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RP - HPLC Method Development & Validation for the Simultaneous Estimation of Encorafenib and Binimetinib in API & Tablet Dosage Form

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Abstract: ***A new RP-HPLC method was developed, validated and adapted for the estimation of encorafenib and binimetinib in bulk and tablet formulation. In this method, separation and assay of encorafenib and binimetinib was done in stationary phase using Agilent C18 column with mobile phase of 0.1M dipotassium hydrogen phosphate (pH 4.0) and methanol in 50:50 vol/vol ratio. The Binimetinib was eluted at 3.448 min and encorafenib at 5.795 min. Linearity ranges are 7.5-22.5 µg/ml and 37.5-112.50 µg/ml with regression coefficient values of 0.9996 and 0.9997 for binimetinib and encorafenib respectively. The LOD values found were binimetinib – 0.017 µg/ml and encorafenib – 0.114 µg/ml, and the LOQ values of binimetinib – 0.058 µg/ml and encorafenib – 0.381 µg/ml. Validation parameters examined following suggestions of ICH are accurate ample for the supposed assay. The approach is confirmed as splendid method for assay of encorafenib and binimetinib in tablet formula with excellent assay percentage values.***

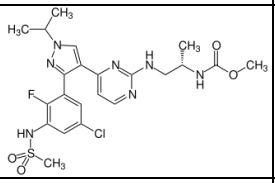
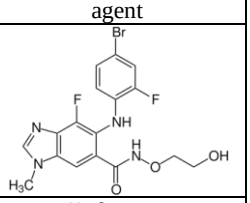
Keywords: RP-HPLC, Encorafenib, Binimetinib, Antineoplastic drugs

1. Introduction

Encorafenib and Binimetanib belong to the class of antineoplastic agents. They are used to treat Melanoma. It is a type of skin tumour that is produced in cells producing pigments, melanocytes mutate and turn out cancerous. BRAF is a constituent of the mitogen-activated protein kinase (MAPK) signaling pathway, which serves to activate downstream MEK, and is one of the most commonly mutated oncogenes in human tumors. Indeed, BRAF V600 mutations are present in approximately 40% of metastatic melanoma tumors. Encorafenib and binimetinib are small-molecule inhibitors of BRAF and MEK, respectively. BRAF and MEK inhibitors have been shown to improve overall and progression-free survival among patients with metastatic melanoma. Of these inhibitors, encorafenib and binimetinib are the newest combination. Proteins in pathways work together to do specific jobs within the cell. Some of the other proteins in this pathway include RAS, MAP2K1 and ERK. It passes signals from outside the cell to the cell's nucleus. The nucleus is the control center of the cell. These signals may tell the cell to grow, divide, or die. These are all normal cell functions. The body turns the signals on and off as needed. The V600E mutation is in the part of BRAF that passes along the cell growth signal. In cells with this mutation, BRAF can always turn on MAP2K1. This keeps the growth pathway on. Cells with this mutation can grow out of control, which can lead to cancer. BRAF inhibitors work well. But, after a while, your cancer cells may stop responding to these drugs. So, your doctor may also prescribe a MAP2K1 inhibitor drug. MAP2K1 inhibitors keep MAP2K1 from turning on ERK. Trametinib, selumetinib, and cobimetinib are common MAP2K1 inhibitors that may work well in cancer cells with BRAF mutations.

The Food and Drug Administration approved encorafenib and binimetinib in combination for patients with unresectable or metastatic melanoma with a BRAF V600E or V600K mutation on June 27th, 2018. The recommended dose of binimetinib is 45 mg orally twice daily and of encorafenib is 450 mg orally once daily. Approval by FDA was based on a randomized, active-controlled, open-label, multicenter trial in 577 patients with BRAF V600E or V600K mutation-positive or metastatic melanoma.Treatment continued until disease progression or unacceptable toxicity. Taking both a BRAF and a MAP2K1 inhibitor helps make sure this growth pathway is off. The drugs inhibit the growth of cells with this mutation and may lead to cell death. Combination treatment with a BRAF inhibitor and MEK inhibitor is the standard of care for patients with advanced $BRAF^{V600}$ mutation-positive melanoma.

