraparib, etc. in human plasma are also reported.[12-15] There are various analytical methods available (HPLC as well as LC- MS) but no method discussed about ten degradation products and their separation in short run time.[16-26] Separation, identification and characterization of degradation products of few anticancer drugs using the UPLC-MSMS technique have been reported.[27-30]

HPLC is now an outdated technology in terms of sensitivity and cost-effectiveness. Till date, no study is reported on the degradation and stability of drugs under varied conditions such as hydrolytic, oxidative, thermal and photolytic. Herein we report (i) A comprehensive forced degradation study of dabrafenib under hydrolytic, oxidative, thermal and photolytic conditions as prescribed by ICH guidelines; (ii) to separate the degradation products by UPLC (ten degradation products were separated); (iii) validation of rapid, accurate, highly sensitive and economical RP- UPLC method as per ICH guidelines.

MATERIALS AND METHODS

Chemicals and Reagents

Dabrafenib pure sample (99.8%, B.N-BD10051016) and standard (99.7%, B.N- DBRN/A255/5A/09) were kindly provided by MSN laboratory limited, Hyderabad as a gift sample. Ultra-pure LC-MS grade acetonitrile (CH3CN) and methanol (CH3OH) were procured from JT baker (Bangalore, India). Sodium hydroxide (NaOH) pallets and formic acid (HCOOH) of Analytical Reagent (AR) grade were procured from Merck (Mumbai, India). Finar chemicals (Hyderabad, India) provided the analytical grade Hydrochloric acid (HCl, 37%). Hydrogen peroxide (H202, 30%) was procured from S.D Fine chemicals (New Delhi, India). ElIX-10 system (Merck Millipore) was used to collect the Milli-Q-water. The solutions used in the study were filtered through 0.22 μm filter paper (Millipore, India).

Equipment

The Waters ACQUITY H Class UPLC system coupled with Quaternary Solvent Manager (QSM), sample manager and photodiode array (PDA) detector was used. All weighing operations were carried out on analytical balance (Mettler Toledo, Switzerland). Other instruments used in the study were Photostability chamber (thermo lab scientific instruments, India), sonicator (Equitron, 5 L), hot air oven (Medline Scientific, India) and digital pH meter (Metrohm, India). Empower PRO 2.0 software was used for monitoring and integration of chromatographic peaks.

UPLC Chromatographic Conditions

Various parameters were taken into consideration while developing and optimizing the chromatographic separation conditions including mobile phase, stationary phase, flow rate and detector wavelength.

Table 1: Summary of optimization of solvent.

% Eluent A	% Eluent B	Observation
ammonium acetate (pH-2.5)	MeOH	Very broad peak with peak splitting
ammonium acetate (pH-2.5)	ACN	Peak splitting
Potassium dihydrogen phosphate (pH-2.5 with 10% OPA)	MeOH	Broad peak shape
Potassium dihydrogen phosphate (pH 2.5 with 10% OPA)	ACN	Separation not achieved
0.05% OPA	MeOH	Good peak shape with base t base separation
0.05% OPA	ACN	Peak shape was sharpbut separation not observed
0.05 % HCOOH in water	MeOH	Peak shape broadenings
0.05 % HCOOH in water	ACN	Less theoretical plates and peak fronting

Chromatographic separation was achieved using reverse-phase gradient mode of elution with ACQUITY BEH C_{18} (100 mm × 2.1 mm, 1.8µm) column using mobile phase that contains 0.05% *ortho*-phosphoric acid in water and methanol at a flow rate of 0.3 mL min^{-1}. The mobile phase was prepared freshly and filtered through 0.22 µm filter paper. A Photo-array detector (PDA) was used and 225 nm was set for detector wavelength with an injection volume of 5 µL. The optimized developed conditions of chromatography are shown in supplementary Table 1.

Diluent Preparation

An equal ratio of water and methanol was taken; mixed properly and used as diluent. All the sample preparation was sonicated and filtered through 0.22 µm filter paper before injection in UPLC.

Sample Solution

Main stock of 1000µg mL^{-1} was prepared with diluent. Ten milligrams of drug substance was transferred in a 10 mL volumetric flask and dissolved in the diluent. This solution was then diluted further in order to get the nominal concentration of 50µg mL^{-1}. This solution is further diluted to perform various parameters like LOD (Detection limit), QL (Quantitation limit), specificity, recovery, precision and linearity precision, linearity.

