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Determination of Valsartan, Candesartan, and Carvedilol in Pure and Pharmaceutical Forms Using RP-HPLC with Wavelength Programming

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ABSTRACT

A straightforward, quick, and reliable reverse-phase high-performance liquid chromatographic method for the simultaneous determination of three antihypertensive drugs (Valsartan, Candesartan, and Carvedilol) was established and validated. The drugs were separated using a C18 column with an isocratic mobile phase of methanol and water (84:16, v/v) at a temperature of 40 °C with a flow rate of 1.2 mL/min. The three drugs were detected under optimum conditions with two wavelengths; Valsartan and Candesartan were detected at 250 nm (0 to 3.0 minutes), while Carvedilol was detected at 240 nm (3.1 to 10.0 minutes). The total run time was less than 9.5 minutes, and the three drugs were well separated.

The method had a wide linear response range for each drug (1 to 25 µg/mL) with a correlation coefficient of 0.9983. The method limits of detection showed Valsartan 0.5539, Candesartan 0.2228, and Carvedilol 0.1716 µg/mL, respectively. The limits of quantitation were Valsartan 1.6785, Candesartan 0.6751, and Carvedilol 0.5201 µg/mL, respectively. The method had good accuracy (Recovery 101.32-104.25%) and precision (Relative standard deviation ≤ 3.87%).

The method was successfully used on six different commercial preparations and showed no interference from the excipients in these commercial products; the recoveries of the three drugs were between 90.22% and 99.78%. This method is a highly reliable, cost-effective, environmentally friendly alternative to common approaches for the routine quality control testing of these antihypertensive medications and improves analyte separation by using isocratic elution and wavelength programming without using a gradient.

Keywords: High-Performance Liquid Chromatography, Valsartan, Candesartan, Carvedilol, wavelength programming.

INTRODUCTION

Hypertension remains a major global public health challenge and is one of the leading risk factors for cardiovascular morbidity and mortality worldwide. Consequently, the development and quality control of effective antihypertensive medications are of great importance in pharmaceutical analysis and healthcare practice [1,2]. Analytical quality control plays a crucial role in ensuring the safety, efficacy, and consistency of pharmaceutical products throughout their lifecycle [3]. Among the most commonly prescribed antihypertensive drugs are valsartan (VAL), candesartan (CAN), and carvedilol (CAR). VAL and CAN belong to the class of angiotensin II receptor blockers (ARBs), whereas CAR is a non-selective β-blocker with additional α1-blocking activity. Due to their widespread clinical use, reliable analytical methods

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are required for their routine quality control and quantitative determination in pharmaceutical formulations [4–6]. The physicochemical properties and chemical structures of these drugs influence their chromatographic behavior and separation characteristics. VAL, chemically known as (S)-N-(1-oxopentyl)-N-[[2'-(1H-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl]-L-valine, has a molecular weight of 435.52 g/mol [7]. CAN, systematically named (±)-1-hydroxyethyl 2-ethoxy-1-[[2'-(1H-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl]-1H-benzimidazole-7-carboxylate, has a molecular weight of approximately 440.45 g/mol [8]. CAR, or (±)-1-(carbazol-4-yloxy)-3-[[2-(2-methoxyphenoxy)ethyl]amino]-2-propanol, has a molecular weight of 406.47 g/mol [9]. Various analytical techniques have been employed for drug determination, including spectrophotometric methods because of their simplicity, low cost, and applicability in routine analysis [10]. Flow injection analysis has also been successfully applied in pharmaceutical analysis [11], while several environmentally friendly analytical approaches have recently been introduced to reduce solvent consumption and improve sustainability [12]. Diazotization–coupling reactions have been used for the determination of pharmaceutical compounds in different matrices [13], and green extraction techniques such as dispersive liquid–liquid microextraction (DLLME) have further enhanced the environmental sustainability of analytical procedures [14].

Despite the advantages of these techniques, the simultaneous determination of complex antihypertensive drug mixtures often requires higher selectivity and chromatographic resolution. High-performance liquid chromatography (HPLC) remains one of the most reliable and widely applied techniques for pharmaceutical analysis because of its excellent separation efficiency and analytical performance [4–6]. Although several studies have reported the determination of individual antihypertensive drugs, only limited attention has been given to the simultaneous determination of valsartan, candesartan, and carvedilol in a single analytical run. Therefore, the present study aimed to develop and validate a simple, rapid, and reliable RP-HPLC method for the simultaneous determination of VAL, CAN, and CAR in pure and pharmaceutical dosage forms using wavelength programming.

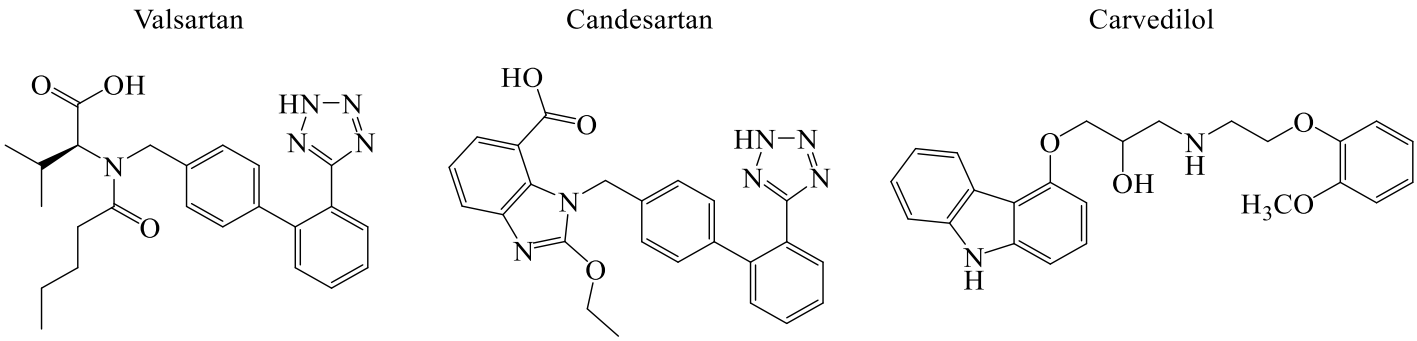


Figure 1. Chemical structures of VAL, CAN, and CAR.

MATERIALS AND METHODS

Methanol of HPLC grade and pure pharmaceutical reference standards (VAL, CAN, and CAR) were procured from Sigma-Aldrich USA. The respective commercial formulations of all three drugs were purchased from the local retail stores and are identified in Table 1, along with their specifications.

Table 1: The commercial pharmaceutical formulations.