Table 1: Profile of Encorafenib and Binimetinib

Overview of Drug	ENCORAFENIB	BINIMETINIB
Group	BRAF inhibitor, antineoplastic agent	Anticancer agent, MEK inhibitor, Antineoplastic agent
Structure		
IUPAC term	Methyl[(2S)-1-{[4-(3-{5-chloro-2-fluoro-3-[(methylsulfonyl)amino]phenyl}-1-isopropyl-1H-pyrazol-4-yl)-2-pyrimidinyl]amino}-2-propanyl]carbamate	5-((4-bromo-2-fluorophenyl)amino)-4-fluoro-N-(2-hydroxyethoxy)-1-methyl-1H-benzo[d]imidazole-6-carboxamide
Molecular formula	$C_{22}H_{27}ClFN_7O_4S$	$C_{17}H_{15}BrF_2N_4O_3$
Molecular weight	540.011 gm/mol	441.23 gm/mol
Solubility	Ethanol, Dimethyl formamide, DMSO	Ethanol, Dimethyl formamide, DMSO;
Excretion	47% in feces and 47 % in urine	62% in faeces; 31% urine
Half life	3.5 hr	3.5 hr
Intended for	Metastatic melanoma treatment	Treatment of metastatic melanoma with the

		mutations in BRAF V600E or V600K
Mode of action	Raf kinase enzyme, an important enzyme in RAF/MEK/ERK signalling pathway, was inhibited by encorafenib which decreased proliferation of tumourcells .	MEK1/2 inhibition stops the activation of transcription factors dependent on MEK1/2 and inhibits tumour cells.

2. Experimental

2.1 Materials and Reagents

Encorafenib and Binimetinib were gifted samples from Acron Pharma.. Remaining all reagents werehplc grade. Hydrogen peroxide, hydrochloric acid, sodium hydroxide, orthophosphoric acid were obtained from MERCK. Dipotassium hydrogen phosphate was obtained from FINAR chemical Ltd.

2.2 Instruments

Waters Alliance 2695 model hplc system was used in the present study, Empower 2 software used for data interpretation, Agilent, C18, 250 mm × 4.6 mm, 5µm colomn and PDA detector was used in RP-HPLC.

2.3 HPLC conditions

Column oven temp:25°C, volume of injection: 10 µl, flow rate: 1ml/min, wavelength for detection: 268, PH: 4.0

2.4 Mobile Phase

0.1M di-potassium hydrogen orthophosphate and methanol mixed in 50:50 volume/volume ratio. Orthophosphoric acid was used for pH (4.0) adjustment.

2.5 Standard Solution

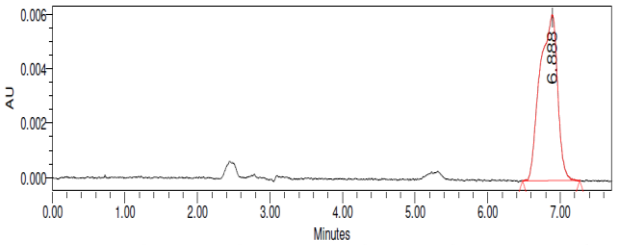
15 mg binimetinib and 75 mg encorafenibwere taken in 100ml volumetric flask. 30 ml of diluent was added and sonicated for 20 minutes. To produce 100 ml volume (Concentration -150 µg/ml binimetinib and 750 µg/ml encorafenib), the diluent was added. This solution is the stock solution for binimetinib and encorafenib. To test out validation parameters, one ml binimetinib and encorafenib stock solution was diluted to ten ml by diluent.

3. Results and Discussion

3.1 Method development

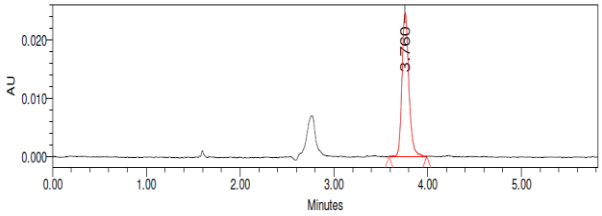
Table 2: Trial 1,2,3 conditions

	Trial 1	Trial 2	Trial 3
Combination and ratio of solvents in mobile phase	0.1 M KH_2PO_4 : methanol (60:40, *vol/vol*)	0.1 M KH_2PO_4 : methanol (60:40, *vol/vol*)	0.1 M K_2HPO_4 : methanol (50:50, *vol/vol*)
Column checked	Zodiac, C18, 250 mm × 4.6 mm, particle size 5 µm	Agilent, C18, 250 mm × 4.6 mm, particle size 5 µm	Agilent, C18, 250 mm × 4.6 mm, particle size 5 µm
Flow rate in column	1.0 ml/min	1.0 ml/min	1.0 ml/min
Sample size injected	10 µl	10 µl	10 µl
Column's set temperature	25˚C	25˚C	25˚C
Detection at	PDA	PDA	268
Time of run	7 min	5 min	9 min



