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Development and Validation of a Novel RP-HPLC Method for Quantitative Estimation of Dabrafenib in Bulk Drug and Pharmaceutical Dosage Forms

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ABSTRACT: The measurement of Dabrafenib in bulk and pharmaceutical dose form was achieved using a reverse phase high-performance liquid chromatographic approach that was quick, straightforward, accurate, and specific. A mobile phase consisting of Methanol: Phosphate Buffer (35:65%) v/v was used to execute the separation on a Symmetry ODS C18 (4.6x250mm, 5µm) column. We used a flow rate of 1 mL/min and a UV detection wavelength of 235 nm. Room temperature was maintained for the column. The run length was under 8 minutes at these chromatographic conditions. A duration of 2.257minutes was determined for Dabrafenib. Across the concentration range of 6-14 µg mL-1, the calibration curve remained linear. Both the quantification and detection limits were 3.6 ng mL−1 and 1.2 ng mL−1, respectively. A commercial product's recovery percentage ranged from 98% to 102%, while the average test percentage was 99.86%. Results from the investigation of accuracy indicated a relative standard deviation of less than 2%. Dabrafenib in bulk and pharmaceutical dosage forms that are commercially available may be determined using the novel method, which is user-friendly, accurate, quick, and specific.

Keywords: Dabrafenib, RP-HPLC, Validation, Technique Development, and ICH Guidelines are the main terms here.

INTRODUCTION

Method development:

Preparation of Regular solution:

Transfer 10 milligrams of Dabrafenib, the usual operating procedure, to a dry, clean 10-milliliter volumetric flask. Add around seven milliliters of methanol, sonicate to eliminate any air, and then fill the rest of the way up with the same methanol.

Fill a 10-milliliter volumetric flask with 0.1 milliliters of the Dabrafenib stock solutions mentioned above. The precise quantity of methanol should be added.

Steps to take:

You need to change the chromatographic parameters, add the samples, record the chromatograms, and note the conditions for optimum peak release in order to meet the validation criteria required by ICH.

Improving the Efficiency of the Mobile Phase:

To begin, the mobile phase consisted of varying concentrations of methanol and a mixture of methanol and water. Lastly, a methanol and phosphate buffer combination of 35:65% v/v was used to modify the mobile phase.

Column Optimization: C18 columns, Xterra columns, and the X-bridge column were all used in the optimization process. Peak form and clarity were found to be good at a flow rate of 1 ml/min using Symmetry ODS C18 (4.6 x 250 mm, 5 m).

Equipped With A pH of 3.6, Potassium Dihydrogen Phosphate (KH2PO4) Was Used to Create a Buffer:

It is recommended to dissolve 6.8043 gof potassium dihydrogen phosphate in 1000 ml of HPLC water. After that, use diluted orthophosphoric acid to bring the pH down to 3.6. Ultrasonication and suction filtration may be used to clean and sonicate the combination.

Setting Up the Mobile Portion:

Utilizing a digital ultrasonicate, 350 ml of 35% methanol and 650 ml of 65% phosphate buffer were subjected to air expulsion. The next step was to apply pressure and filter the mixture through a 0.45-inch filter.

Preparation of Diluting agent:

There was a diluting phase known as the mobile phase.

Technique Authentication

Test Parameters for System Suitability

We used high-performance liquid chromatography to measure the area of five separate injections of the reference solution. It was found that the % RSD for the area of five repeated injections was within the allowable range.

Specificity:

Following three injections of both the standard and sample solutions, the assay was computed using the following formula:

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

Coverage and Size (Linearity):

Calculate the chromatographic system 'speak area after the addition of each level.

Create a graph showing the peak areas as a function of both concentration and X-and Y-axis coordinates. Finding the correlation coefficient is the next step.

