Research Article

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A Comprehensive New Approach to Method Development and Validation of Encorafenib Using UV Spectroscopy in Bulk and Pharmaceutical Formulation

Anusha GANDI[1]*
ORCID: 0000-0002-0474-546X
Pavan Kumar DUDI[1]
ORCID: 0009-0000-7256-4540
Eswar Sandeep PANTALA[1]
ORCID: 0009-0005-4998-6493
Varaprasada Rao KOLLABATHULA[1]
ORCID: 0000-0003-2967-1169
Srinivasa Rao YARGUNTLA[1]
ORCID: 0000-0002-8803-9668

[1]Department of Pharmaceutical Analysis, Vignan Institute of Pharmaceutical Technology, Besides VSEZ, Kapujaggraju peta, Duvvada, Visakhapatnam, Andhra Pradesh 530049, India

Corresponding author:
Anusha GANDI
Department of Pharmaceutical Analysis, Vignan Institute of Pharmaceutical Technology, Beside VSEZ, Kapujaggraju peta, Duvvada, Visakhapatnam, Andhra Pradesh 530049, India

E-mail: gandianusha11@gmail.com
Tel: + 917330809694

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ABSTRACT

Encorafenib is a class of anti-cancer medication used to treat cancer infections. It is a selective BRAF inhibitor, has emerged as a promising therapeutic agent in the treatment of metastatic melanoma. This research endeavors to introduce a fresh approach and authenticate a UV spectrophotometric technique for examining encorafenib in pharmaceutical formulations. A unique, precise, and cost-effective UV spectrophotometric procedure has been devised using a blend of phosphate buffer and acetonitrile 90:10 v/v. Various standard solutions ranging from 10 to 70 µg/ml of the drug were evaluated for their absorbance at 235nm against a blank solution. The method exhibited a linear dynamic range of 10.0–70.0 µg/ml with an impressive correlation coefficient (R^2=0.9996) and a regression equation of y =0.0102x-0.0049. Intra- and inter-precisions displayed relative standard deviations (RSD) below 2.0%. The limits of detection (LOD) and quantification (LOQ) were determined as 0.159 µg/ml and 0.483 µg/ml respectively. All validation parameters adhered to the stipulated limits outlined in ICH guidelines. This approach has the potential to be a valuable asset for regular quality assessment in pharmaceutical labs contributing to the assurance of safety and efficacy in medications containing Encorafenib.

Keywords: Encorafenib, UV Spectrophotometric method, Phosphate Buffer, Acetonitrile, Method validation

1. Introduction

Encorafenib was approved by FDA in 2020, sold under the brand name Braftovi, is a type of oral medication known as a small molecule BRAF inhibitor.It is a neoplastic agent used in the treatment of certain types of cancer in adults[1]. Encorafenib inhibits the expression and signalling of the BRAF V600E gene mutation in addition to the normal (wild-type) BRAF and CRAF genes. The BRAF gene encodes kinases known as BRAF enzymes, which stimulate cell growth and proliferation. BRAF mutations, on the other hand, cause unchecked cell growth and proliferation. Encorafenib suppressed BRAF V600E, D, and K mutations in laboratory experiments [2]. The median time to reach maximum concentration (Tmax) of encorafenib is 2 hours, with at least 86% of the dose being absorbed. The apparent volume of distribution has a geometric mean of 164 L, with a coefficient of variation of 70%. Encorafenib exhibits 86% protein binding in vitro and the blood-to-plasma concentration ratio is measured at 0.58. The main metabolic pathway for encorafenib involves CYP3A4 (83%), with CYP2C19 (16%) and CYP2D6 (1%) playing minor roles in its metabolism. Chemically it is "Methyl N-[(2S)-1-({4-[3-(5-chloro-2-fluoro-3-methanesulfonamidophenyl)-1-(propan-2-yl)-1H-pyrazol-4-yl] pyrimidin -2-yl}amino)propan-2-yl] carbamate" with molecular formula $C_{22}H_{27}ClFN_7O_4S$. It is soluble in DMSO,DMF, Ethanol.The structure of drug is shown in Figure 1. No documented UV-based method currently exists for this specific drug. This method is time efficient compare with LCMS/MS and UPLC.This technique introduces a novel approach for analyzing encorafenib in both its bulk form and in pharmaceutical formulations. Other methods documented involve the simultaneous estimation of encorafenib alongside other drugs through the utilization of hyphenated and analytical techniques like LCMS/MS [3-6] and UPLC[7].