Retention Time	Area	USP Resolution	USP Tailing	USP Plate Count
6.888	102706	-	0.82	3598

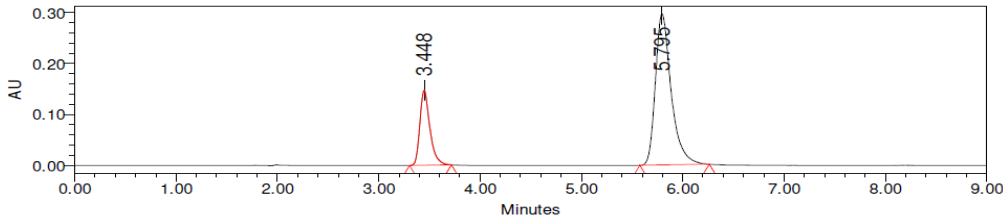
Figure 1: Trial 1 conditions chromatogram
Remarks: one peak only eluted



Retention Time	Area	USP Resolution	USP Tailing	USP Plate Count
3.760	125010	-	1.22	13566

Figure 2: Trial 2 conditions chromatogram

Remarks: Two peaks eluted but first peak was not properly eluted.



Retention Time	Area	% Area	Height	USP Resolution	USP Tailing	USP Plate Count
3.448	1011473	23.94	146967	-	1.36	6117
5.795	3214256	76.06	296834	10.07	1.41	7141

Figure 3: Trail three conditions chromatogram

Remarks: Two peaks with all satisfied system suitability values eluted and these conditions are choosen. Binimetinib – 3.448 min and Encorafenib – 5.795 min.

4. Validation

4.1 Selectivity

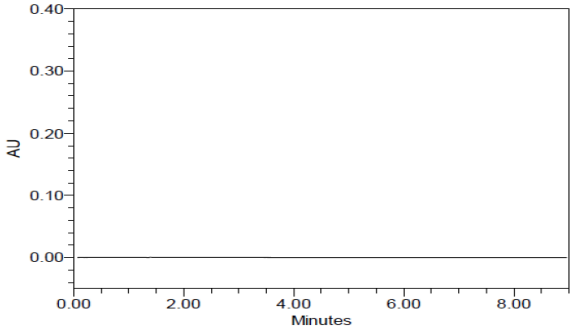


Figure 4: Chromatogram showing blank

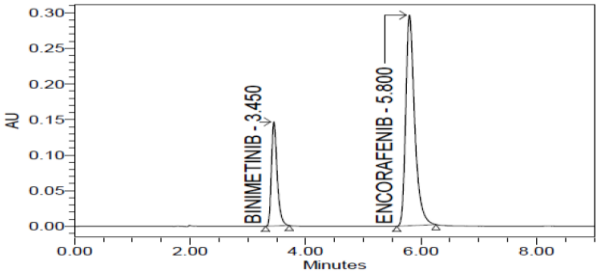


Figure 5: Chromatogram showing standard

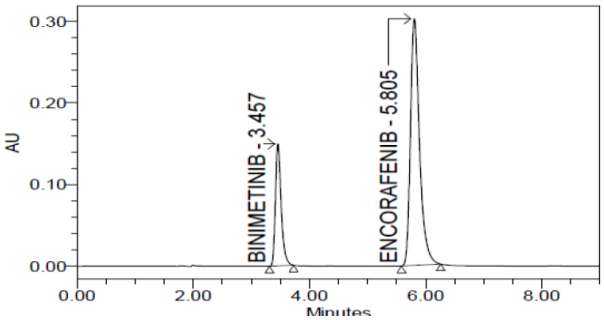


Figure 6: Chromatogram showing sample

4.2 Linearity

Table 3: Linearity evidence of binimetinib and encorafenib

Binimetinib quantity (µg/ml)	Binimetinib peak area	Encorafenib quantity (µg/ml)	Encorafenib peak area
7.5	502414	37.5	1593150
11.25	753977	56.25	2388527
15.00	1002994	75	3186011
18.75	1259567	93.75	3977371
22.5	1507324	112.50	4778022

Linear equation: y = 67043x - 324.5; regression coefficient - R^2 = 0.9996 - binimetinib

Linear equation: y = 42455x + 421.7; regression coefficient - R^2 = 0.9997 - encorafenib

