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DEVELOPMENT AND VALIDATION OF A RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF VANZACAFTOR, TEZACAFTOR, AND DEUTIVACAFTOR IN PHARMACEUTICAL FORMULATION

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Keywords:

Vanzacaftor, Tezacaftor, Deutivacaftor, RP- HPLC, Method Development, Validation

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ABSTRACT: The present study reports the development and validation of a simple, precise, and robust RP- HPLC method for the simultaneous estimation of Vanzacaftor (VNC), Deutivacaftor (DEC), and Tezacaftor (TZC) in the fixed-dose combination formulation Alyftrek. Chromatographic separation was achieved using an XTERRAC18 column (250×4.6mm, 5µm) with a mobile phase consisting of potassium dihydrogen phosphate buffer and methanol (80:20, v/v). The analytes were detected at 247nm, and well-resolved peaks were obtained at retention times of 3.206 min (VNC), 5.148 min (DEC), and 6.823 min (TZC). The method was validated according to ICHQ2 (R1) guidelines. Linearity was established over the ranges of 2–6 µg/mL for VNC, 25–75µg/mL for DEC, and 10–30µg/mL for TZC with correlation coefficients (R^2) close to 1. The limits of detection were 0.045 µg/mL (VNC), 0.431 µg/mL (DEC), and 0.073 µg/mL (TZC), while the limits of quantification were 0.150 µg/mL, 1.436 µg/mL, and 0.245 µg/mL, respectively. Precision studies showed %RSD values below 1%, indicating excellent repeatability. Accuracy was confirmed with recovery values between 99–100% for all three drugs. Overall, the method demonstrated suitability for routine quality control analysis, offering accurate quantification and reliable performance for simultaneous estimation of VNC, DEC, and TZC in pharmaceutical formulations.

INTRODUCTION: Cystic fibrosis (CF) is a genetic disorder characterized by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, leading to impaired chloride transport and progressive multisystem complications [1, 2, 3]. CFTR modulators including correctors and potentiators have significantly improved therapeutic outcomes by enhancing the quantity and functional activity of the CFTR protein at the cell surface [1].

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Vanzacaftor (VNC) and Tezacaftor (TZC) are CFTR correctors that facilitate proper protein folding and trafficking, while Deutivacaftor (DEC) is a CFTR potentiator that enhances channel gating [5, 6]. The combination of VNC, TZC, and DEC has been recently introduced in the pharmaceutical formulation Alyftrek for patients with responsive CFTR mutations [5, 6].

Despite the growing clinical use of this triple-combination therapy, no simple and validated RP-HPLC method has been reported for the simultaneous quantification of VNC, DEC, and TZC in a single run [7, 8, 9]. Available analytical methods primarily focus on two-drug combinations or individual components, making them inadequate for routine quality control of this multi-component

formulation [7, 8]. Therefore, the present study aims to develop and validate a rapid, sensitive, and reliable RP- HPLC method for the simultaneous estimation of VNC, DEC, and TZC in bulk and combined dosage forms [7, 8, 9, 10].

The method is optimized for adequate peak resolution, accuracy, precision, and robustness, ensuring suitability for routine quality control and stability testing applications [16, 17, 18, 19, 20].