Specificity-Forced Degradation Studies

Stress studies were performed to understand the chemical stability, degradation pathways and DPs of drug substances and drug products. It engages exposure of drug substances under hydrolytic, photolytic, oxidative and thermal degradation conditions. These studies depict the specificity and stability-indicating capability of the developed method. Dabrafenib was exposed to a different types of stress conditions as stated by ICH recommended guidelines.[31-32] PDA detector was run to ensure peak purity of dabrafenib and its DPs produced during stress study. Stress studies were performed by initially preparing 1 mg mL^{-1} stock solution of a drug substance. Sample of acid hydrolysis was neutralized with sodium hydroxide and a sample of alkaline hydrolysis was neutralized with hydrochloric acid. Before performing analysis, these solutions were filtered through 0.22 µm filter paper.

Hydrolytic Degradation

Hydrolytic degradation covers a wide range of pH (acidic, basic and neutral). Functional groups that may undergo hydrolysis are esters, amides, alcohol, aryl amines, carbamates etc. The sample was exposed individually with 5 mL of 1M HCl, 5 mL of 1M NaOH and water maintained at 80°C for 24 hr. After the desired time, treated samples were allowed to attain room temperature and then neutralized with acid and base. Further diluted with diluent in order to achieve final concentration of 50µg mL^{-1}.

Oxidative Degradation

Though many oxidizing agents for instance metal ions and radical initiators (e.g., AIBN) can be taken to carry out degradation studies but hydrogen peroxide (H_2O_2) is most widely used to perform oxidation degradation. The sample was treated with hydrogen peroxide (10% H_2O_2) and kept at room temperature for 8 hr. The stressed sample was further diluted with diluent to achieve final concentration of 50µg mL^{-1}.

Degradation Due to Photolysis

Photolytic stability was carried out to check the influence of light when a drug substance is exposed to UV or fluorescent conditions. Functional groups like aryl chloride, nitro aromatic, N- oxide, carbonyl and sulfides are likely to introduce drug photosensitivity. An adequate amount of drug was kept in Petri-dish and treated with 1.2 million lx h of fluorescent light and 200 W h/m^2 of UV light. After the desired time-period, the sample was taken out from the chamber to prepare a final concentration of 50µg mL^{-1} with diluent.

Degradation Due to Thermal Conditions

Thermal degradation studies were carried as per ICH Q1A guidelines suggested at accelerated conditions. Arrhenius equation is used to study the outcome of temperature upon thermal degradation of a drug. The equation is as under:

$$k = Ae - Ea\ RT^{-1}$$

where A is frequency factor, k is specific reaction rate, R is gas constant (1.987 cal deg mole^{-1}), Ea is energy of activation and T is absolute temperature.

An adequate amount of drug substance was kept in Petri dish and treated to dry oven at temperature 105°C for 24 hr. After the desired time-period, the sample was taken out to prepare a final concentration of 50µg mL^{-1} with diluent.

Chromatographic Analysis of Forced Degraded Samples

After degradation, every stressed sample was diluted to get the final nominal concentration of 50µg/mL. Degradation of every condition was checked by comparing the purity of every stressed condition with that of control sample (unstressed condition). The % degradation was calculated as Percentage drug degraded=

$$\% \text{ Drug degraded} = \frac{\text{Area under unstressed sample} - \text{Area under stressed sample}}{\text{area under unstressed sample}} \times 100$$

Method validation

Method validation was carried out as per ICH recommended Q2 (R1) guidelines.[33] Various parameters like selectivity/specificity, accuracy/recovery, linearity, precision; DL, QL, Ruggedness/Robustness and stability of analyte in solution were performed for the method validation. All the validation parameter and their acceptance criteria as stated by ICH guidelines are shown in Supplementary Table 2.

System Suitability

System suitability parameter is a very crucial part of all analytical methods. A standard solution of dabrafenib at a concentration of 75ng mL^{-1} was injected six times. System suitability was tested by calculating the parameter like USP tailing factor, % RSD and theoretical plate count. The % RSD of the area of DBR peak from six replicate injections was calculated.

Specificity and Selectivity

The capability of analytical procedure to determine the response of pure drug substances in the existence of potential impurities and DPs formed during stress study is called specificity. The specificity was examined by determining mixtures of the acidic, basic and oxidative degradation products with known concentrations of dabrafenib. Specificity parameter was performed by establishing the desired USP resolution between the dabrafenib peak and nearest DP peak formed during the degradation study and establish the resolution of all DPs from each other. PDA detector was used to check the peak purity of dabrafenib peak and DPs peak to check the method selectivity.

Table 2: Summary of optimization of stationary phase.