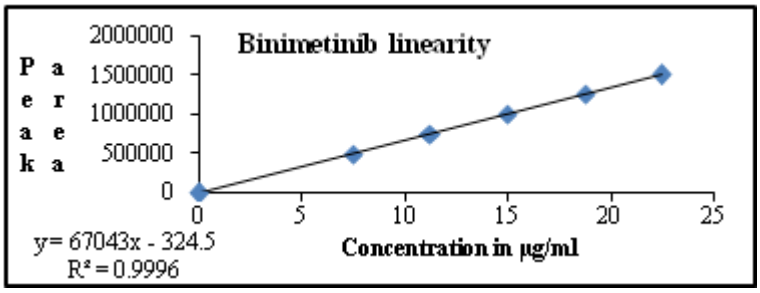


Figure 7: Binimetinib linearity confirmation curve

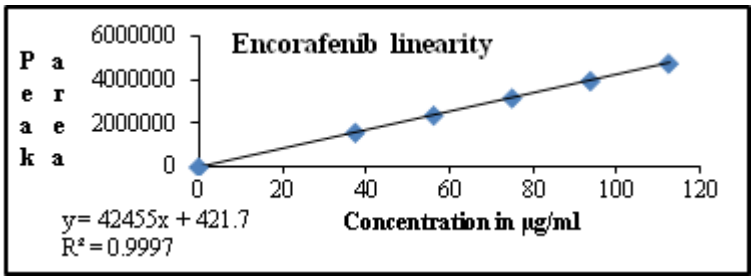


Figure 8: Encorafenib linearity confirmation curves

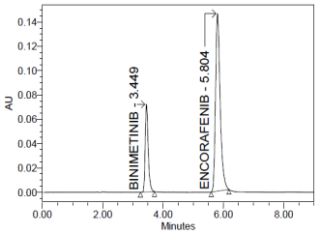


Figure 9: Chromatogram showing linearity level-1

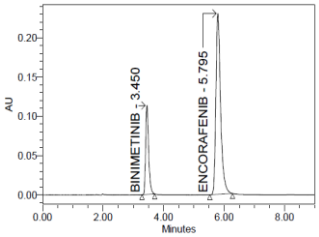


Figure 10: Chromatogram showing linearity level-2

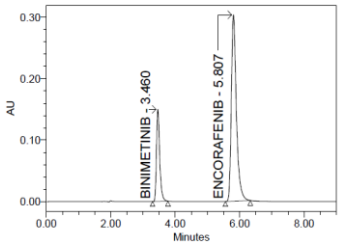


Figure 11: Chromatogram showing linearity level-3

4.3 Limit of Detection and limit of Quantification

Signal-to-noise level at 3 times is utilized to determine LOD (binimetinib – 0.017 µg/ml and encorafenib – 0.114 µg/ml) and Signal-to-noise level at 10 times is utilized to determine LOQ (binimetinib – 0.058 µg/ml and encorafenib – 0.381 µg/ml).

4.4 System Suitability

Table 4: System suitability evidence for binimetinib and encorafenib

Peak Name: ENCORAFENIB

	SampleName	Peak Name	RT	Area	USP Plate Count	USP Resolution	USP Tailing
1	STD2	ENCORAFENIB	5.794	3212858	7137	10.07	1.41
2	STD2	ENCORAFENIB	5.796	3196595	7154	10.03	1.41
3	STD2	ENCORAFENIB	5.800	3195331	7150	10.08	1.40
4	STD2	ENCORAFENIB	5.797	3174512	7158	10.08	1.40
5	STD2	ENCORAFENIB	5.798	3136869	7268	10.13	1.40
Mean				3183233.1			
% RSD				0.9			

Peak Name: BINIMETINIB

	SampleName	Peak Name	RT	Area	USP Plate Count	USP Tailing
1	STD2	BINIMETINIB	3.447	1012130	6110	1.36
2	STD2	BINIMETINIB	3.452	1006706	6048	1.37
3	STD2	BINIMETINIB	3.450	1008040	6101	1.36
4	STD2	BINIMETINIB	3.453	1001379	6136	1.36
5	STD2	BINIMETINIB	3.456	992637	6208	1.36
Mean				1004178.5		
% RSD				0.7		