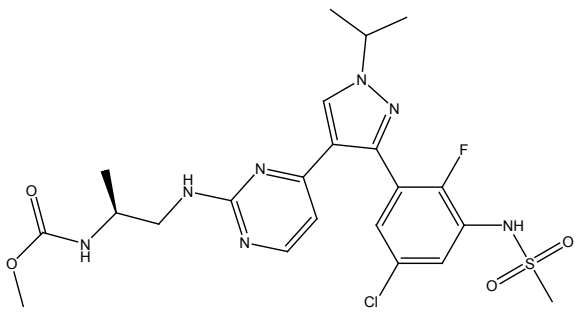

Figure 1. Structure of Encorafenib

2. Material and Methods

2.1. Drug and chemicals

Encorafenib, sourced as a gift sample with a purity of 99.67%, was generously provided by Aurbindo Pharma Pvt. Ltd., Hyderabad. Braftovi capsules each containing 75 mg of Encorafenib were procured from Indian mart. The chemicals Disodium Hydrogen phosphate, Potassium dihydrogen phosphate, acetonitrile, and glacial acetic acid with a purity range of 99.5-100.5% were obtained from Qualigens.

2.2. Instruments used

A Lab India double beam UV spectrophotometer (model T60) paired with M.Wave Professional software is employed for UV measurements, while a Bruker Alpha Compact FT-IR spectrometer is utilized for recording IR spectra. Sample weights are determined using a high-precision balance and an ultrasonicator ensures thorough mixing of solutions. Additionally, a pH meter is used to accurately adjust the pH of buffer solutions.

2.3. Method Development

Solubility testing for encorafenib was conducted using various polar solvents, including water, methanol, ethanol, acetonitrile, methanol-water mixtures, acetonitrile-methanol mixtures, and phosphate buffer-acetonitrile mixtures [8]. Following these studies, a phosphate buffer and acetonitrile mixture in a ration of 90:10 v/v was identified as the most suitable solvent. Utilizing the solubility data and physical characteristics of the drug, a standard stock solution of encorafenib was prepared and λ max was determined to be 235nm was shown in Figure 2.

2.3.1. Characterization of Encorafenib

IR spectroscopy were conducted as per standard procedure.IR spectra of pure encorafenib were noted from 4000 to 400cm^{-1} with Bruker Alpha FTIR. Sample is mixed with 100times its weight of powdered potassium bromide (KBR). This finely ground mixture is the pressed under high pressure in minipress to form a pellet and it is placed in path of the beam of IR spectrometer.

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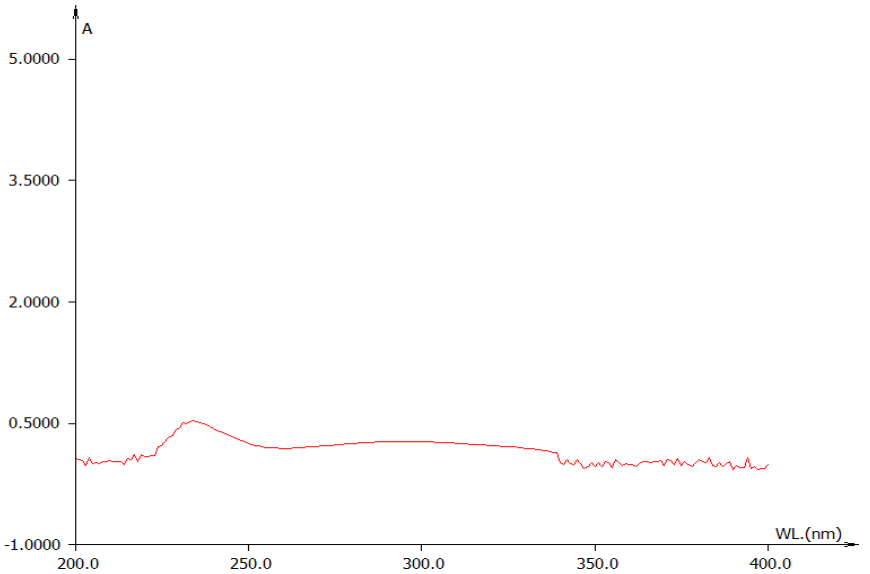

Figure 2. Absorption maxima of Encorafenib at 235nm

2.3.2. Preparation of Reagents

Preparation of Phosphate buffer pH 4.0 (Mixed)

Weigh accurately di-sodium hydrogen phosphate and 1.26g and potassium di -hydrogen phosphate 0.75g in a 250 ml graduated flask. The pH was modified to 4.0 using glacial acetic acid.