Stationary phase	Dimension	Observation
CSH C_{18} (waters)	(100 × 2.1) mm, 1.7 µ	Peak fronting observed
HSS T3 (waters)	(100 × 2.1) mm, 1.7 µ	Peak tailing observed
BEH Shield (waters)	(100 × 2.1) mm, 1.7 µ	Peak eluted early
CSH Phenyl hexyl (waters)	(100 × 2.1) mm, 1.7 µ	Peak eluted early
Eclipse plus C_{18}, RRHD (agilent)	(50 × 2.1) mm, 1.8 µ	Peak eluted before 1 min
BEH C_{18} (waters)	(100 × 2.1) mm, 1.7 µ	Satisfactory peak shape observed

Linearity

The calibration curve was obtained by plotting the response of dabrafenib against their respective concentration. Seven different concentrations were prepared by serial dilution and used to obtained the calibration curve data ranging from 12.50-125ng mL^{-1} keeping the injection volume same throughout the study. The correlation coefficient (R^2), slope and y-intercept of dabrafenib were calculated.

Precision

System precision was evaluated in terms of inter-day precision (reproducibility) and intra-day (repeatability). The precision study was performed by injecting different three concentrations (25, 75 and 125ng mL^{-1}) in triplicate in RP-UPLC on the same day and the next day. The value of standard deviation (SD) and % RSD was assessed for reproducibility and repeatability.

Accuracy/Recovery

The accuracy of an analytical method is the closeness of test results obtained by that method compared with the true values. Method accuracy was assessed by performing recovery studies for the pure drug by standard addition process. A known concentration of

drug substance (75ng mL^{-1}) was added to three different concentration levels (QL, 100 and 150 %). Control sample and recovery samples were injected in triplicate and % recovery was calculated at each level.

Limits of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ capability determine the sensitivity of the method. The calibration curve method was used to establish LOD and LOQ. Series of the dilute solution having known concentration were injected to determine the LOD and LOQ values. Signal to noise ratio was calculated for LOD and LOQ. % RSD of the peak area was calculated at the QL concentration level.

$$\text{LOD} = \frac{3 \times \text{standard deviation of } y - \text{intercept}}{\text{slope of calibration curve}}$$

$$\text{LOQ} = \frac{10 \times \text{standard deviation of } y - \text{intercept}}{\text{slope of calibration curve}}$$

Robustness

The measurement of the capacity of the analytical procedure to remain unaffected by small but deliberate variations in developed experimental conditions is called robustness. This parameter provides an indication that the developed method is robust and rugged. Different variables like column temperature, flow rate and initial mobile phase composition were evaluated in the robustness study. Specificity solution containing dabrafenib drug substance and its DPs was injected in actual condition as well as in all variable condition to check the robustness of the method. Effect on system suitability parameters like USP resolution between closely related compounds and USP tailing were observed in the initial condition and each deliberate variation.

Solution Stability

Solution stability was established at room temperature. These solutions were injected serially at respective time intervals and the stability of these solutions was evaluated for 24 hr. The comparison of these solutions was evaluated against the freshly prepared sample solution.

RESULTS AND DISCUSSION

Method Development

Green Analytical Chemistry Approach: In RP-UPLC Method Development

Different solvents are harmful in terms of eco-friendly. Acetonitrile and isopropyl are among those solvents that could harm the environment. To develop a green UPLC method, Methanol was used throughout the analysis which enhances the eco-friendly property of the method. We have developed a very eco-friendly method by using methanol as an organic solvent throughout the study. Another factor that contributes towards the greenness of the method is its run time. Since the developed method is the UPLC method and having a very short run time of 15 min to separate out the ten DPs with DBR peak, therefore consumption of solvent is very less due to short run time and flow rate is also less as compared to convention HPLC method.

Development and Optimization of UPLC Chromatographic Conditions

The main objective of stability-indicating method development was to achieve the separation and quantification of dabrafenib and its impurities (process-related or degradation impurities). The absorbance maximum of the drug substance and its DPs is 225 nm. Therefore, the detection was carried out at 225 nm. Depending upon the physicochemical properties, solubility, molecular weight and structure of dabrafenib, different systematic trials such as selection of stationary phase, buffer selection, organic modifier and different pH conditions were taken for the development of robust, linear, precise and accurate UPLC method for estimation of dabrafenib.