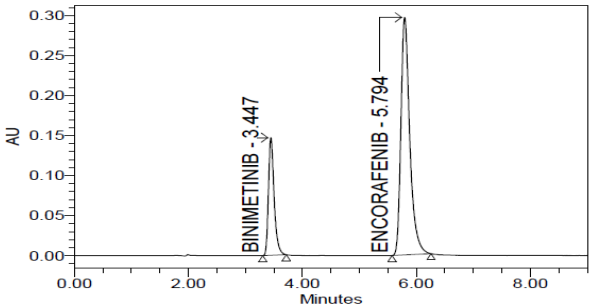


Figure 12: System suitability chromatogram for std-1

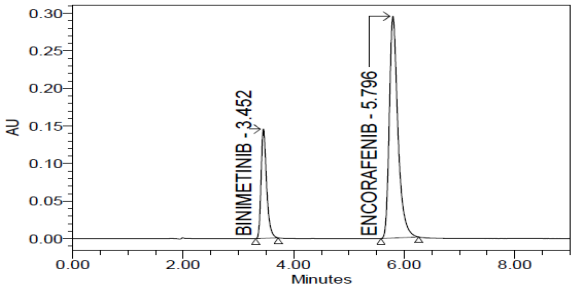


Figure 13: System suitability chromatogram for std-2

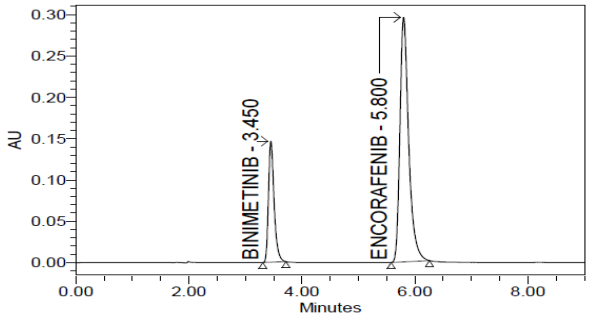


Figure 14: System suitability chromatogram for std-3

4.5 Precision

Table 5: Precision test findings for binimetinib and encorafenib

Binimetinib peak area	Statistical validation	Encorafenib peak area	Statistical validation
1002950	Mean value:	3185151	Mean value:
1007057	1008959	3187397	3178027
1013745	SD value:	3172312	SD value:
1016506	0.579	3162881	0.304
1007130	RSD value:	3186038	RSD value:
1001566	0.585	3174385	0.308

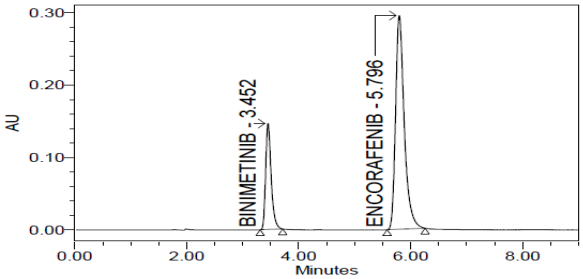


Figure 15: Chromatogram showing precision (Injection 1)

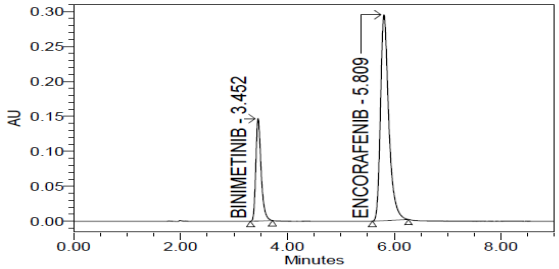


Figure 16: Chromatogram showing precision (Injection 2)

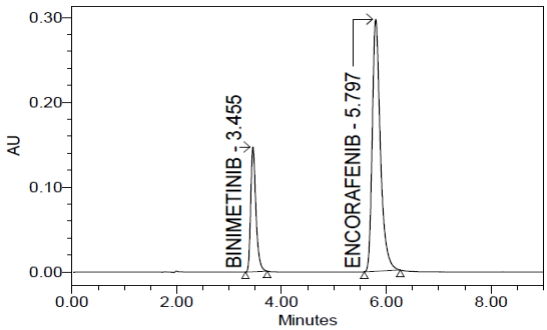


Figure 17: Chromatogram showing precision (Injection 3)