Name	Dosage (mg)	Company	Active material
VALSART 160	160	Multiple generic manufacturers	VAL
Tabuvan	160	Tabuk Pharmaceutical Manufacturing Co. (Saudi Arabia)	
Candelong-16	16	Generic manufacturers (varies) (China)	CAN
Gardia	16	Generic pharmaceutical companies (India)	
BICOL 25mg	25	Biocon Ltd. (India)	CAR
Carvedi-Denk25	25	Denk Pharma GmbH & Co. KG (Germany)	

Chromatographic system

The HPLC configuration had the following components: two LC-20AD pumps; SPD-20A UV-Vis detector; manual injector; DGU-20A5 degasser; CBM-20A system controller; CTO-20A column oven (manufacturer: Shimadzu). A Fortis C18 column (250 mm length, 4.6 mm i.d., 5 µm particle size) (Fortis Technologies Ltd., U.K.) (with an EC guard column cartridge (length 4 mm, i.d. 3 mm) used for separation) was employed. Guard column holder part number REF 718966. Shimadzu LCsolution was used for data acquisition and data analysis.

Separation conditions and mobile phase

Separation was accomplished using a mobile phase of a methanol and deionized water (84:16, v/v) mixture. Each solvent was filtered using a 0.45-µm diameter membrane filter and degassed by ultrasonication before being used for chromatography. The flow rate, temperature, and volume of the column were maintained at 1.2 mL/min, 40°C, and 20 µL, respectively, throughout chromatography. The mobile phase was provided continuously (isocratically) during run time. Wavelengths were programmed throughout the procedure for detection purposes; peak area and peak height were measured for quantitative analysis of analytes.

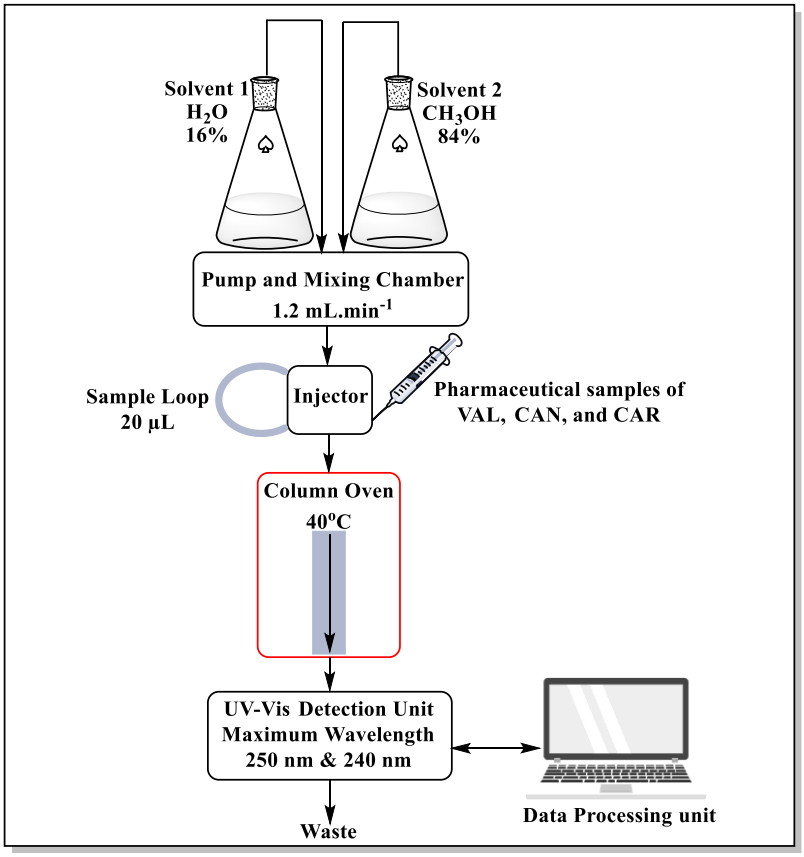


Figure 2. The HPLC system.

Preparation of Stock and Working Solutions (VAL, CAN, and CAR)

Each drug (VAL, CAN, and CAR) was prepared separately by weighing the appropriate amounts of reference standards and dissolving them in HPLC-grade methanol to form 100 µg/mL stock solutions. These solutions were stored until needed. For optimizing the chromatographic conditions, mixed working solutions were created from aliquots taken from stock solutions to create a mixed working solution of the drugs at their respective final concentrations of VAL 10, CAN 10, and CAR 5 µg/mL. Calibration of the analytical method was accomplished using mixed standard solutions prepared from the stock solutions at the following concentration levels: 1, 3, 5, 8, 10, 12, 15, 20, and 25 µg/mL for each analyte, using equal concentrations for all analytes at each concentration. For the analysis of the pharmaceutical formulations, stock solutions of the six commercially available products were prepared at a concentration of 100µg/mL and then diluted to obtain the respective working concentrations based on the optimized chromatographic conditions.

Method validation

The suggested HPLC technique has been validated with respect to its system suitability and analytical performance. The validation criteria involved precision, linearity, accuracy, and limits of quantification (LOQ) and limits of detection (LOD). Linearity was determined by producing calibration curves from measurements of antihypertensive drug substances plotted against the concentration of peak area. The linear relationship was quantified by calculating the correlation coefficient r^2, data were processed through linear regression techniques complemented with statistical analysis. Accuracy measurements were evaluated by percentage relative error (Er%) based on drug substance concentrations at both 8 and 12 µg/mL; three replicates per concentration (n=3). Similarly, the two concentration levels were also examined in order to assess accuracy. The precision was determined by measuring drug concentration at both 8 and 12 µg/mL three times each day during

the determination. Intra-day precision (accuracy) was reported as a relative standard deviation (RSD%), representing the variability between replicates of each measured analyte. The LOD and LOQ were established through S/N ratios of signal to noise using acceptable values equal to or greater than 10 and 3, respectively, using blank injection values (n=1).

Mobile phase optimization protocol

The final separation of a mixture of (VAL, CAN and CAR) on a C18 column using RP-HPLC can be affected by many factors, including the degree to which the mobile phase was saturated with HPLC-grade methanol before use, variation in flow rate during analysis, and variation in column temperature during analysis in order to eliminate potential problems with poor resolution due to analytes interacting with residual silanol groups on the stationary phase. In order to obtain optimal separation of all three drugs and decrease analysis time, we performed a systematic optimization study based on the various parameters previously discussed (i.e., mobile phase component(s), flow rate, and temperature) in order to produce optimal peak shape and improve separation efficiency for the drugs being studied.