Preparation of blank

Add buffer solution to the acetonitrile with a ratio (90:10) v/v and sonicate for 5 minutes

Preparation of stock standard solution

Precisely measure 10 mg of Encorafenib and dissolve it in a 10 ml graduated flask using a phosphate buffer and acetonitrile mixture in a ratio of 90:10 v/v until reaching the mark resulting in a concentration of 1000 µg/ml.

Preparation of working standard solution

Transfer 1 ml of the previously prepared stock standard solution and dilute it with phosphate buffer and acetonitrile (90:10) v/v in a 10 ml graduated flask to achieve a concentration of 100 µg/ml.

Preparation of sample solution

Encorafenib capsules with brand name Braftovi (75mg). Ten capsules were weighed, removed capsule shell, powdered, and an amount equivalent to 100mg of Encorafenib was accurately weighed and dispense into a 100 ml volumetric flask containing phosphate buffer and acetonitrile (90:10) v/v. The mixture was then sonicated for 15 minutes with vigorous sonic and the volume was adjusted to 100mL with phosphate buffer and acetonitrile (90:10) v/v. The resulting solution was filtered through Whatmann filter paper #44. The filtrate was appropriately diluted with phosphate buffer and acetonitrile (90:10) v/v to obtain a final solution with a concentration of 1000µg/ml. Subsequently, 1ml of this stock standard solution was diluted with phosphate buffer and acetonitrile (90:10) v/v in a 10ml volumetric flask to achieve a concentration of 100µg/ml. The diluted solution was then analyzed using a Double beam UV-VIS spectrophotometer with phosphate buffer and acetonitrile (90:10) v/v serving as the blank in the UV range of 200-400 nm.

2.4. Method validation parameters

2.4.1. Linearity

Working standard solutions of encorafenib ranging from 10 to 70µg/ml, n=1 for each concentration prepared from a 100µg/ml stock solution were trans-

ferred into ten millilitre volumetric flasks and topped up to the mark with phosphate buffer and acetonitrile (90:10) v/v. The lamba max of these solutions at 235nm was measured against a blank[9,10].

Preparation of Linearity solutions from 10µg/ml to 70µg/ml

From the working standard solution, aliquots of 1.0ml, 2.0ml, 3.0ml, 4.0ml, 5.0ml, 6.0ml, and 7.0ml were individually transferred into 10ml volumetric flasks. These aliquots were then diluted with phosphate buffer and acetonitrile (90:10) v/v to achieve concentrations of 10, 20, 30, 40, 50, 60, and 70µg/ml, respectively [11,12].

2.4.2. Accuracy

To assess the accuracy of the method, n=3 for each recovery level, solutions of varying concentrations (i.e., 80%, 100%, 120%) were prepared while maintaining a constant amount of marketed formulation Encorafenib (40 µg/ml), with the amount of pure drug being adjusted (32, 40, 48 µg/ml, respectively) [13].

Preparation for 80% accuracy

Add 4.0ml of working standard solution (100µg/ml) and 3.2ml of the sample solution into a 10ml graduated flask, then brim up to the label with phosphate buffer & acetonitrile (90:10) v/v.

Preparation for 100% accuracy

Transfer 4.0ml of working standard solution and 4.0ml of the sample solution into a 10ml graduated flask, then brim up to the label with phosphate buffer & acetonitrile (90:10) v/v.

Preparation for 120% accuracy

Add 4.0ml of working standard solution and 4.8ml of the sample solution into a 10ml graduated flask, then brim up to the label with phosphate buffer & acetonitrile (90:10) v/v.

2.4.3. Precision

In the intra-day and inter-day variation studies, six different solutions(n=6) of the same concentration (40µg/ml) were prepared and analyzed three times a day (morning, afternoon, and evening) [14,15].

Preparation of 40µg/ml concentration

Transfer 4.0ml of working standard solution into a 10ml graduated flask and dilute it with phosphate buffer and acetonitrile (90:10) v/v up to the mark.

2.4.4. Robustness

It was assessed by analyzing the sample at two different wavelengths, and the corresponding absorbance values were recorded [16,17].