UPLC Column Selection

Various UPLC columns (stationary phase) were tried for separation of dabrafenib peak and its DPs such as AQUITY HSS C_{18} (2.1 mm × 100 mm, 1.8 µm), AQUITY BEH C_{18} (2.1 mm × 100 mm, 1.7 µm), AQUITY CSH C_{18} (2.1 mm × 100 mm, 1.7 µm), AQUITY BEH phenyl (2.1 mm × 100 mm, 1.7 µm) and AQUITY CSH phenyl hexyl (2.1 mm × 100 mm, 1.7 µm). Based on various parameters like USP resolution between closely related compounds (DP-III and DP-IV), USP tailing and theoretical plate count, AQUITY BEH C_{18} (2.1 mm × 100 mm, 1.8 µm) was selected as a suitable column. Summary of optimization of stationary phase has shown in Table 1.

pH and Selection of Buffer

Various buffers like formate, phosphate, acetate, ammonia covering pH ranging from 2 to 10 were tried based on the pKa value. It was observed that in basic pH 8 to 10, DPs were merged using different buffers and in neutral pH, peak shape was distorted. Best separation was observed at acidic pH (0.05% OPA in water). The peak shape of dabrafenib and its all- degradation products

was also very sharp. Therefore, *ortho*-phosphoric acid was selected as a buffer for analysis.

Organic Modifier Selection

Both methanol and acetonitrile were evaluated as organic solvents for the mobile phase. The study was observed better resolution and peak shapes with methanol when compared with acetonitrile. Therefore, methanol was utilized as an organic modifier for the analysis (enhance greenness of method). The composition of the mobile phase was optimized to get a better peak shape and decrease the retention time (Rt) of the drug. The summary of optimization of solvent has shown in Table 2.

Optimization of Chromatographic Conditions

Various gradient trials were taken to achieve the best peak shape and separation of all DPs with dabrafenib in a very short run time. The detection was carried out at 225 nm wavelength based on absorbance maxima of dabrafenib. Different column temperature and flow rate trials were also taken to get desired peak shape and resolution. The optimized column temperature was 45°C with a flow rate of 0.3 mL min^{-1} to achieve good peak shape and separation between closely eluted peaks. The final optimized UPLC method using the typical retention times of DP-I, DP-II, DP-III, DP- IV, DP-V, DP-VI, DBR, DP-VII, DP-VIII, DP-IX and DP-X were about 4.18, 4.99, 5.41, 8.27, 8.69, 9.05, 9.47, 10.65, 11.09, 11.45 and 11.71 min respectively. The optimized method was capable of separating all DPs with dabrafenib and for estimation of dabrafenib at a very low level. Overlay chromatograms of Dabrafenib (50µg mL^{-1}) and its ten degradation products under different stressed conditions have shown in Figure 2.

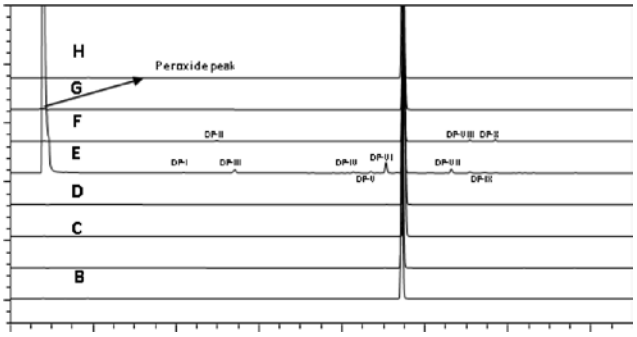

Figure 2: Overlay chromatograms of DBR (50 µg mL^{-1}), untreated sample (A), neutral sample (B), acidic sample (C), basic sample(D), oxidative sample(E), photolytic sample (F), UV sample (G) and thermal sample (H) degradations.

Method Validation

System Suitability

The average theoretical plate count of six injections was 86870 and the average USP tailing of six injections was 1.0 (<2.0) and % RSD of the area of dabrafenib peak in six replicate injection of system suitability solution was 0.30 (<5.0). The method was suitable for use as all the parameters were within the limit. The outcome of system suitability parameters was summarized in Supplementary Table 3.

Linearity

Linearity of the method was performed by determining standard solutions at seven different concentration levels covering the range of 12.5 to 125ng mL^{-1}. Slope, coefficient of correlation (r2) and intercept for dabrafenib were 107.0722, 1.0000, and 99.1730 respectively. Linearity data are shown in Table 3. The calibration curve of dabrafenib is shown in supplementary Figure 1.

Precision

The % RSD values were 0.07%-0.64% and 0.33-1.17%, respectively for inter-day and intra- day precision. Results of Intra and inter-day precision of dabrafenib are presented in Table 4.