4.6 Accuracy

Table 6: Recovery outcomes of binimetinib and encorafenib

	Spiking Level (%)	quantity spiked (µg/ml)	quantity recovered (µg/ml)	Recovered percent (%)
Binimetinib	50	7.425	7.394	99.58
Encorafenib	50	37.125	36.874	99.32
Binimetinib	50	7.425	7.407	99.75
Encorafenib	50	37.125	37.107	99.94
Binimetinib	50	7.425	7.405	99.73
Encorafenib	50	37.125	37.091	99.91
Binimetinib	100	14.850	14.742	99.27
Encorafenib	100	74.250	74.225	99.97
Binimetinib	100	14.850	14.786	99.57
Encorafenib	100	74.250	74.009	99.68
Binimetinib	100	14.850	14.866	100.1
Encorafenib	100	74.250	74.389	100.191
Binimetinib	150	22.275	22.155	99.46
Encorafenib	150	111.375	110.820	99.50
Binimetinib	150	22.275	22.200	99.66
Encorafenib	150	111.375	110.894	99.61
Binimetinib	150	22.275	22.163	99.50
Encorafenib	150	111.375	111.178	99.82

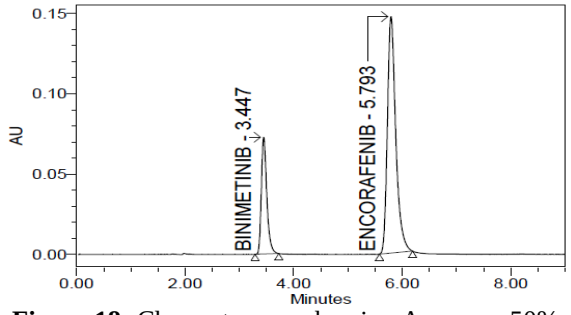


Figure 18: Chromatogram showing Accuracy 50% (Injection 1)

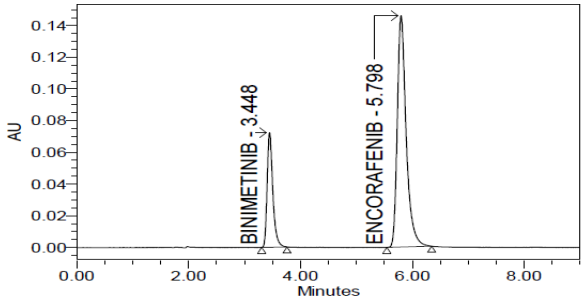


Figure 19: Chromatogram showing Accuracy 50% (Injection 2)

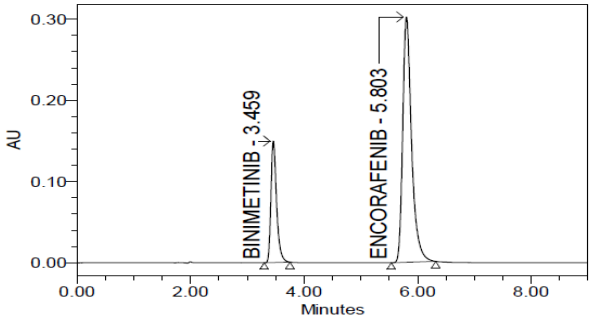


Figure 20: Chromatogram showing Accuracy 100% (Injection 1)

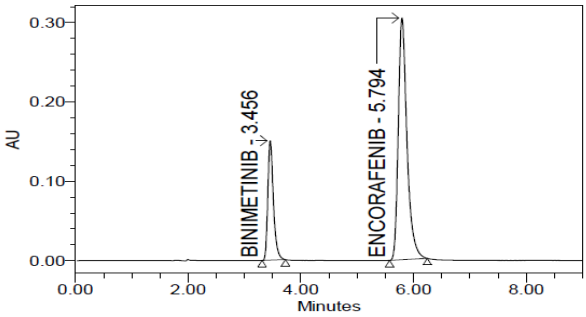


Figure 21: Chromatogram showing Accuracy 100% (Injection 2)

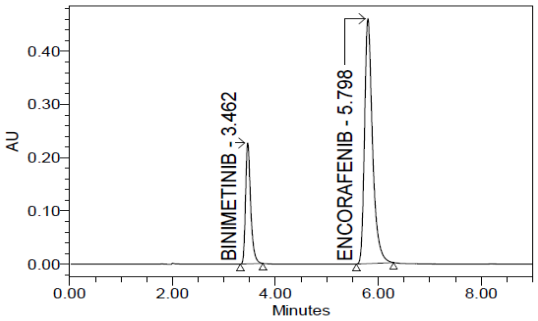


Figure 22: Chromatogram showing Accuracy 150% (Injection 1)