2.4.5. Ruggedness

This method was evaluated by analyzing the sample with the involvement of two different analysts [18].

Preparation of 40µg/ml concentration:

Transfer 4.0ml of the working standard solution into a 10ml graduated flask and dilute it with a mixture of phosphate buffer and acetonitrile (90:10) v/v until reaching the mark.

3. Results and Discussion

3.1. Linearity

It was observed that the absorbance values exhibited linearity within the concentration range of 10-70µg/ml. A calibration curve was constructed by plotting concentration against absorbance, as depicted in Figure 3. The resulting curve displayed linearity across the concentration range of 10 to 70µg/ml. Detailed results are presented in Table 1.

3.2. Accuracy

These solutions were primed in table and the accuracy was determined by calculating the percentage recovery as shown in Table 2.

3.3. Precision

To assess precision, both intra-day and inter-day variation studies were conducted.For the intra-day study, (40µg/ml) were prepared and analyzed six times within day (morning,afternoon,evening).For the inter-day variation study, solutions of identical concentration (40µg/ml) were prepared and analyzed six times over three consecutive days (1^{st},2^{nd} ,3^{rd} day) with absorbance recorded each time in Table 3. The percentage of relative standard deviation (%RSD) was then calculated and reported in Tables 4 and 5.

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Table 1. Results of linearity

S.No.	Concentration (µg/ml)	Absorbance
1	10	0.0999
2	20	0.1972
3	30	0.2964
4	40	0.3971
5	50	0.5036
6	60	0.6149
7	70	0.7123
Slope	0.0102	
Intercept	0.0049	
Correlation coefficient(R^2)	0.9996	

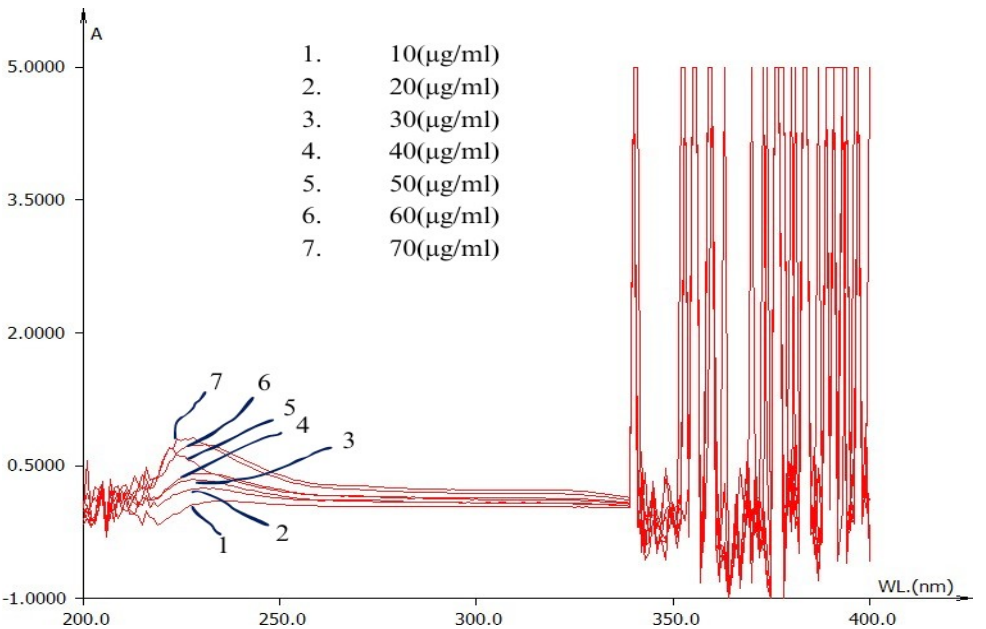

Figure 3. Linearity overlap spectrum of Encorafenib

3.4. Robustness

These findings are presented in Table 6.

3.5. Ruggedness

The absorbance values recorded by each analyst are presented in Table 7.