Results and Discussion:

The goal of this study was to create and validate an RP-HPLC technique for the simultaneous measurement of VAL, CAN, and CAR in their pure forms and in commercial products. The optimized HPLC conditions were determined by examining the impact of the proportion of organic modifier in the mobile phase, the mobile phase flow rate, and the column temperature.

Determination of common detection wavelength

Using a Shimadzu UV-Visible spectrophotometer (UV-1800, Japan), the 20 µg/mL VAL, CAN, and CAR samples were subjected to UV spectrum analysis between 200 and 350 nm. These solutions were prepared by diluting accurately weighed amounts of each analyte into HPLC-grade methanol. The resulting spectrum exhibited an overlap of the three analytes, making simultaneous determination achievable. A common wavelength of 250 nm was selected as a compromise wavelength for detection via this HPLC method to give acceptable absorbance values for all analytes. While this compromise wavelength does not reflect the λmax for each of the analytes, it results in adequate sensitivity for the simultaneous determination, with improved baseline stability, and decreased potential interferences from components found within the mobile phase.

Effect of organic modifier

The Fortis C18 (250 mm × 4.6 mm) column was at an ambient temperature of 30 ºC for all tests performed in isocratic mode with the organic modifier. Methanol (HPLC-grade) instead of acetonitrile was utilized as the organic phase because methanol was more suitable for the antihypertensive medications studied, with better solubility and sufficient interaction with the analyte, producing better shape and resolution than acetonitrile. Also, methanol is much less expensive and friendlier to the ecology than acetonitrile, which makes it a logical choice for daily pharmaceutical testing procedures. While methanol is generally thicker (more viscous) than acetonitrile [15], the optimal conditions produced good separations in a reasonable time period. Further studies were conducted to examine the effect of changing the concentration of methanol on the separation efficiency of antihypertensive drugs and to establish the optimal resolution and symmetry of the peaks for the compounds under consideration. The optimum organic modifier concentration was determined using mixtures of methanol and water with different proportions of total organic solvent (i.e., 80%, 82%, 84%, 86%, 88%, and 90%) as the mobile phase. The separation was monitored by calculating chromatographic factors (i.e., retention factor (K′), number of theoretical plates (N), resolution (Rs), and selectivity coefficient (α)) along with the chromatographic peak shape.

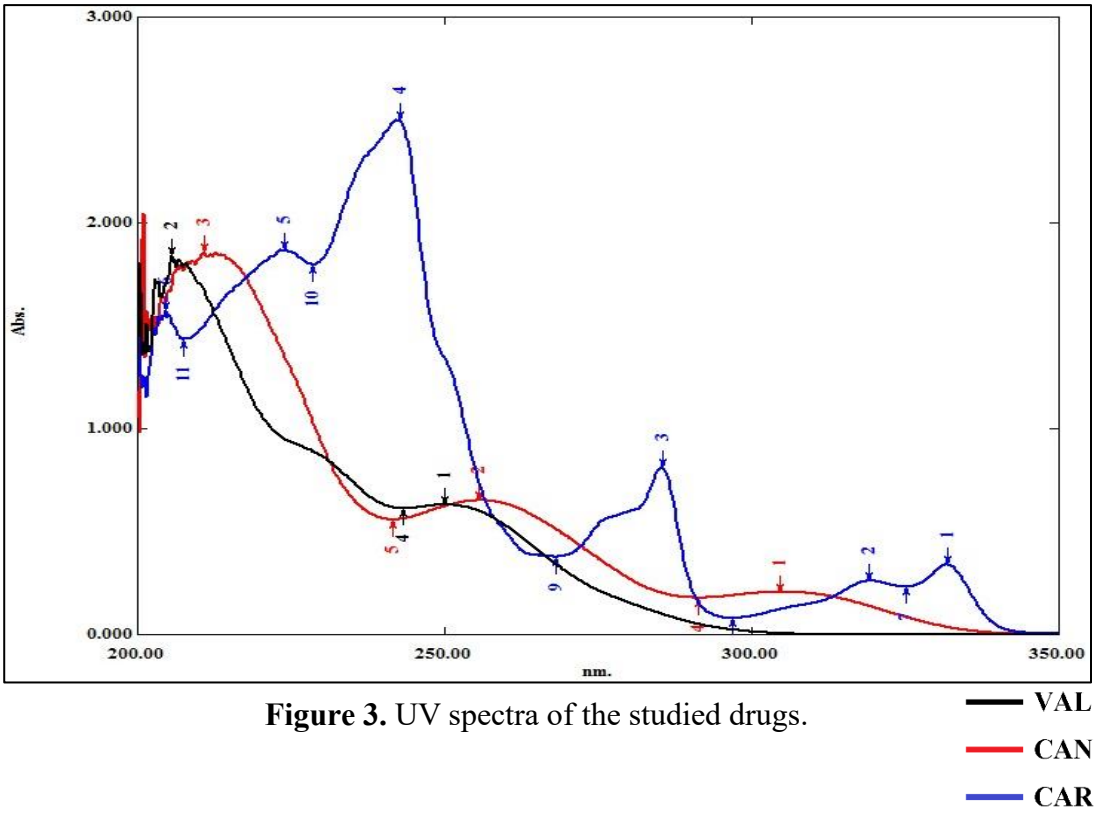


Figure 3. UV spectra of the studied drugs.

Table 2. The effect of organic modifier on efficiency and retention factors.

Organic modifier (Methanol) (%)	VAL		CAN		CAR		α_{12}	α_{23}	Rs_{12}	Rs_{23}
	K`	N	K`	N	K`	N				
80	0.48	136.34	1.13	279.10	7.84	376.98	2.34	6.95	1.27	5.76
82	0.50	145.80	0.99	168.70	7.19	153.13	1.96	7.30	0.87	3.81
84	**0.50**	**191.41**	**0.86**	**304.66**	**6.62**	**163.64**	**1.73**	**7.70**	**0.85**	**4.10**
86	0.50	293.14	0.76	513.93	6.09	218.60	1.51	8.04	0.77	4.79
88	0.49	349.54	0.69	889.79	5.83	276.38	1.42	8.40	0.75	5.49
90	0.49	483.50	0.64	837.03	5.39	187.04	1.30	8.45	0.59	4.54