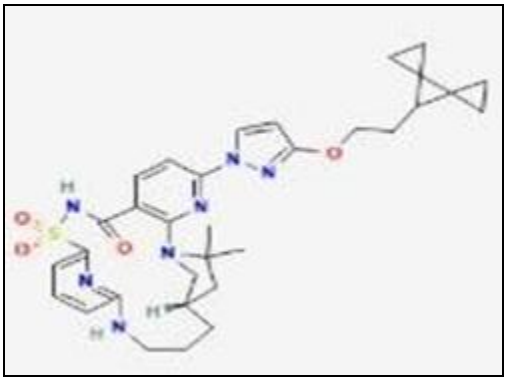

FIG. 1: STRUCTURE OF VANZACAFTOR

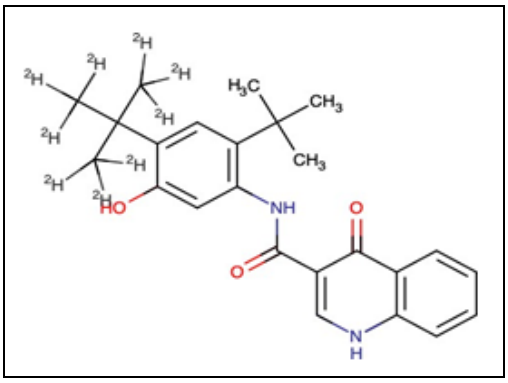

FIG. 2: STRUCTURE OF DEUTIVACAFTOR

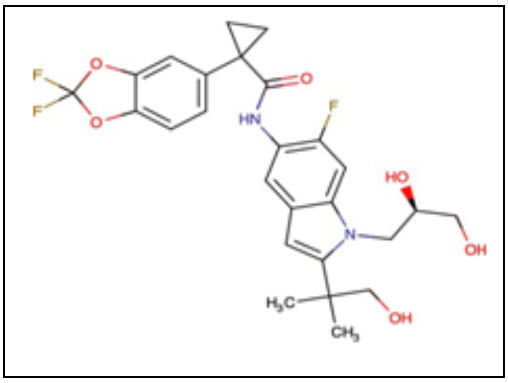

FIG. 3: STRUCTURE OF TEZACAFTOR

Chemicals and Reagents: Vanzacaftor (VNC), Deutivacaftor (DEC), and Tezacaftor (TZC) were used as reference standards for the analysis. Methanol, sodium hydroxide (NaOH), potassium dihydrogen phosphate (KH_2PO_4), acetonitrile, and hydrochloric acid (HCl) of HPLC grade were employed throughout the study [16, 17]. All solutions were prepared using HPLC-grade water to ensure purity and minimize interference [16, 17].

Instrumentation: Chromatographic analysis was performed on a Waters Alliance HPLC system equipped with a Photodiode Array (PDA) detector and operated using Empower 2 software (Waters Corporation, USA) [16]. The system provided stable baseline performance and consistent retention for all analytes [16].

Chromatographic Conditions: Separation was achieved on an XTERRAC18 column (250×4.6mm, 5µm) using a mobile phase of potassium dihydrogenphosphate buffer and methanol (80:20, v/v). The buffer was adjusted to pH 4.8 and filtered through a 0.45µm membrane prior to use. The mobile phase was degassed by sonication. Detection was carried out at 247 nm [16].

Stockvnc, Dec & Tzcsolution: A primary stock solution was prepared by accurately weighing 4 mg of VNC, 50 mg of DEC, and 20 mg of TZC into a 100 mL volumetric flask and dissolving the mixture with a diluent consisting of $0.1MK_2HPO_4$ buffer (pH3.2) and methanol in a 60:40(v/v) ratio. The flask was then made up to volume to obtain stock concentrations of40 µg/mL for VNC, 500µg/mL for DEC, and 200 µg/mL for TZC [16].

From this solution, a working standard was prepared by transferring 10 mL of the primary stock into a separate 100 mL volumetric flask and diluting to volume with the same diluent, yielding final concentrations of 4 µg/mL (VNC), 50 µg/mL (DEC), and 20 µg/mL (TZC) [16]. These working concentrations were used throughout the method

development and validation studies, including linearity, accuracy, precision, and sensitivity assessments [19, 20].

Preparation of Sample Stock Solution: Crush 20 tablets into a fine powder. Accuratelyweigh175 mg (containing 4 mg Vanzacaftor, 20mg Tezacaftor, and 50mg Deutivacaftor) and transfer into a100Ml volumetric flask. Add 10 mL methanol, sonicate for 20 minutes or shake for 10 minutes, then make up the volume with water.

Transfer 1 mL of this solution into a 10 mL volumetric flask and dilute with methanol. Filter through a 0.45 µm filter before HPLC injection.

Method Validation: The developed RP-HPLC method was validated in accordance with ICHQ2 (R1) guidelines [19, 20] to confirm its suitability for the quantitative determination of VNC, DEC, and TZC. The parameters evaluated included system suitability, linearity, accuracy, precision, sensitivity, and robustness [16, 17].

Linearity: Linearity was assessed at five concentration levels (2–6 µg/mL for VNC, 25–75 µg/mL for DEC, 10–30 µg/mL for TZC). Calibration curves were constructed, and regression analysis demonstrated excellent linearity across the tested ranges [7, 8, 9].