3.6. Limit of Detection (LOD) and Limit of Quantification (LOQ)

The Limit of detection (LOD) and limit of quantification (LOQ) was determined according to the ICH guidelines for the validation of analytical procedure. The formulae used were:LOD = 3.3σ/S, LOQ = 10σ/S.Where, σ = standard deviation of the response

Table 2. Results of accuracy data

Accuracy	Concentration of standard (µg/ml)	Concentration of marketed formulation (µg/ml)	Amount Found	%Recovery	Statistical Parameters
80%	40	32	31.8	99.375%	Mean= 99.63 SD=0.194 Relative error value =0.1947
	40	32	31.9	99.68%	
	40	32	31.95	99.84%	
100%	40	40	39.8	99.5%	Mean= 99.69 SD=0.1532 Relative error value =0.1536
	40	40	39.91	99.7%	
	40	40	39.95	99.87%	
120%	40	48	47.2	98.3%	Mean= 98.93 SD=0.532 Relative error value =0.5377
	40	48	47.5	98.9%	
	40	48	47.85	99.6%	

Table 3. Results of precision

Concentration (µg/ml)	S.No.	Absorbance
40 (µg/ml)	1	0.3315
	2	0.3310
	3	0.3300
	4	0.3315
	5	0.3315
	6	0.333
Statistical Analysis	Mean	0.33141667
	SD	0.0009704
	%RSD	0.29

Table 4. Results of Intra-day study

Concentration (µg/ml)	%RSD			Avg. %RSD
	morning	afternoon	evening	
40	0.9258	0.9288	0.913	0.9225

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Table 5. Results of Inter-day study

Concentration (µg/ml)	%RSD			Avg. %RSD
	1st Day	2nd Day	3rd Day	
40	0.9234	0.9423	0.918	0.9279

Table 6. Results of robustness data

Concentration(µg/ml)	Absorbance		
	Change in wavelength(nm)		
	234	235	236
40 (µg/ml)	0.3738	0.3938	0.3991
	0.3753	0.3943	0.3995
	0.3743	0.3953	0.3992
	0.3738	0.3956	0.4015
	0.3743	0.3965	0.4010
	0.3756	0.3970	0.3995
Mean	0.3745166	0.39541	0.39996
SD	0.0007626	0.00123	0.00101
%RSD	0.203	0.311	0.25

Table 7. Results of ruggedness data

Concentration(µg/ml)	Absorbance			
	Analyst 1		Analyst 2	
40	0.3936		0.3932	
40	0.3941		0.3946	
40	0.3939		0.3933	
40	0.3944		0.3956	
40	0.3952		0.3997	
40	0.3560		0.3902	
Statistical Analysis	Mean	0.3945333	Mean	0.394433
	SD	0.000903	SD	0.003158
	%RSD	0.228	%RSD	0.8

(intercept),S = slope of the calibration curve. The results are mentioned in Table 8.

3.7. Assay of Encorafenib

The assay is conducted according to the procedure as mentioned in preparation the sample solution. The absorbance of the solution was measured at 235nm against a blank. The percentage assay was determined to be 99.33%. Details of these results are presented in Table 9.

3.8. IR Spectroscopy

The pure Encorafenib spectra indicated intensive bands 3685cm^{-1} N-H streching amines, 3387.72 cm^{-1} N-H streching primary and secondary amines and amides, 1730.05 cm^{-1} C=O streching α,β unsaturated esters, 1700.77 cm^{-1} carboxylic acids C=O streching,1584.16 cm^{-1} C-C streching aromatic,1534 cm^{-1} N-O asymmetric streching nitro compound,1329.96 cm^{-1} C-N streching aromatic amines,1157.64 cm^{-1} C-H alkylhalides at ideal wavenumber is shown in Figure 4.

The method was developed and validated following the guidelines set by ICH. Validation was conducted for various parameters including linearity, precision, accuracy, robustness, ruggedness, LOD, and LOQ. Beer's law was found to be applicable across the concentration range of 10-70 µg/ml, as determined by regression analysis yielding a linear equation of y=0.0102x-0.0049 with a high correlation coefficient (R^2=0.9996). Precision results indicated %RSD values below 2% at each level, signifying precision at all tested concentrations. Accuracy was assessed through recovery studies, yielding values within the

Table 8. Results of LOD and LOQ

Limit of detection (µg/ml)	Limit of Quantification (µg/ml)
0.159	0.483

Table 9. Assay results of Encorafenib

Brand Name	Drug name	Label Claim (mg)	Amount found (mg)	% Recovery
BRAFTOVI	Encorafenib	75mg	74.5mg	99.333%