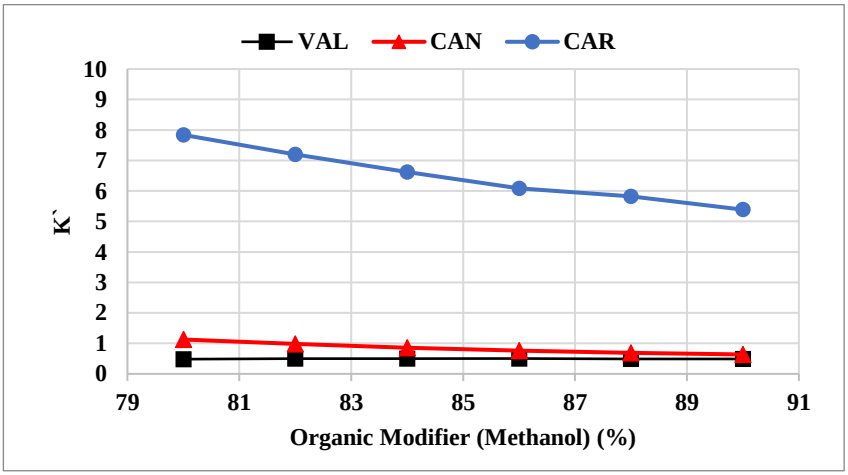


Figure 4. Effect of methanol percentage on retention factors (K′) of VAL, CAN, and CAR.

Effect of flow rate of the mobile phase

Multiple mobile phase flow rates were tested (0.8, 0.9, 1.0, 1.1, and 1.2 mL/min) to determine the optimal flow rate yielding complete separation in a short amount of time with adequate sensitivity and high column efficiency. As shown in Figure 5, the optimal flow rate for the mobile phase is 1.2 mL/minute.

Table 3. The effect of flow rate on efficiency and retention factors.

Flow Rate (mL/min)	VAL		CAN		CAR		α_{12}	α_{23}	Rs_{12}	Rs_{23}
	K`	N	K`	N	K`	N				
0.8	0.84	165.46	1.41	333.57	9.87	335.60	1.69	6.98	1.05	5.83
0.9	0.63	158.38	1.19	178.70	8.63	501.45	1.88	7.25	0.95	6.26
1	0.49	135.29	0.95	157.72	7.09	93.76	1.93	7.45	0.81	3.10
1.1	0.35	154.63	0.78	181.18	6.54	157.56	2.21	8.39	0.89	3.93
1.2	**0.27**	**139.88**	**0.67**	**210.71**	**6.11**	**199.02**	**2.46**	**9.15**	**0.89**	**4.40**

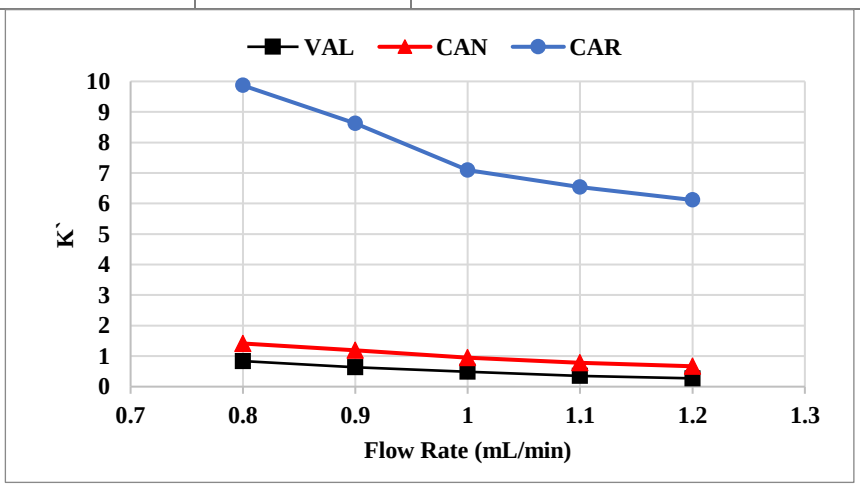


Figure 5. Effect of mobile phase flow rate on retention factors (K′) of VAL, CAN, and CAR.

Effect of Temperature

The temperature effect of the column was studied at (30, 35, 40, 45, and 50°C) using an optimized chromatographic set of conditions. It was noted that the retention factors (K′) of CAN and CAR decreased as temperature increased, the viscosity of the mobile phase decreased, while the viscosity of the mobile phase remained approximately constant for VAL. Also, column efficiencies (N) improved significantly with increasing temperature for all compounds analysed. The selectivities (α) and resolutions (Rs) of all compounds decreased when the temperature was increased, especially between VAL and CAN. However, at the temperature of 40 °C, there was an acceptable balance between column efficiency, analysis time, and resolution. Therefore, based on all of the data, 40 °C was selected as the optimum column temperature providing adequate chromatographic performance, shorter analysis time, and higher efficiency.

Table 4. The effect of temperature on retention factors, selectivity, and resolution under optimized conditions.

Temperature (ºC)	VAL		CAN		CAR		α_{12}	α_{23}	Rs_{12}	Rs_{23}
	K`	N	K`	N	K`	N				
30	0.42	149.00	0.87	282.99	7.48	143.97	2.08	8.58	0.99	4.04
35	0.41	209.79	0.79	293.95	6.65	150.55	1.90	8.44	0.92	4.03
40	**0.44**	**227.20**	**0.76**	**364.84**	**5.72**	**128.92**	**1.74**	**7.51**	**0.87**	**3.62**
45	0.47	252.26	0.74	441.39	5.00	153.68	1.58	6.74	0.77	3.76
50	0.44	365.02	0.68	493.07	4.42	209.46	1.54	6.52	0.79	4.16

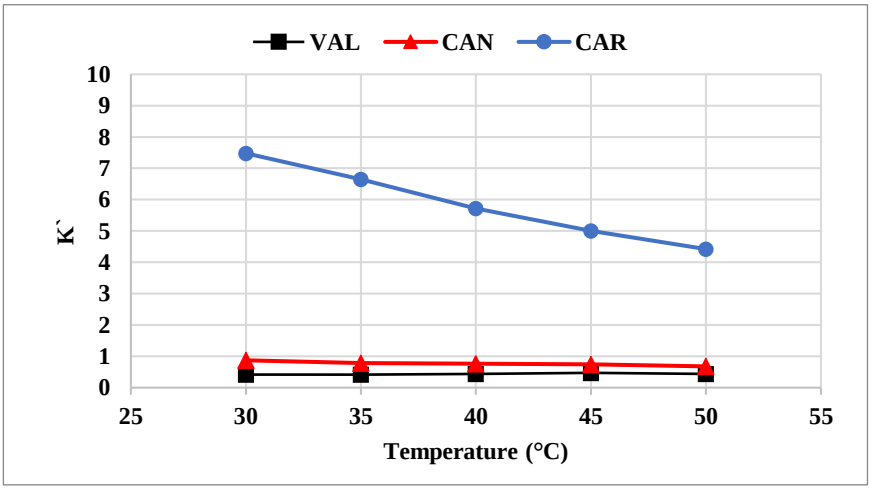


Figure 6. Effect of column temperature on retention factors (K′) of VAL, CAN, and CAR.