Table 3: Linearity Data for dabrafenib

Concentration (ng mL^{-1})	Peak Area	Slope (m)	Intercept Value (b)	Correlation coefficient (R^2)
12.5	1440	107.0722	99.1730	1.0000
25	2771			
50	5498			
60	6500			
75	8100			
100	10800			
125	13500			

Table 4: Intra-day and Inter-day precision study of developed method of dabrafenib.

Sample No	Concentration (ng mL^{-1})	Intra-day Precision		Inter-day Precision	
		Mean±SD	RSD (%)	Mean±SD	RSD (%)
1	25	2788±17.93	0.64	2703±31.51	1.17
2	75	8208±6.08	0.07	8313±27.54	0.33
3	125	13287±11.59	0.09	13341±52.20	0.39

[a] Mean of three replicate

Table 5: Recovery data of Dabrafenib (*n*=3).

Spiked Concentration (ng/mL)	Found Concentration (ng/mL, Mean[a] ± SD)	RSD (%)	Recovery (%)
25 (QL)	25.33±52.69	0.50	101.3
75 (100%)	77.49±42.50	0.20	103.3
125 (150%)	131.11±30.30	0.10	104.9

[a] Mean of three replicate

Table 6: Robustness data of Dabrafenib and its degradation products.

Parameter	Value	Level	tRc (min)	Rs	Tc	USP plate count
	0.27	-0.03	9.94	2.4	1.0	96547
Flow rate (mL/min)	0.30	0	9.47	2.5	1.0	88840
	0.33	+0.03	9.03	2.6	1.0	80600
Column temperature (°C)	40	-5	9.60	2.4	1.0	87490
	45	0	9.47	2.5	1.0	88840
	50	+5	9.35	2.5	1.0	93352
% Eluent B initial composition	28	-3	9.74	2.3	1.0	107514
	30	0	9.47	2.5	1.0	88840
	32	+3	9.15	2.7	1.0	71182
Buffer Concentration (%)	0.04	-0.01	9.54	2.4	1.0	86790
	0.05	0	9.47	2.5	1.0	88840
	0.06	+0.01	9.40	2.6	1.0	87760

R_S = resolution between closely related degradation products (DP-IX and DP-X)
T_C = tailing factor of dabrafenib peak
[c] R_t = retention time of dabrafenib peak

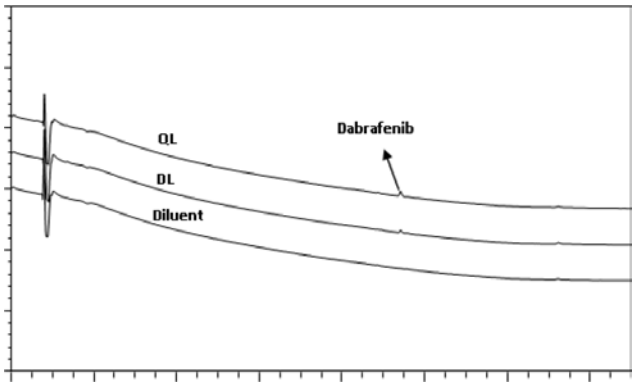

Figure 3: Overlaid chromatogram of Diluent and QL solution.

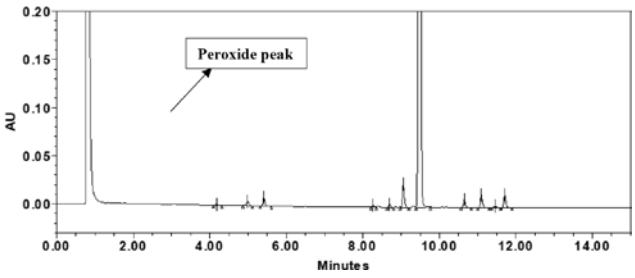

Figure 4: Specificity chromatogram of dabrafenib and ten degradation products.

Table 7: Solution stability of dabrafenib.

% Assay	Initial	6 hrs.	12 hrs.	18 hrs.	24 hrs.
Solution stability	99.5	100.1	99.6	99.8	99.4
Mobile phase stability	99.9	100.2`	99.5	99.4	99.6

Accuracy/Recovery

The %recovery was obtained to be in the range of 101.3% - 104.9%. The recovery results showed that the developed method is precise and also observed that there was no intervention due to the existence of DPs. The results are shown in Table 5.

Robustness

A robustness study was performed by creating small, deliberate variations in the flow rate (±0.03 mL min^{-1}), temperature conditions (±5°C) and change in initial buffer composition in gradient and found to be unaffected upon these deliberate variations in parameters. Results are shown in Table 6.