Results and Discussion:

Optimized Chromatogram (Standard):

Mobile phase ratio : Methanol: Phosphate Buffer (35:65) V/V

Column : Symmetry ODS C18(4.6×250mm, 5µm)

Column temperature: Ambient

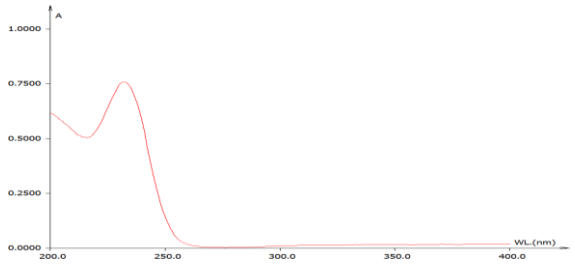
Wavelength : 235nm

Flowrate : 1ml/min

Injection volume : 10µl

Run time : 8min

UV-Spectrophotometer Investigation:



UV Spectrum for Dabrafenib (235nm)

Outcomes of System Suitability for Dabrafenib:

System Suitability:

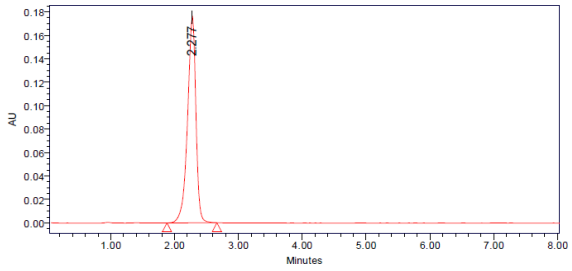


Fig. 1: Chromatogram of system suitability

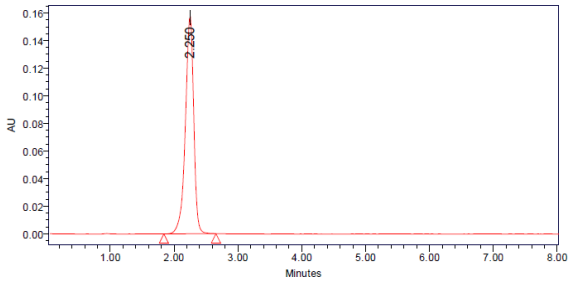
Table 1: Outcomes of System Suitability for Dabrafenib

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Dabrafenib	2.277	1652847	185647	6589	1.24
2	Dabrafenib	2.277	1653658	186254	6587	1.26
3	Dabrafenib	2.267	1654521	185475	6584	1.28
4	Dabrafenib	2.265	1653564	186594	6582	1.29
5	Dabrafenib	2.277	1658745		6895	1.24
Mean			1654667			
Std. Dev.			2355.764			
% RSD			0.142371			

LINEARITY

Fig.2 : Chromatogram of Linearity

Table 2:



Statistics for Linearity of Dabrafenib

Concentration µg/ml	Average Peak Area
6	1078475
8	1461129
10	1808358
12	2211573
14	2593778

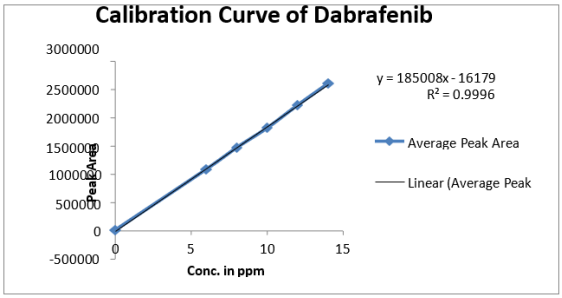


Fig. 3: Linearity Curvature of Dabrafenib

Precision: Repeatability:

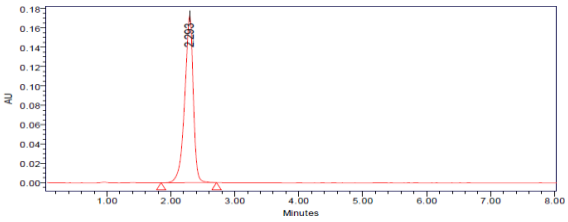


Fig.4: Chromatogram of Precision

Table 3: Results of Repeatability:

Results of repeatability for Dabrafenib:

S. No	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Dabrafenib	2.293	1658954	186958	1.26	6785
2	Dabrafenib	2.276	1658745	187548	1.27	6854
3	Dabrafenib	2.286	1659865	189854	1.26	6852
4	Dabrafenib	2.277	1653254	186985	1.25	6784
5	Dabrafenib	2.280	1654781	189542	1.24	6895
6	Dabrafenib	2.293	1661324	187586	1.28	6965
Mean			1657821			
Std.dev			3120.433			
%RSD			0.188225			

Accuracy:

50% of Accuracy:

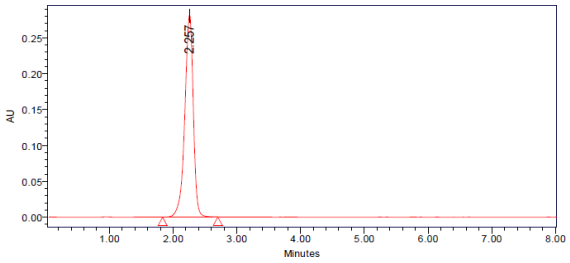


Fig. 5: Chromatogram of 50% accuracy

Table 4: Accuracy-50% of Injection

Results of Accuracy for Concentration-50%

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Dabrafenib	2.257	108982	92715	0.99	4695	1
2	Dabrafenib	2.253	108659	92548	1.02	4658	2
3	Dabrafenib	2.258	109564	92685	1.00	4785	3

Table 5: Results of overall accuracy:

The Accuracy Results for Dabrafenib

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	109068.3	5	5.021	100.420%	100.72%
100%	202187	10	10.054	100.540%	
150%	297032.3	15	15.181	101.206%	

LOD and LOQ: The new analytical approach shown high sensitivity with the Detection Limitation (LOD) of 0.95 µg/mL and Quantification Limit (LOQ) of 2.90 µg/Ml

Table 6: Results of Stability studies:

Results of Forced Degradation Studies for Dabrafenib

S.No.	Stress Condition	Peak Area	% of Degraded Amount	% of Active Amount	Total % of Amount
1	Standard	1658242	0	100%	100%
2	Acidic	1331734.15	19.69	80.31	100%
3	Basic	1594233.85	3.86	96.14	100%
4	Oxidative	1394747.34	15.89	84.11	100%
5	Thermal	1575827.37	4.97	95.03	100%
6	Photolytic	1345331.73	18.87	81.13	100%
7	Water	1360090.08	17.98	82.02	100%

SUMMARY:

Dabrafenib in medical tablet and bulk pharmaceutical forms was effectively isolated using an RP-HPLC technique that is

easy to use, fast, accurate, exact, and shows stability indicators. We used a Symmetry ODS C18 column (4.6 × 250 mm,5 µm), a Methanol: Phosphate Buffer (35:65, v/v), a flow rate of 1.0 mL/min, and UV detection at 235 nm in order to get the optimal outcomes. To ensure system appropriateness, the approach produced a sharp, symmetrical peak in these cases. The suggested approach was established to be effective based on the following ICH criteria: system applicability; specificity; linearity; precision; accuracy; robustness; and limit of detection (LOD) and limit of quantification (LOQ). The approach performed admirably within the specified concentration range, as shown by a correlation value of r = 0.999. Levels of %RSD below 2% were shown by the precision tests. The analytical approach was determined to be accurate, reliable, and repeatable with recovery rates ranging from 98% to 102%. Due to its high sensitivity, the novel approach has a LOD of 0.95 µg/mL and a LOQ of 2.90 µg/mL. Because of this, it was possible to detect and quantify even trace levels of Dabrafenib. The method's robust performance in the face of intentionally tweaked chromatographic parameters indicates its suitability for routine analysis.