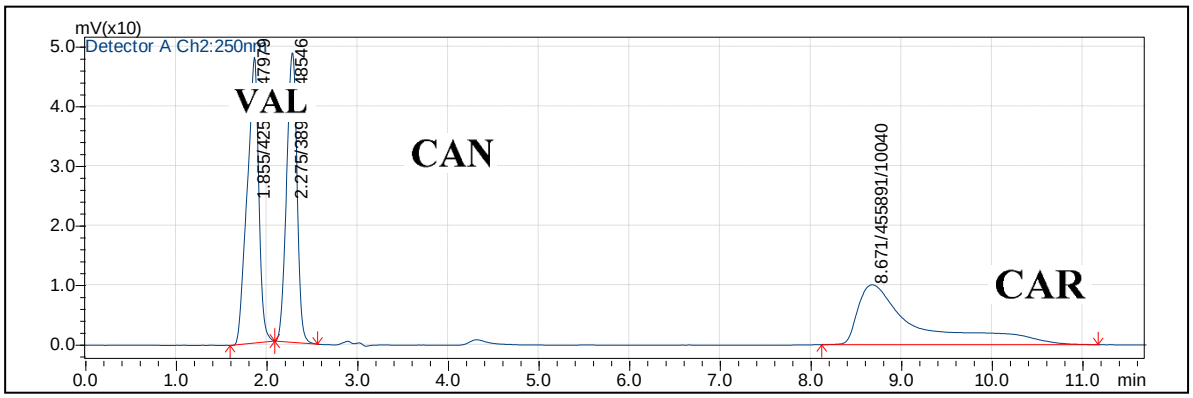


Figure 7: Chromatograms of a mixture of 10 µg/mL VAL, 10 µg/mL CAN, and 5 µg/mL CAR at 40ºC.

Development of the isocratic HPLC method using wavelength programming

The use of gradient elution in HPLC systems has traditionally been used to provide good separation. However, this study utilized an alternative method of wavelength programming to allow the analysis without any variation in the composition of the mobile phase. Previous work has utilized the same method for the chromatographic analysis of complex multicomponent mixtures using either gradient RPC or wavelength programming, showing umpteen advantages including increased chromatographic resolution, enhanced detection sensitivity, and faster analysis [15]. Given that all three analytes contain adequate absorbance at 250 nm, the 250 nm monitoring of VAL and CAN between 0 and 3 minutes was performed. The monitoring of CAR would therefore require a change in monitoring wavelength to 240 nm from 3.1 to 10 minutes due to its higher absorbance at this wavelength. The wavelength programming provides improved detection sensitivity to individual analytes during the separation of samples within one analytical run and improved accuracy of quantification without the need to run standards for each analyte.

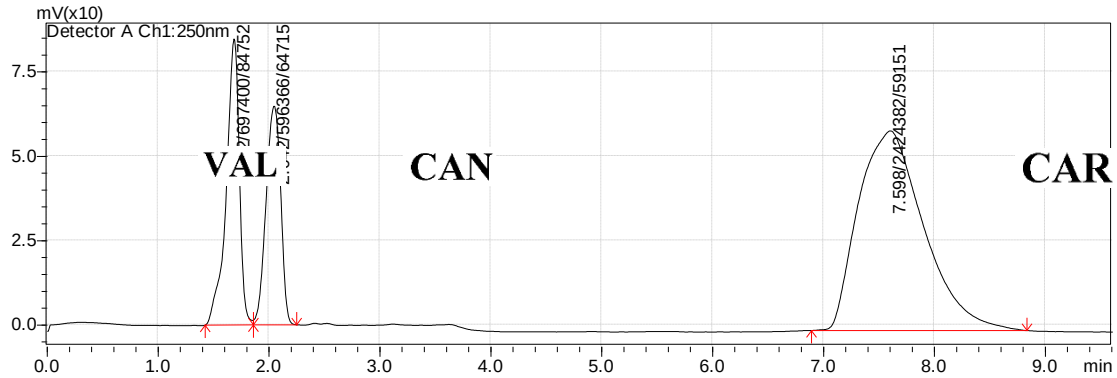


Figure 8. Chromatogram of mixture of 20 µg/mL VAL, CAN, and CAR under optimized conditions.

Table 5. Wavelength programming schedule for separation of the drugs using RP-HPLC.

Time (min)	Module	Action	Value (nm)	Drug
0	(DET. A)	Wavelength Ch1	250	VAL and CAN
3	(DET. A)	Wavelength Ch1	250	
3.1	(DET. A)	Wavelength Ch1	240	CAR
10	(DET. A)	Wavelength Ch1	240	
10.50	Controller	Stop		

System suitability and HPLC parameters

The chromatographic system was evaluated for performance based on the optimization parameters of K', Rs, and α. for VAL, CAN, and CAR. The separation of these three analytes was demonstrated in the chromatograms in Figure 8 with adequately shaped and symmetrical peaks. The calculated system suitability characteristics shown in Table 7 show that the sample separation efficiency is acceptable, and the chromatographic system meets all required analytical specifications. The resolution values for adjacent peak pairs are sufficient to ensure complete separation. The selectivity values support good discrimination of the three analytes. The results of this study indicated that the novel RP-HPLC method developed for this study can consistently provide an accurate, reproducible, and efficient means of separating the analytes studied, thus making it an excellent candidate for routine quantitative analyses of drug formulations.

Table 6. System suitability data and HPLC parameters of the studied drugs.

VAL			CAN			CAR			α_{12}	α_{23}	Rs_{12}	Rs_{23}
Rt	K`	N	Rt	K`	N	Rt	K`	N				
1.682	0.30	241.43	2.042	0.58	436.39	7.598	4.89	244.41	1.92	8.39	0.87	4.76

Validation

Assessment of the calibration curve linearity was done via linear regression analysis using Microsoft Excel 2016 to determine the relationship between concentration and peak area for three drugs (VAL, CAN, and CAR) in concentrations ranging from 1 to 25 µg/mL. The regression equation and correlation coefficient (R^2) for each of the three drugs indicated very good linearity within the studied concentration range. Therefore, the analytical system is applicable and has been shown to be very effective for the quantification of drugs at concentrations in the range of those examined drugs. The data from each of the studies were subjected to statistical analysis, including recovery (Rec.%), standard deviation (SD), relative standard deviation (RSD%), relative error, and detection and quantification limits (LOD and LOQ), shown in Table 8. The results of these analyses indicated that the method is highly accurate and precise, having a recovery of nearly 100% and an acceptable level of standard deviation, confirming the accuracy of the method as developed. Table 9 confirms the accuracy and consistency of the methods' statistical parameters and demonstrates that the method developed is suitable for use in quantitatively analyzing all three drugs in the studied ranges with good stability and reproducibility.