Accuracy: Evaluated using standard-addition at 80%, 100%, and 120% levels. Percent recoveries were within 98–102% with %RSD < 2%, indicating good trueness of the method [7, 8].

Precision: Repeatability (intra-day) and intermediate precision (inter-day) showed %RSD values <2%, confirming adequate precision [7, 8].

Sensitivity (LOD and LOQ): Determined based on signal-to-noise ratios of 3:1 and 10:1, respectively, demonstrating high sensitivity [7, 8].

System Suitability: Theoretical plates, tailing factor, and resolution met USP criteria, confirming chromatographic performance [16].

RESULTS AND DISCUSSION:

System Suitability Parameters:

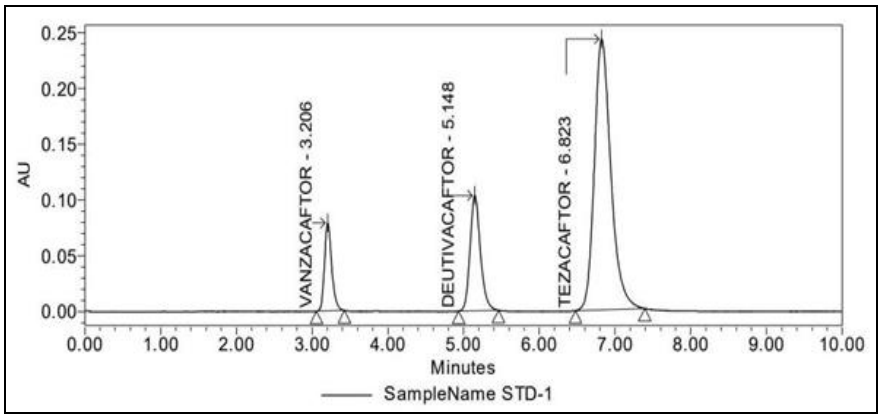

FIG. 4: SYSTEM SUITABILITY CHROMATOGRAM

TABLE1: SYSTEM SUITABILITY PARAMETERS

Parameter	Vanzacaftor	Deutivacaftor	Tezacaftor	eptance Criteria (USP)
Retention Time (min)	3.206	5.148	6.823	—
Area	526592	984770	3686996	—
%Area	10.13	18.94	70.93	—
Height	79051	103575	243136	—
USP Resolution (Rs)	—	9.02(vs VNC)	5.15(vs DEC)	Rs≥ 2.0
USP Tailing Factor (Tf)	1.25	1.20	1.27	Tf≤2.0
USP Plate Count (N)	5,471	6,989	4,932	N≥ 2000

Validation Parameters:

Linearity: Calibration curves for VNC, DEC, and TZC were generated by plotting their peak areas against corresponding concentrations at five different levels. The data **Table 2** and **Fig. 5** were analyzed using least-squares regression, confirming strong linear relationship for VNC (2–6µg/mL), DEC (25–75µg/mL), and TZC (10–30 µg/mL).

TABLE 2: LINEARITY DATA

VNC		DEC		TZC	
µg/mL	**Area**	**µg/mL**	**Area**	**µg/mL**	**Area**
2	264435	25	495443	10	1820726
3	398612	37.5	744666	15	2742740
4	531907	50	993241	20	3669625
5	665822	62.5	1228405	25	4587290
6	798803	75	1473790	30	5500232