LOD and LOQ

LOD and LOQ of drug substances were obtained to be 12.5ng mL^{-1} and 25ng mL^{-1}, respectively. Signal to noise ratio for LOD was more than 3 and for LOQ was more than 10. Results of LOD and LOQ are given in Supplementary Table 5 and Table 6 respectively. Overlay chromatogram of diluent, DL and QL has shown in Figure 3.

Solution Stability and Mobile phase Stability

Percent RSD of dabrafenib was found less than 1.0 % and there was no major change observed in peak area indicates solution and mobile phase stability up to 24 hr. Results are tabulated in Table 7.

Forced Degradation Studies

Specificity and Selectivity

In optimized UPLC methods, all DPs formed were well separated with drug substance indicates that the method is specific. A photo array detector (PDA) was used to ensure peak purity. The purity angle of each DP including dabrafenib was less than the purity threshold. There was no interference of any other peak of DP, impurity, or matrix observed which ensure the selectivity

of the method. Assay study of stressed samples was performed to perform the mass balance study. Assay results were more than 98.0 % in every stressed condition. Specificity chromatogram of dabrafenib and its ten DPs are given in Figure 4. Method specificity and peak purity data are given in supplementary Table 7. Diluent/Blank chromatogram has shown in Figure 5.

Degradation in Neutral Condition

A neutral degradation study was carried out at different time intervals such as 0 min, 1 hr, 4 hr and 8 hr. At each interval, before injection, the sample was filtered through 0.22 µm filter. The drug substance was found to stable in neutral degradation conditions. The results are given in Table 8 and Figure 6.

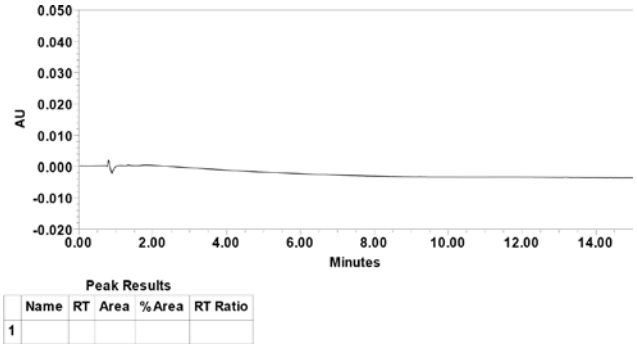

Figure 5: Blank/Diluent chromatogram.

Degradation in Acidic Condition

Acid degradation study was carried out at different time intervals such as 0 min, 1 hr, 4 hr and 8 hr. At each interval, the stressed sample was neutralized with 1M NaOH and filtered through 0.22 µm filter before analysis. There was no significant degradation observed the results are given in Table 8 and Figure 7.

Degradation in Basic Condition

Alkaline degradation study was carried out at different time intervals such as 0 min, 1 hr, 4 hr and 8 hr. At each interval, the stressed sample was neutralized with 1M HCl and filtered through 0.22 µm filter before analysis. There was no signification degradation observed The results are given in Table 8 and Figure 8.

Oxidative Degradation

Oxidative degradation study was carried out at different time intervals such as 0 min, 1 hr, 4 hr and 8 hr. At

Table 8: Optimized stress degradation conditions for dabrafenib.

Stress Condition	Concentration of stressor	Exposed conditions	Duration	Degradation products formed	% of DPs	Assay of dabrafenib (%)	aMass Balance
Neutral degradation	H_2O	80°C	8 hr	No DPs	ND	99.47	99.62
Acid degradation	1N HCl	80°C	24 hr	No DPs	ND	99.65	99.80
Basic degradation	N NaOH	80°C	24 hr	No DPs	ND	99.57	99.72
Oxidative degradation	10% H_2O_2	RT	8 hr	All DPs except DP-II	12.92	86.78	99.70
Photolytic degradation	Solid state	40°C, 75% RH	3 days	DP-II, VIII & X	2.94	96.78	99.72
UV degradation	Solid state	40°C, 75% RH	3 days	No DPs	ND	99.37	99.52
Thermal degradation	Solid state	105°C	24 h	No DPs	ND	99.45	99.60

[a] Mass balance =% Assay +% Sum of all impurities +% Sum of all degradants
RT: Room temperature, RH: Relative humidity, ND: Not detected

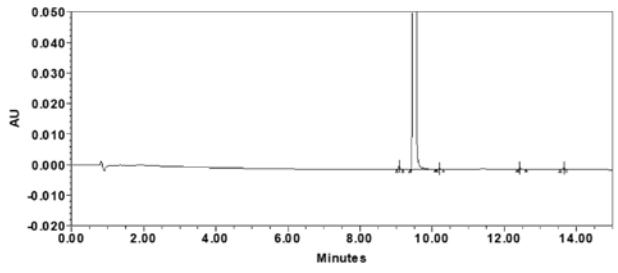

Figure 6: Chromatogram of Neutral degradation.