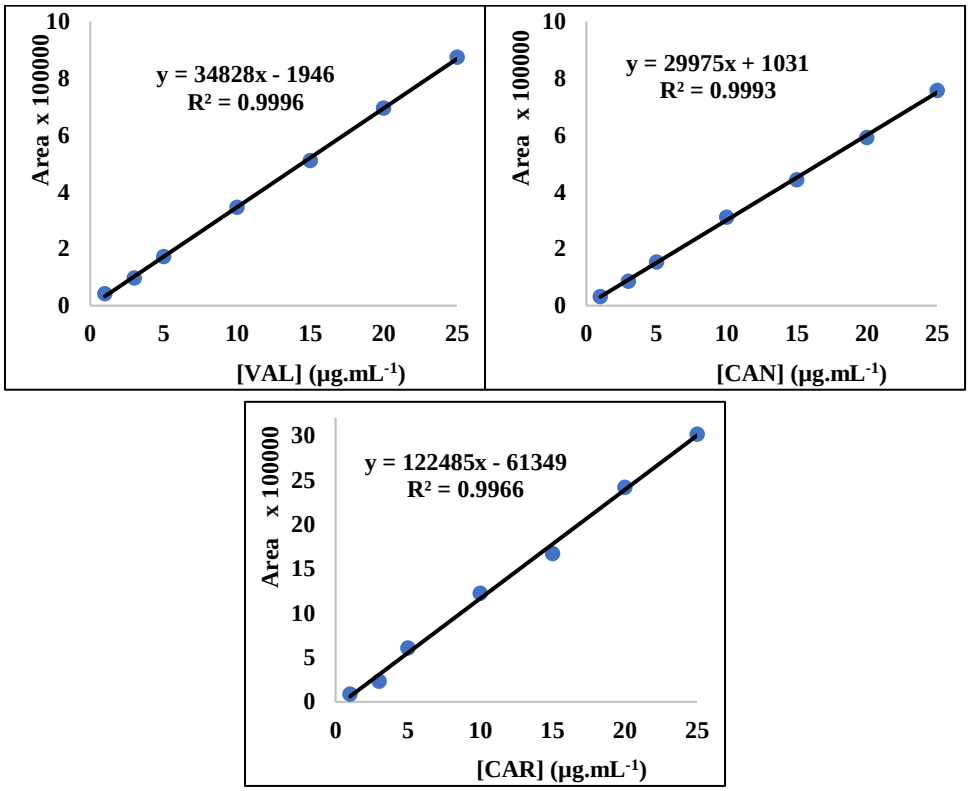


Figure 9. Calibration graphs, concentration (µg/mL) *vs.* peak area for VAL, CAN and CAR.

Table 7. Calibration curve data.

Parameters	Value		
	VAL	CAN	CAR
λ_{max} (nm)	250	250	240
Regression equation (y = bx+a)	y = 34828x - 1946	y = 29975x + 1031	y = 122485x – 61349
Linear range (µg/mL)	1-25	1-25	1-25
Average of recovery (Rec%)	101.77	102.37	103.35
Average of Relative Error (E_{rel}%)	1.77	2.37	3.35
Average of Relative standard deviation (RSD%)	2.65	1.54	1.48
Average of Standard deviation (SD)	8486	4892	20878
Slope (b)	34828	29975	122485
Intercept (a)	-1946	1031	-61349
Linearity R^2	0.9996	0.9993	0.9966
Correlation coefficient (r)	0.9998	0.9996	0.9983
Standard deviation of slope (S_b)	314	367	3185
Standard deviation of intercept (S_a)	4411	5157	44806
Standard deviation of the residuals; Sy/x	6966	8144	70758
Limit of detection (LOD) (µg/mL)	0.5539	0.2228	0.1716
Limit of quantification (LOQ) (µg/mL)	1.6785	0.6751	0.5201

Table 8. Accuracy and precision.

Drug	Present µ (µg/mL)	Found $\bar{x}$ (µg/mL)	Error	Rec%	Erel%	RSD% (peak area)	SD (peak area)
VAL	8	8.18	0.18	102.22	2.22	3.87	10944
	12	12.16	0.16	101.32	1.32	1.43	6028
CAN	8	8.30	0.30	103.73	3.73	1.29	3210
	12	12.12	0.12	101.01	1.01	1.80	6574
CAR	8	8.34	0.34	104.25	4.25	0.19	1831
	12	12.29	0.29	102.45	2.45	2.76	39925

*SD: Standard deviation calculated from peak area values (in area units)

Analysis of standard drugs and commercial pharmaceutical formulations

The developed procedure was successfully used for the analysis of VAL, CAN, and CAR in the aforementioned pharmaceutical preparations. Results obtained are presented in Table (10), whereas the corresponding chromatograms of the tested samples are shown in Figure (10). The values of drug recoveries ranged from 90.22% to 99.78%, suggesting an acceptable accuracy of the procedure. Values of RSD% were found to vary from 0.44% to 3.76%, which suggests a good precision of the method. Data obtained show a high correlation between declared and experimental concentrations of all investigated drugs. This proves that the developed method can be considered reliable, accurate, and precise enough for routine analytical purposes. Besides, the proposed approach shows excellent selectivity and effectiveness in determining the analyzed pharmaceutical substances simultaneously.

Table 9. Application of the proposed method for the determination of commercial pharmaceutical formulations at 10 µg/mL.