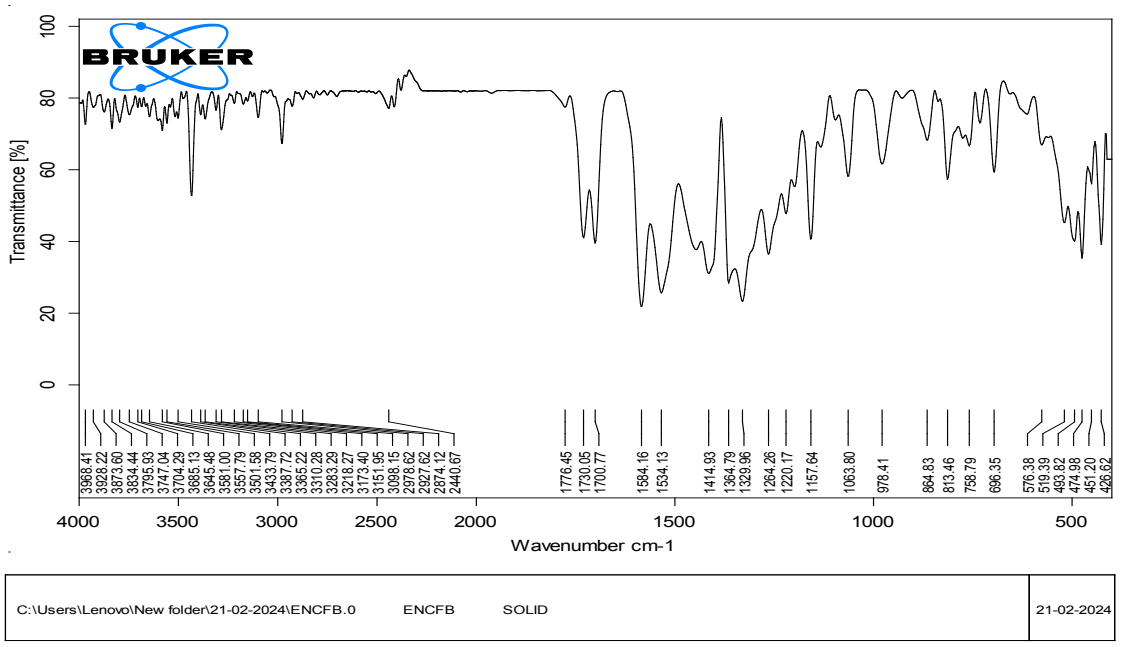

Figure 4. IR spectrum of Encorafenib

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acceptable range of 98-102%. Robustness and ruggedness assessments indicated heightened sensitivity of the method. Additionally, no interference was observed from the formulation excipients, indicating specificity in the determination of Encorafenib in tablet formulations. The content of Encorafenib in tablets closely matched the label amount. %RSD values across all parameters remained within the acceptable limit of <2%. The advantages of this developed method over reported works, With no observed interference from excipients, the method demonstrates a high degree of specificity which is essential for reliable results in quality control settings. This level of precision is particularly advantageous in ensuring product uniformity and quality during routine analysis. LOD and LOQ is particularly advantageous in stability studies where detecting degradation products at low levels is crucial. Currently, no UV-based analytical method has been reported for Encorafenib. This technique offers an innovative approach for quantifying Encorafenib in both bulk material and in pharmaceutical formulations.

4. Conclusion

In the current scenario, there's been a noticeable rise in the prevalence of diseases. However, before a drug can be introduced to the market, it must undergo various procedures. Validation and analytical methods play crucial roles in ensuring the purity and reliability of the drug. The comprehensive approach to method development and validation outlined in this study offers a robust analytical method for quantifying Encorafenib using UV spectroscopy with a phosphate buffer and acetonitrile (90:10) v/v mobile phase. This method holds promise as a valuable tool for routine quality control analysis in pharmaceutical laboratories, thereby ensuring the safety and efficacy of Encorafenib-based medications. The analytical procedure has been validated in accordance with ICH criteria. Ultra-Violet spectroscopy is employed for the estimation of Encorafenib, capitalizing on its maximum absorbance at 235 nm within the wavelength range of 200 to 400 nm. The method adheres to Beer's law within the concentration range of 10 to 70 µg/ml of Encorafenib. All validation parameters were found to meet the allowable limits set by ICH guidelines, suggesting its suitability for routine quality control purposes.

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Conflict of Interest

The authors have no conflicts of interest regarding this investigation.

Statement of Contribution of Researchers

Concept – A.G.; Design – P.K.D.; Materials –A.G.; Data Collection and/or Processing – V.R.K,SRY.; Literature Search –P.K.D,E.S.P; Writing – A.G.

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