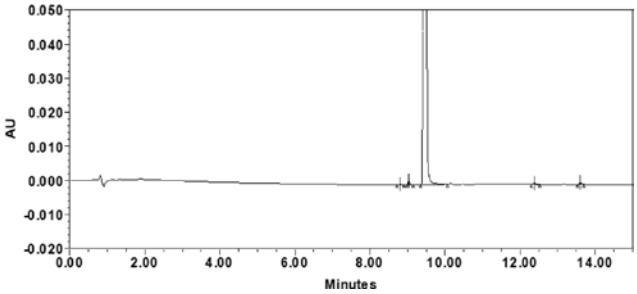

Figure 7: Chromatogram of Acidic degradation.

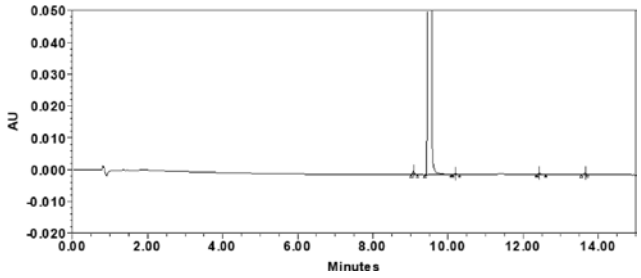

Figure 8: Chromatogram of Basic degradation.

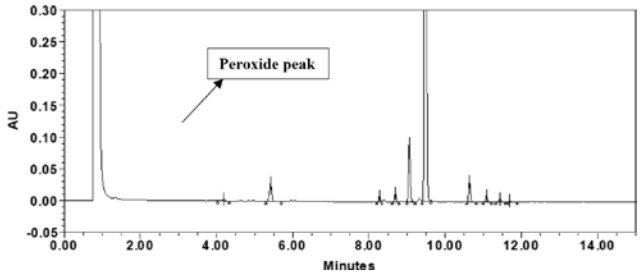

Figure 9: Chromatogram of Oxidative degradation.

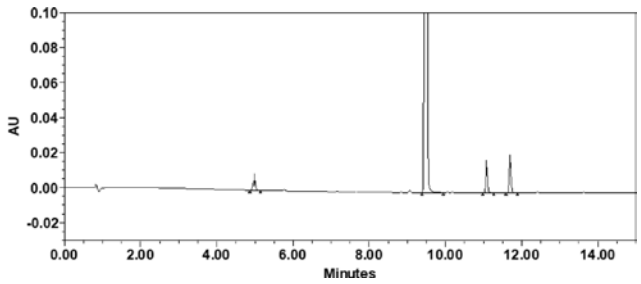

Figure 10: Chromatogram of Photolytic degradation.

each interval, the stressed sample was filtered through 0.22 µm filter before analysis. Nine major DP's (Rt 4.19, 5.42, 8.27, 8.69, 9.06, 10.63, 11.08, 11.44 and 11.70 min) of 12.92% were formed during oxidative degradation. The results are shown in Table 8 and Figure 9.

Photolytic Degradation

Dabrafenib drug substance was obtained to the stable when exposed to photostability chamber (1.2 million lux h) and UV light. Three major DP's (Rt 4.99, 11.08 and 11.69 min) of 2.94% were formed during photolytic degradation. The results are given in Table 8 and Figure 10.

Thermal Degradation

Thermal degradation parameter was carried out by keeping 100 mg sample in duplicate in Petri dish and sealed. One Petri dish exposed to the dry oven at a temperature of 105°C for 24 hr and the other kept as control. After desired time period (24 hr), the stressed sample and control sample was diluted with diluent to produce a final concentration of 50µg/mL and filtered with filter paper before injection. Dabrafenib was found to be stable under thermal (105°C for 24 hr) stress conditions. The results are given in Table 8 and Figure 11.

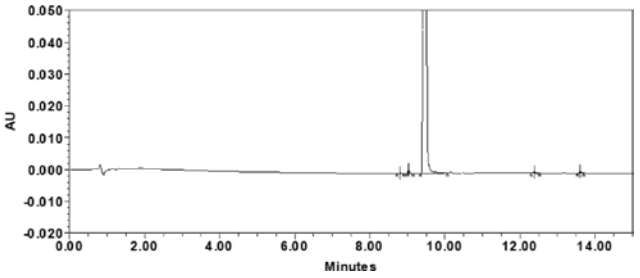

Figure 11: Chromatogram of Thermal degradation.