Active material	The commercial pharmaceutical formulations	Found Conc. (µg/mL) *	Rec.%	RSD%
VAL	VALSART 160	9.02	90.22	3.76
	Tabuvan	9.67	96.69	0.44
CAN	Candelong-16	9.52	95.23	0.95
	Gardia	9.98	99.78	0.44
CAR	BICOL 25mg	9.58	95.75	1.41
	Carvedi-Denk25	9.74	97.36	0.60

**Average of three determinations.*

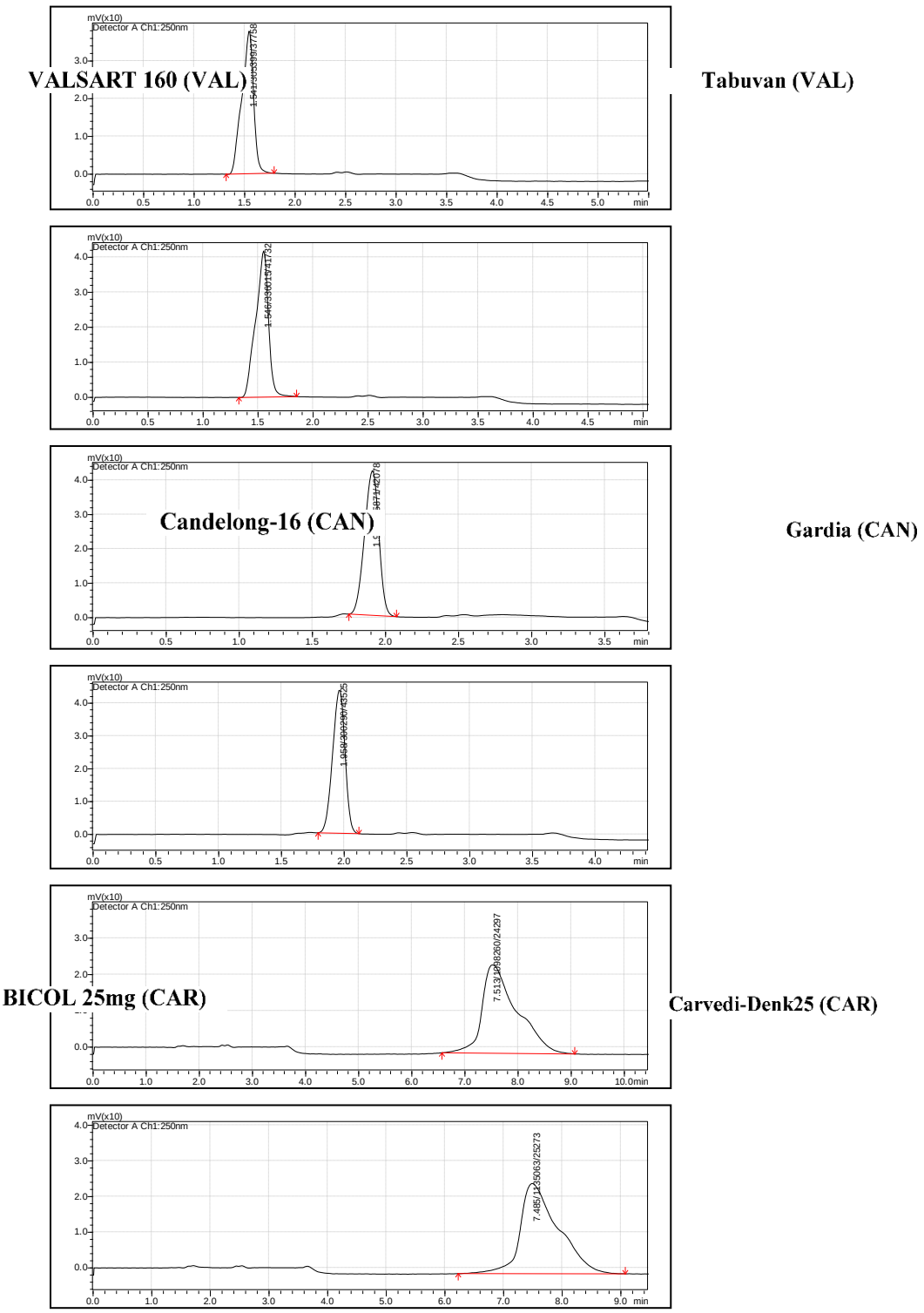


Figure 10**.** Overlaid chromatograms of commercial pharmaceutical formulations.

A comparison with previously reported analytical methods.

In comparison with the recently reported chromatographic approaches (2024-2025), the suggested RP-HPLC technique possesses a number of substantial benefits. The described approach enables the simultaneous detection of VAL, CAN, and CAR within a shorter period of time (analysis time < 9.5 min) based on the application of the isocratic elution approach, thus avoiding more complex and solvent-consuming gradient elution conditions. On the contrary to a great number of previously described methods that were performed by utilizing acetonitrile as an organic component, the current approach was accomplished with the use of a methanol-water mixture (84:16, v/v). Methanol is considered to be less toxic, less expensive, and environmentally acceptable than acetonitrile. The use of wavelength programming in addition improved the sensitivity and selectivity of the method without changing mobile phase composition during the course of the analysis. Moreover, the developed analytical approach was characterized by high linearity ($R^2 \geq 0.9966$), moderate sensitivity (LOD 0.17-0.55 µg/mL), and sufficient recovery rate (101-104%). These features, along with a high AGREE value (0.83) and a perfect Eco-Scale score show that the suggested method can be considered to be greener, simpler, and cheaper than previous techniques.

Table 10. Comparison with published methods.

Drug(s)	Analytical method	Column used	Analysis time	Linear range (µg/mL)	LOD (µg/mL)	LOQ (µg/mL)	Ref.
Valsartan, Candesartan, Carvedilol	RP-HPLC with wavelength programming	C18 (250 × 4.6 mm, 5 µm)	< 9.5 min	1-25 for each drug	VAL: 0.5539; CAN: 0.2228; CAR: 0.1716	VAL: 1.6785; CAN: 0.6751; CAR: 0.5201	
Hydrochlorothiazide, Captopril, Valsartan, Atorvastatin, Bisoprolol, Amlodipine, Carvedilol, Vincamine	HPLC-DAD	C18 column	NR	VAL: 2-200; CAR: 0.5-50	0.11-0.57	0.34-1.72	16
Valsartan, Candesartan and five other ARBs	HPLC method transfer	C18 (250 × 4.6 mm, 5 µm)	….	VAL: 1-30; CAN: 1-30	0.04 for each drug	0.16 for each drug	17
Candesartan, Valsartan, Carvedilol and other antihypertensive drugs	UPLC-MS/MS in human plasma	C18 (2.1 × 50 mm, 1.7 µm)	5 min	0.1-200 for each drug	CAN: 0.02; VAL: 0.005; CAR: 0.01	CAN: 0.06; VAL: 0.015; CAR: 0.03	18
Valsartan	Stability-indicating RP-HPLC	Agilent C18	….	0.1-50	0.0291	0.0897	19
Carvedilol	RP-HPLC	C18 (250 × 4.6 mm, 5 µm)	4.18 min	5–30	0.61	1.86	20