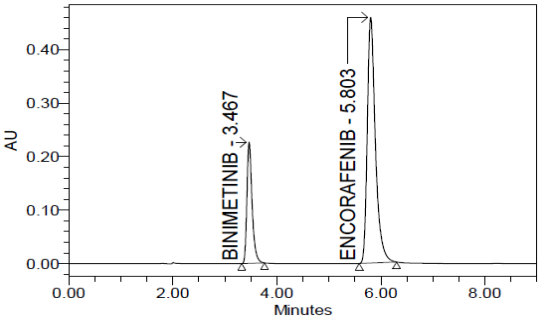


Figure 23: Chromatogram showing Accuracy 150% (Injection 2)

4.7 Degradation study

Table 7: Stability study outcomes for binimetinib and encorafenib

Condition	% Binimetinib assay	% Binimetinib degradation	% Encorafenib assay	% Encorafenib degradation
Acid	95.2	4.8	94.39	5.61
Base	94.64	5.36	95.79	4.21
Peroxide	97.26	2.74	96.07	3.93
Heat	93.93	6.07	93.5	6.5
Sunlight	97.02	2.98	94.78	5.22

4.8 Robustness

Table 8: Robustness of binimetinib and Encoforafenib

	Intentional alternation made	Peak tailing	Area response	Plate count
Binimetinib	Rate of flow:	1.36	843060	5636
Encorafenib	0.9 (ml/min)	1.40	2679710	6579
Binimetinib	Rate of flow:	1.35	914122	5841
Encorafenib	1.1 (ml/min)	1.39	2900015	6832
Binimetinib	pH: 3.8	1.36	1002950	6186
Encorafenib		1.40	3185151	7156
Binimetinib	pH: 4.2	1.36	997057	6132

Encorafenib		1.40	3167397	7129
Binimetinib Encorafenib	Temperature: 23 (˚C)	1.37 1.42	1168781 3715809	6472 7437
Binimetinib Encorafenib	Temperature: 27 (˚C)	1.37 1.42	1369263 4337249	6778 7852
Binimetinib Encorafenib	Methanol ratio: 55 (%)	1.36 1.40	843060 2679710	5636 6579
Binimetinib Encorafenib	Methanol ratio: 45 (%)	1.37 1.42	1168781 3715809	6472 7437