CONCLUSION

A novel, linear, accurate, specific, reliable and specific stability-indicating UPLC method was developed and fully validated for the determination of dabrafenib in the presence of its DPs. It is first reported highly sensitive UPLC method capable of separating dabrafenib and its ten DPs up to nanogram level.

The drug under investigation was found to be more sensitive to peroxide degradation conditions as it degraded by 12.92% when exposed to hydrogen peroxide. The drug was also found to be sensitive in photolytic degradation conditions as it degraded to 2.94% in photo-degradation conditions. Dabrafenib is found to be stable when exposed to hydrolytic (acidic, basic and neutral) and thermal degradation conditions as there was no degradation observed under these conditions. Validation of the RP-UPLC method as per the ICH guidelines demonstrates that the method is highly sensitive, linear, rapid, robust and stability-indicating. Therefore, work may be useful for estimation of dabrafenib in bulk and pharmaceutical dosage form and also very useful in further identification and characterization of different DPs when exposed to stress conditions.

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MSN Laboratory, Hyderabad for proving us with a pure drug as a gift sample to carry out the research.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

ABBREVIATIONS

°C: Degree Celsius; **ACN:** Acetonitrile; **BEH:** Ethylene Bridged Hybrid; **BRAF:** v-raf murine sarcoma viral oncogene homolog B1; **DBR:** Dabrafenib; **g:** Gram; **HPLC:** High Performance Liquid Chromatography; **h:** hour; **LC:** Liquid chromatography; **min:** Minute; **mL:** Milliliter; **NaOH:** Sodium hydroxide; **RP:** Reverse-Phase; **RT:** Retention time; **UPLC:** Ultra Performance Liquid Chromatography; **UV:** Ultra Violet.

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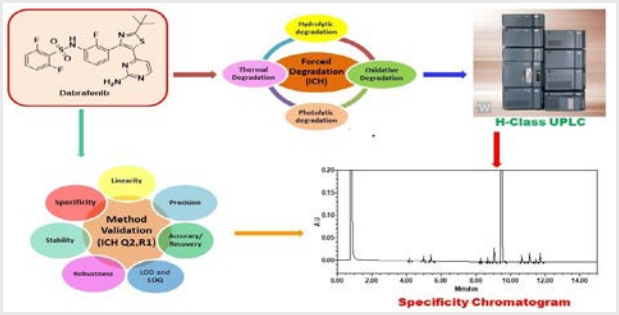
PICTORIAL ABSTRACT



SUMMARY

The research work in the paper presents the novel stability-indicating UPLC method was for the estimation of dabrafenib in the presence of its ten DPs. The mobile phase used for separation of drug substance and its DPs was orthophosphoric acid and methanol with flow rate of 0.3 mL min-1. The retention time for dabrafenib was found to be at 9.47 minutes. The regression at linearity 12.5 to 125ng mL-1 was $r^2 = 1.0000$. The method was validated as per ICH guidelines. LOD and LOQ of drug substances were obtained to be 12.5ng mL-1 and 25ng mL-1, respectively. The forced degradation studies under different conditions obtained and the results shows that the degradation is observed in oxidative and photolytic condition whereas drug substance was found to be stable when exposed to hydrolytic (acidic, basic and neutral) and thermal degradation conditions. It is first reported highly sensitive UPLC method capable of separating dabrafenib and its ten DPs up to nanogram level.

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Dr. Parul Grover has completed her Ph.D from Punjabi University, Patiala. She is presently serving as Assistant Professor in KIET School of Pharmacy, KIET Group of Institutions, Ghaziabad, Uttar Pradesh. She has more than 10 years of experience in teaching and research. She is positive thinker, scientific and self-determined research professional. She has more than 45 publications in journals of high repute, out of which 25 are SCI/Scopus indexed. She has presented and attended various research papers at national and international conferences. She has published 3 books and 5 book chapters. She has published 8 patents. She received Young Scientist Award-2014 for best research paper publication by Biotechnology Society of India and has been honoured with prestigious C V Raman Award in recognition of Best Researcher at Institute level in 2021. She has an ongoing consultancy project on preformulation and docking studies. She is Co-PI in AICTE RPS project. She is lifetime member of APTI. Her areas of interest include Pharmaceutical Analysis, Natural Product Chemistry, Carbohydrate Chemistry and Biocatalysis.

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