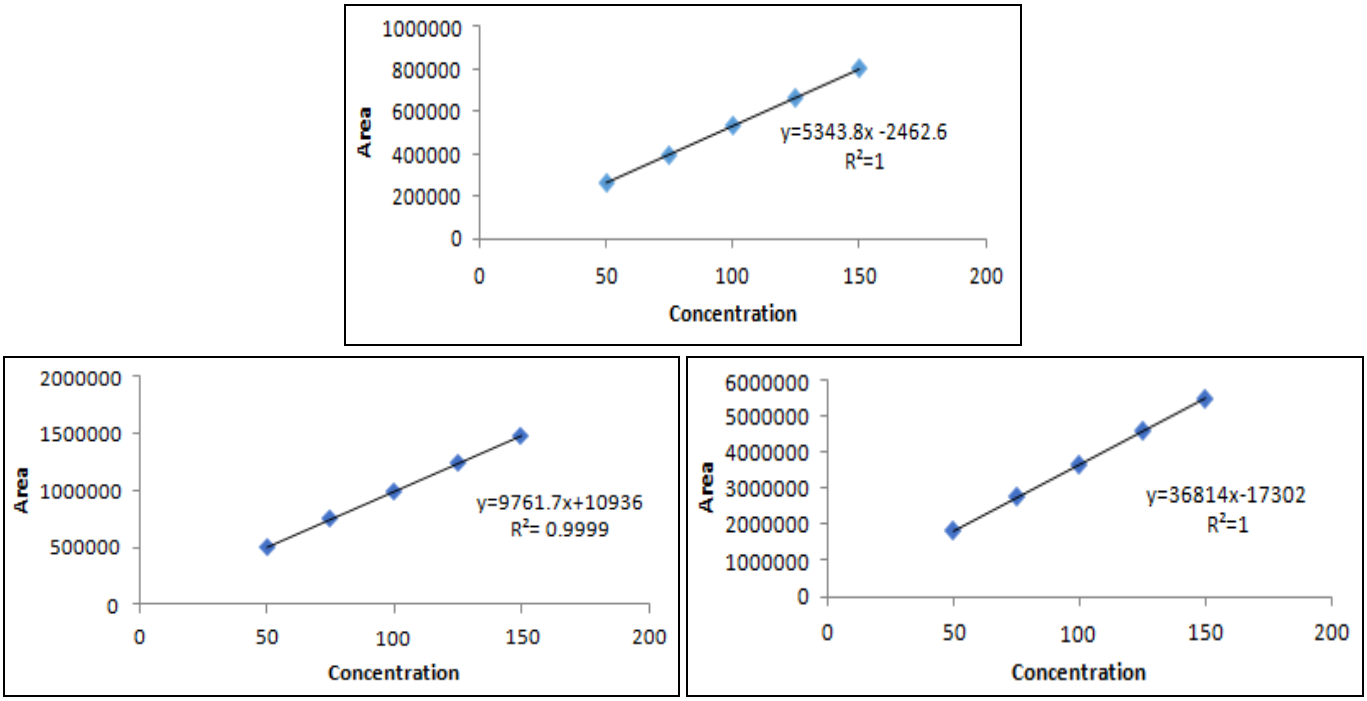

FIG. 5: LINEARITY OF VNC, DEC, &TZC

Limit of Detection: The LOD for VNC, DEC, and TZC was determined based on a signal to noise ratio (S/N) of approximately 3:1, as per ICH Q2(R1). The LOD values obtained were:

VNC: 0.045µg/mL

DEC: 0.431µg/mL

TZC: 0.073µg/mL

TABLE 3: LOD DATA

Sample Name	Peak Name	RT (min)	Area (µV·sec)	S/N
LOD	Vanzacaftor	3.212	5485	3.3
LOD	Deutivacaftor	5.169	9608	3.1
LOD	Tezacaftor	6.893	66154	3.2

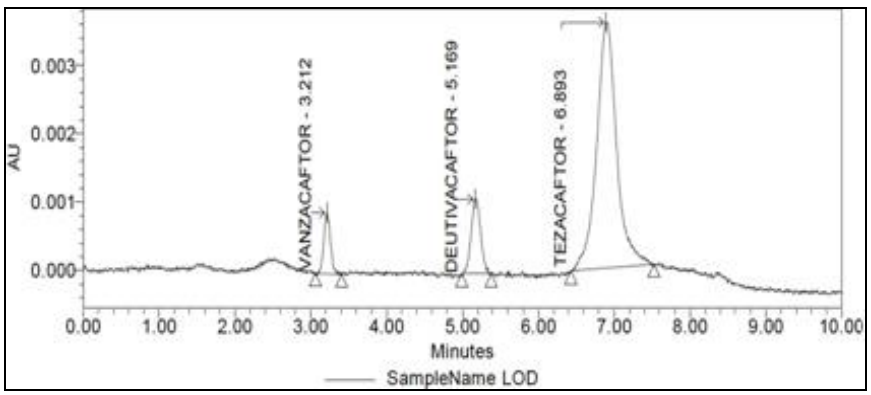

FIG. 6: CHROMATOGRAM OF LOD

Limit of Quantification: The LOQ was calculated using the formula LOQ = 10 × (SD / slope) and confirmed experimentally using signal to noise ratio of approximately 10:1. The LOQ values obtained were:

VNC: 0.150µg/mL

DEC: 1.436µg/mL

TZC: 0.245µg/mL

TABLE 4: LOQ DATA

Sample Name	Peak Name	RT (min)	Area (µV·sec)	S/N
LOQ	Vanzacaftor	3.212	14188	10.6
LOQ	Deutivacaftor	5.181	26331	10.6
LOQ	Tezacaftor	6.901	125995	10.1

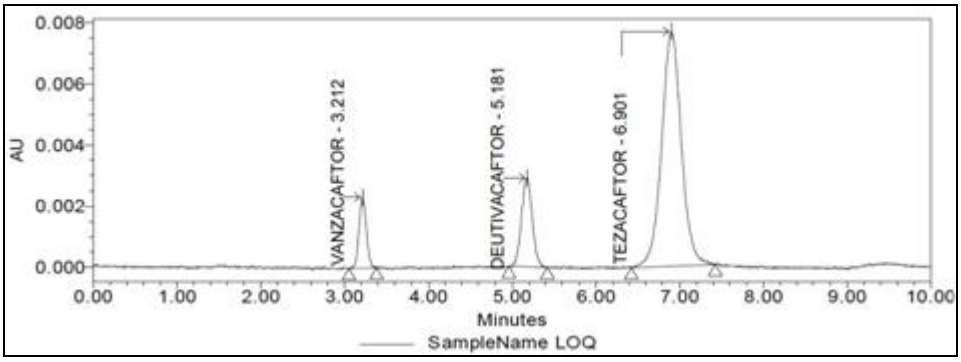

FIG. 7: CHROMATOGRAM OF LOQ

Precision: Solutions containing VNC (4 µg/mL), DEC (50 µg/mL), and TZC (20 µg/mL) were injected six consecutive times. The standard deviation (SD), relative standard deviation (RSD), and chromatograms for the peak responses of VNC, DEC, and TZC were calculated. The results demonstrated high accuracy in the combined analysis of VNC, DEC, and TZC.

TABLE 5: VNC, DEC & TZC PRECISION

Injection	VNC Area	DEC Area	TZC Area
1	531789	993126	3662883
2	530688	992205	3656348
3	531493	992133	3662130
4	530943	992966	3668665
5	531158	993257	3653296
6	531367	992619	3657127
Mean	531240	992718	3660075
SD	395.3	476.2	5557.6
%RSD	0.07% (≈0.1%)	0.05% (≈0.0%)	0.15% (≈0.2%)
Acceptance Criteria	%RSD≤2%	%RSD≤2%	%RSD≤2%

Accuracy: Solutions of VNC (4µg/mL), DEC (50µg/mL), and TZC (20 µg/mL) were injected six times consecutively. Peak responses, assay values, and chromatograms for VNC, DEC, and TZC were calculated, showing high accuracy in their combined analysis.

TABLE 6: ACCURACY TABLE OF VNC

Level	Analyzed (µg/mL)	Determined (µg/mL)	%Recovery
50%	1.980	1.98	100.0
	1.980	1.97	99.5
	1.980	1.98	100.0
100%	3.960	3.97	100.3
	3.960	3.96	100.0
	3.960	3.97	100.3
150%	5.940	5.97	100.5
	5.940	5.96	100.3

	5.940	5.96	100.3
Mean Recovery (%)	—	—	100.2
%RSD	—	—	0.0
Acceptance Criteria	98–102% recovery; %RSD ≤ 2%		

TABLE 7: ACCURACY TABLE OF TZC

Level	Analyzed (µg/mL)	Determined (µg/mL)	%Recovery
50%	9.900	9.90	100.0
	9.900	9.89	99.9
	9.900	9.84	99.4
100%	19.800	19.86	100.3
	19.800	19.79	99.9
	19.800	19.83	100.2
150%	29.700	29.80	100.3
	29.700	29.73	100.1
	29.700	29.83	100.4
Recovery (%)	—	—	**100.1**
%RSD	—	—	**0.0**
Acceptance Criteria	98–102%recovery; %RSD ≤ 2%		

TABLE 8: ACCURACY TABLE OF DEC

Level	Analyzed (µg/mL)	Determined (µg/mL)	%Recovery
50%	25.0	24.84	99.4
	25.0	24.82	99.3
	25.0	24.86	99.4
100%	50.0	49.78	99.6
	50.0	49.78	99.6
	50.0	49.73	99.5
150%	75.0	73.99	98.7
	75.0	73.70	98.3
	75.0	73.31	97.7
Recovery (%)	—	—	99.0
%RSD	—	—	0.9
Acceptance Criteria	98–102%recovery; %RSD ≤ 2%		

Robustness: In order to figure out how robust it was, the most important chromatographic settings were changed, and the chromatographic equipment suitability profile was watched and written down at the same time.