CONCLUSION:

For the purpose of quantifying Dabrafenib in therapeutic dosage forms and bulk medications, the proposed RP-HPLC technique is easy, sensitive, accurate, precise, robust, and stable. All validation parameters for the technique were found to meet the acceptance requirements set forth by the ICH guidelines, indicating that it is stable and good enough for frequent quality control and test analysis. Under acidic, alkaline, oxidative, thermal, photolytic, and water stress settings, forced degradation experiments validated the method's stability-indicating nature. There was no interference and the medicine was completely isolated from its byproducts. This means that the new RP-HPLC technique is a great tool for studying the stability of Dabrafenib, checking its quality, and determining its concentration in pharmacological formulations.

REFERENCES:

[1]. Henry RA. The early days of HPLC at Dupont. Chromatography Online. Avanstar Communications Inc.; 2009.

[2]. Karger BL, Berry LV. Rapid liquid-chromatographic separation of steroids on columns heavily loaded with stationary phase. Clinical Chemistry. 1971;17(8):757-64.

[3]. Giddings JC. Dynamics of Chromatography, Part I. PrinciplesandTheory.NewYork:MarcelDekker,Inc.; 1965;281.

[4]. Ettre C. Milestones in chromatography: The birth of partition chromatography. LCGC. 2016;19(5):506-12.

[5]. Martin AJP, Synge RLM. Separation of the higher monoamino-acids by counter-current liquid-liquid extraction: the amino-acid composition of wool. Biochemical Journal. 1941;35(1-2):91-121.

[6]. Lindsay S, Kealey D. High-Performance Liquid Chromatography. Wiley. In: Hung LB, Parcher JF, Shores JC, Ward EH. Theoretical and experimental foundation for surface-coverage programming in gas-solid chromatography with an adsorbable carrier gas. Journal of the American Chemical Society. 1987;110(11):1090-6.

[7]. Journal of Pharmaceutical and Biomedical Analysis. 1999 Nov 1;21(2):371-82.

[8]. Tropical Journal of Pharmaceutical Research. 2009;8(5):449-54.

[9]. SankarR.InstrumentalMethodofAnalysis. 3rded.New Delhi: Pharmaceutical Press; 2017;18(3):18-6.

[10]. Snyder LR. Practical HPLC Method Development. 2nd ed. New York: Wiley-Inter science; 1997;503.

[11]. U.S. Department of Health and Human Services FDA. Guidance for Industry: Analytical Procedure and Method Validation. Rockville: FDA; 2000.

[12]. ICH. Validation of Analytical Procedures: Methodology. ICH Harmonized Tripartite Guideline Q2B; 1996.

[13]. Sharma BK. Instrumental Methods of Chemical Analysis. Meerut: Goel Publishing House. 1997;75(8):113-5.

[14]. Ewing GW. Instrumental Methods of Chemical Analysis. 5th ed. New York: McGraw-Hill; 1985;1.

[15]. Higuchi T, Brochmann-Hanffen E, Hanssen H. Pharmaceutical Analysis. 1st ed. New York: Wiley; 1961;1-10.

[16]. Beckett AH, Stenlake JB. Practical Pharmaceutical Chemistry. 4th ed. Vol. 2. London: Athlone Press; 1988;275-98.

[17]. Inforum. Quality Assurance: Worth the Effort. 2003;7(4).

[18]. Sethi PD. Quantitative Analysis of Drugs in Pharmaceutical Formulation. 3rd ed. New Delhi: CBS Publishers. 1997;1(21):51-6.

[19]. ICH. Text on Validation of Analytical Procedures. ICH Harmonized Tripartite Guidelines; 1994.

[20]. ICH. Validation of Analytical Procedures: Methodology. ICH Guidelines Q2B. 1996;11(CPMP/ICH/281/95).

[21]. Gupta V, Rajput S, Sharma A, Khare P. Development and validation of HPLC method-A review. International Research Journal of Pharmaceutical and Applied Sciences. 2012;2(4):17-25.

[22]. Bhardwaj SK, Dwivedi J, Mehta A, Kurmi M. A review: HPLC method development and validation. International Journal of Analytical and Bioanalytical Chemistry. 2015;5(3):1-10.