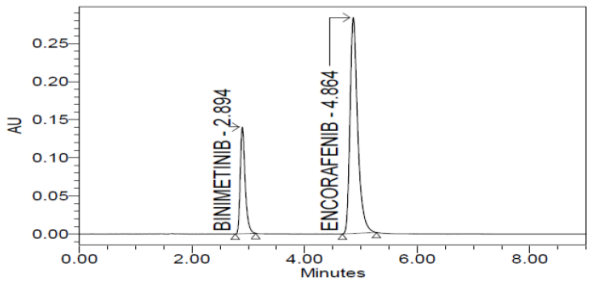


Figure 24: Chromatogram showing less flow of 0.9ml/m

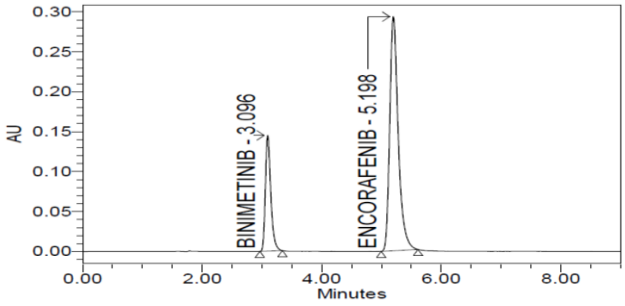


Figure 25: Chromatogram showing more flow of 1.1ml/min

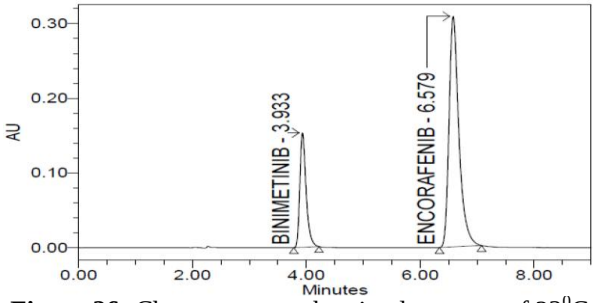


Figure 26: Chromatogram showing less temp of 23^0C

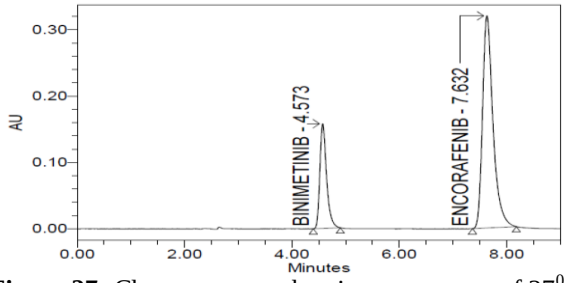


Figure 27: Chromatogram showing more temp of 27^0C

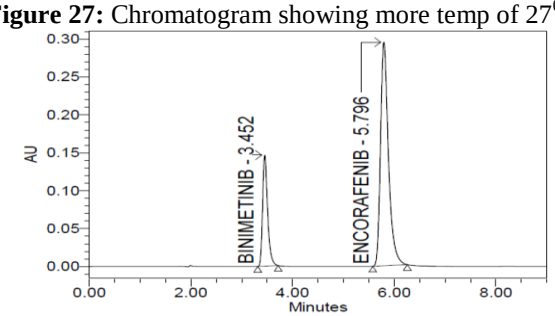


Figure 28 Chromatogram showing less pH 3.8

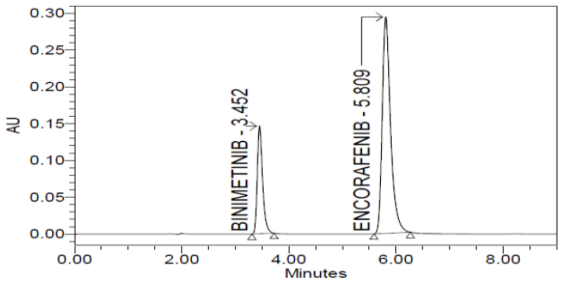


Figure 29: Chromatogram showing more pH 4.2

4.9 Analysis of Binimetinib and Encorafenib in tablets

Table 9: Analysis of encorafenib and binimetinib in tablets

Drug	Claimed strength	Percent assay	Mean	RSD
Binimetinib	15 mg	99.69%	99.63%	0.780%
	15 mg	99.65%		
	15 mg	99.54%		
Encorafenib	75 mg	99.72%	99.77%	0.151%
	75 mg	99.94%		
	75 mg	99.65%		

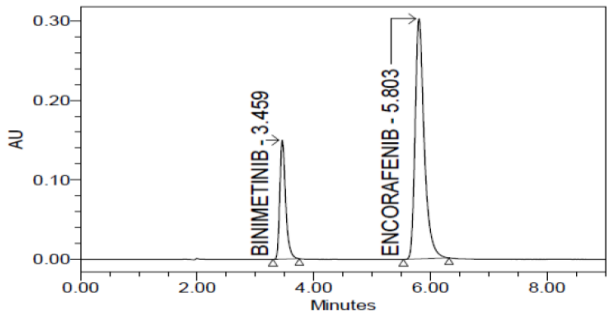


Figure 30: Chromatogram showing tablet sample 1

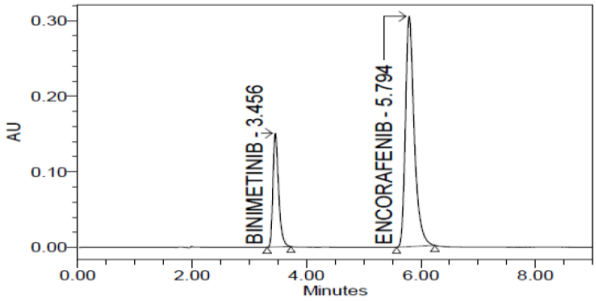


Figure 31: Chromatogram showing tablet sample 2

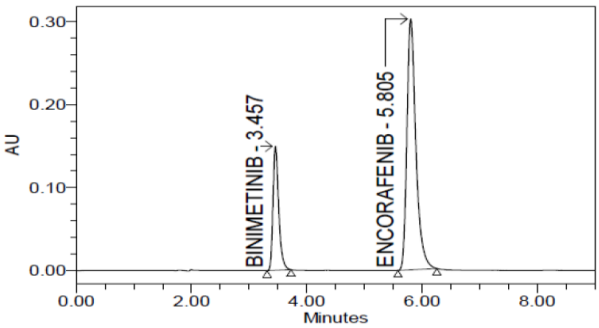


Figure 32: Chromatogram showing tablet sample 3

5. Conclusion

A rapid and sensitive RP-HPLC method was proposed and validated for encorafenib and binimetinib analysis with a short chromatographic runtime. The data assessed that the

procedure is appropriate for the analysis of encorafenib and binimetinib without obstruction from those excipients in the tablets in pharmaceutical formulation. It can be extended to study the encorafenib and binimetinib degradation and also to estimate them in tablet formulations.

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