The following chromatographic settings were thought to be important:

- The amount of acetonitrile,
- The pH value,
- The detector nanometers,
- The rate of stream flow and
- The temperature of the column.

We figured out the peak response, the chromatographic equipment suitability profile, and the chromatograms for VNC, DEC, and TZC. For VNC, DEC, and TZC combinational analysis, the test results were pretty solid.

TABLE 9: ROBUSTNESS DATA AT DIFFERENT FLOW RATES

Drug Sample	Flow Rate (mL/min)	Area	Mean	SD	%RSD
VNC	0.8	875412			
	1.0(Optimized)	876392	876125	506.72	0.0578
	1.2	876571			
DEC	0.8	912684			
	1.0 (Optimized)	913420	913201	431.22	0.0472
	1.2	913499			
TZC	0.8	897522			
	1.0 (Optimized)	898284	898108	496.33	0.0552
	1.2	898518			

TABLE 10: ROBUSTNESS DATA AT DIFFERENT PH

Drug Sample	pH	Area	Mean	SD	%RSD
VNC	3.0	872214			
	3.2 (Optimized)	873192	873008	560.20	0.0641
	3.4	873618			
DEC	3.0	915213			
	3.2 (Optimized)	916054	915978	482.56	0.0526
	3.4	916668			
TZC	3.0	890652			
	3.2 (Optimized)	891412	891306	517.38	0.0580
	3.4	891855			

TABLE 11: ROBUSTNESS DATA AT DIFFERENT TEMPERATURES

Drug Sample	Temperature(°C)	Area	Mean	SD	%RSD
VNC	20	870126			
	25 (Optimized)	871048	871022	640.55	0.0736
	30	871893			
DEC	20	913512			
	25 (Optimized)	914408	914289	539.87	0.0590
	30	914947			
TZC	20	889201			
	25 (Optimized)	890208	890104	620.42	0.0697
	30	890902			

TABLE 12: ROBUSTNESS DATA AT DIFFERENT MOBILE PHASE COMPOSITIONS (ACN %)

Drug Sample	Composition (%ACN)	Area	Mean	SD	%RSD
VNC	15%	874189			
	20% (Optimized)	875316	875092	638.66	0.0729
	25%	875772			
DEC	15%	911426			
	20% (Optimized)	912614	912380	509.47	0.0558
	25%	913101			
TZC	15%	893876			
	20% (Optimized)	894899	894715	607.38	0.0679
	25%	895372			

TABLE 13: ROBUSTNESS DATA AT DIFFERENT WAVELENGTHS (NM)

Drug Sample	Wavelength (nm)	Area	Mean	SD	%RSD
VNC	245	878114			
	247 (Optimized)	879202	879038	652.33	0.0742
	249	879798			
DEC	245	916278			
	247 (Optimized)	917334	917192	541.00	0.0590
	249	917964			
TZC	245	891121			
	247 (Optimized)	892182	892074	661.05	0.0741
	249	892920			

CONCLUSION: In this study, a simple, accurate, and precise RP-HPLC method was successfully developed and validated for the simultaneous estimation of VNC, DEC, and TZC in Alyftrek.

The method exhibited excellent linearity over the selected concentration ranges, with correlation coefficients indicating strong proportionality between concentration and response. Accuracy and precision studies confirmed the reliability of the method, while robustness testing demonstrated that small deliberate variations in chromatographic conditions did not significantly affect performance. The method also showed high sensitivity, enabling the detection and quantification of all three components effectively.

Overall, the validated RP- HPLC method is rapid, reproducible, and suitable for routine quality control analysis of Alyftrek, ensuring consistent assessment of VNC, DEC, and TZC in pharmaceutical formulations.

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CONFLICTS OF INTEREST: Nil

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