Greenness assessment of the proposed RP-HPLC method

The eco-friendly nature of the proposed RP-HPLC method was assessed using green analytical chemistry indices such as the Green Analytical Procedure Index (GAPI), the Analytical Greenness Metric (AGREE) [21], and the Analytical Eco-scale [31]. The use of these indices has become common in the evaluation of the greenness of analytical methods used in pharmaceutical analysis during the last years [22-30], including the analysis method proposed here for the simultaneous determination of valsartan, candesartan, and carvedilol [31]. GAPI offers a graphical depiction of the whole analytical process from sample preparation to determination based on three colors (green, yellow, red) which indicate a low, medium, and high environmental impact, respectively [32]. According to GAPI, the proposed analysis shows high eco-friendliness in most of the analytical processes, as illustrated by the green areas in Figure 12, since the method utilizes methanol (safer than acetonitrile), isocratic elution, a short analysis period (<9.5 min), and minimal waste [22,24,25,27,28]. The quantitative score that was calculated by AGREE using 12 principles of Green Analytical Chemistry was 0.83 for the suggested method [21]. Such a high score reflects good environmental properties of the method and it is explained by the following green characteristics of the process, such as low solvent volume (11.4 mL/run), utilization of less toxic methanol than acetonitrile as an organic modifier, fast analysis time (9.5 min), isocratic mode (which means there is no need to employ gradient mode resulting in greater solvent volume being used), low energy consumption thanks to short run time and reasonable column temperature (40°C), and simplicity of the sample preparation process without hazardous solvents [23,38,29,30].

The above value for the AGREE score can be considered equal to or better than those obtained for some other green analytical methods for antihypertensive drugs [24,25,28,29]. In the case of the Analytical Eco-Scale, a quantitative measure is provided by penalty point allocation, which takes into account the risk of reagents, energy expenditure, generation of wastes, and the risk of the working environment. Subsequently, the penalty points are deducted from 100 to obtain the Eco-Score [31]. As evident from Table 12, the method developed in the present study resulted in an Eco-Score of 93, belonging to the "excellent green analysis" category (>75).

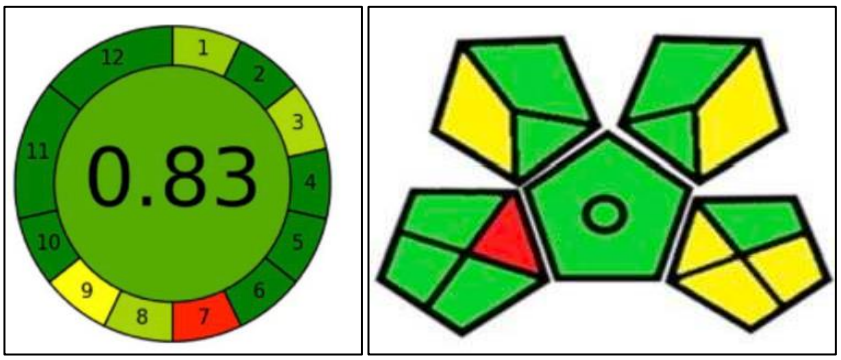


Figure 11. Green Analytical Chemistry assessment of the proposed isocratic RP-HPLC method using AGREE and GAPI metrics for simultaneous analysis of VAL, CAN, and CAR.

Table 11. Analytical Eco-Scale Assessment of the Proposed RP-HPLC Method.

Parameter	Penalty Points
Methanol (HPLC grade, ~9.6 mL per run)	4
Water (deionized, ~1.8 mL per run)	1
HPLC instrument (pumps, detector, column oven)	1
Energy consumption (short run time < 9.5 min, 40°C)	1
Waste generation (total waste < 15 mL per analysis)	1
Occupational hazard (minimal with standard lab practice)	0
Total penalty points	8
Eco-Score (100 – total penalty)	92

Justification of solvent and reagent selection

Even though methanol is not considered green when compared to water or ethanol, there are some aspects that compensate for its usage. To begin with, only a very small volume of this solvent (9.6 mL) is required in the analysis because of the reduced analysis time and isocratic elution. Methanol is chosen rather than acetonitrile because it is less toxic, less costly, and environmentally unfriendly. Moreover, the composition of the mobile phase, consisting of 84% methanol and 16% water, implies a lower volume of the organic solvent than that required by methods involving 100% organic or acetonitrile-containing mobile phases. Finally, the waste volume in the process is also small (11.4 mL). It should be noted that methanol is used in the method to provide sufficient solubility, separation efficiency, and UV detection of VAL, CAN, and CAR. The green nature of the method proved by an AGREE score (0.83) and an Eco-Score (93), implies that the positive aspects of the procedure prevail over its negative features [13].

White Analytical Chemistry (WAC) Assessment

In addition to greening indices, the methodology was also assessed using the concept of White Analytical Chemistry (WAC) with respect to the balance among environmental, technical, and operational aspects [33]. As seen from Table 6, the RP-HPLC method has a rather favorable profile in terms of WAC due to its simplicity (isocratic elution mode), cheapness (use of methanol instead of acetonitrile), small solvent volume consumption (~11.4 mL/analysis run), fast analysis (<9.5 min), high selectivity and resolution, sufficient sensitivity (LOD 0.17-0.55 µg/mL), as well as good accuracy (recovery 101-104%). These characteristics demonstrate its suitability for practical implementation in quality control laboratories. Taken together, the findings of GAPI, AGREE, Analytical Eco-Scale, and White Analytical Chemistry analysis reveal the environmentally friendly nature of the proposed method, which is fully compliant with the tenets of Green Analytical Chemistry [13]. Unlike other methods that use acetonitrile, gradient elution, or longer run times, the proposed RP-HPLC technique represents a more environmentally friendly approach to the analysis of VAL, CAN, and CAR in pharmaceuticals [22,27,29,30].

CONCLUSION

The aim of the research work was the development of an effective, accurate, and reliable RP-HPLC method for determination of VAL, CAN, and CAR in pure form as well as in their pharmaceutical dosage forms. Optimization of the HPLC procedure was carried out through investigation of the various chromatographic conditions such as composition of

the mobile phase, flow rate and column temperature. Introduction of the concept of wavelength programming improved the efficiency of the separation process, and thus increased the sensitivity of the measurement without changing the mobile phase composition. The method developed exhibited excellent sensitivity, accuracy, and reliability. This is evidenced by high correlation coefficients, recovery values, and low relative standard deviation (RSD). Application of the method on pharmaceutical samples provided a close match between the labeled and measured values. It is therefore concluded that the method can be successfully applied in quality control analysis.

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