RP- HPLC-DAD Method for Simultaneous Determination of Carvedilol and Pravastatin Sodium in their Binary Mixture

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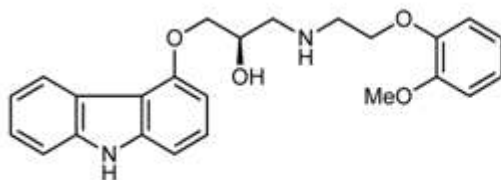General Administration and Medical Affairs, Zagazig University, Egypt

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Abstract: *This new study described a simple, sensitive and rapid isocratic chromatographic separation for simultaneous determination of carvedilol and pravastatin sodium in their binary mixture. A poroshell 120 EC. C18 column and a mobile phase composed of 0.04 mol L^{-1} phosphoric acid: acetonitrile: triethylamine of (40:60:0.15 v/v/v) were applied in the chromatographic separation of the selected drugs using diode array for peaks detection and confirmation. The mobile phase was pumped at a flow rate of 1.0 mL min^{-1} and the peak areas of the analytes were quantified at 238 nm and 240 nm with retention times of 2.19 and 2.82 min, for carvedilol and pravastatin sodium, respectively. The suitability of the chromatographic method was investigated and the constructed calibration graphs were found to be linear over the concentration ranges of 5-120 µg mL^{-1} (r = 0.9997) and 2-100 µg mL^{-1} (r = 0.9995) for carvedilol and pravastatin sodium drugs with lower detection and quantification limits of 2.59 and 0.16 for carvedilol and 3.86, 0.98 µg mL^{-1} for pravastatin sodium, respectively. The proposed method was employed to prove its selectivity as well as specificity by the detection of the two drugs from their laboratory binary mixtures.*

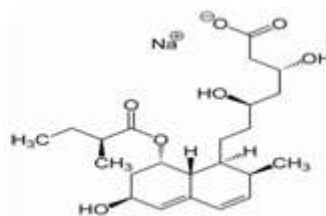**Keywords:** Carvedilol, Pravastatin sodium, RP-HPLC-DAD, Pharmaceutical formulations

1. Introduction

Carvedilol (CRV) is chemically known as 1-(9H-Carbazol-4-yloxy)-3-[2(2-methoxy phenoxy) ethyl amino] propan-2-ol (Figure 1a). It is reported as a beta-blocker which used in the treatment of hypertension and congestive heart failure [1].

Additionally, when used with other medications it improves the blood flow and decrease blood pressure. Owing to the high blood pressure which can cause brain, heart and kidney damage, it should be controlled by blocking the adrenergic receptors on arteries and causes relaxation of blood vessels and decrease the blood pressure [2].

Figure 1: Chemical structures of (a) carvedilol and (b) pravastatin sodium

Several methods have been published describing the spectroscopic detection of carvedilol in different matrices such as spectrophotometry [3-5] and spectrofluorimetry [6]. The electrochemical behavior of carvedilol was determined using voltammetry [7, 8] and potentiometry [9].

Pravastatin sodium (PVS) as depicted in (Figure 1b) is a member of lipid-lowering drugs named statins. Its chemical description known as hexahydro-6-hydroxy -2-methyl-8-(2-methylbutyryloxy)-1-naphthyl-3,5-dihydroxyheptanoate. It is recommended to reduce the risk of heart attack, stroke and death by inhibiting the cholesterol biosynthesis [10].

Many analytical methods have been addressed for the quantification of PVS in its dosage forms and biological samples such as HPLC-UV detection [11-13], reversed phase HPLC [14], HPLC-MS/MS [15], capillary gas chromatography coupled with mass spectrometry [16],

capillary zone electrophoresis [17], high-performance thin layer chromatography [18], spectrophotometry [19-21], Infrared and Raman spectroscopy [22] and square wave voltammetry [23]. The previous literature review revealed no articles concerned with the stability indicating studies, the separation and resolution of the two drugs in their binary mixture.

Due to, the combination of antihypertensive and the cholesterol reducing agents was considered as a very important strategy for treatment of heart diseases, the aim of the present study is to introduce a new simple and validated RP-HPLC study for the separation and detection of both CRV and PVS. The method was also, employed for detection of the investigated drugs in their laboratory mixture. Method validation was employed to evaluate the suitability of the developed chromatographic separation method with respect to the ICH guidelines [24].

2. Experimental

2.1. Instruments

The chromatographic separation using RP-HPLC-DAD system was applied for simultaneous detection of CRV and PVS in their binary mixture. Agilent 1200 series (Agilent Technologies, Santa Clara, CA, USA) was used which comprises of different connected parts such as quaternary pump, diode array, vacuum degasser and multiple wavelength detectors G1315 C/D and G1365 C/D). The system was PC controlled and Agilent ChemStation Software was applied. Poroshell 120 EC. C18 (4.6×250 mm, 2.7 μm particle size) and a Rheodyne manual injector with a 20 μL loop were used.

2.2. Chemicals and reagents

The pure grade CRV was provided as a gift from (Glaxosmithkline, Jeddah, Saudi Arabia), PVS was kindly supplied by Tokyo chemical industry Co., Japan. Sigma-Aldrich Chemie GmbH, Buchs, Switzerland supplied HPLC-grade of methanol, acetonitrile, triethylamine and HPLC pure water. Hydrochloric acid, hydrogen peroxide and orthophosphoric acid were purchased from (Winlab, London, UK). The pharmaceutical formulations Carloc® 25 mg cavedilol/tablet and Pravachol® 80 mg pravastatin sodium /tablet were purchased from local drug stores.

2.3. Preparation of analytical solutions

2.3.1. Preparation of CRV and PVS standard solutions
Stock solution (1000 μg mL^{-1}) of each CRV and PVS were prepared by dissolving 100 mg of each drug separately in 100-mL volumetric flask using methanol. Serial dilutions 5-120 and 2-100 μg mL^{-1} for CRD and PVS respectively, were prepared using the mobile phase.

2.3.2. Preparation of CRV and PVS commercial tablet solutions
Not less than twenty tablets of each Carloc® 25 mg cavedilol/tablet and Pravachol® 80 mg pravastatin sodium /tablet was finely powdered and mixed well. Accurate amount of each drug equivalent to prepare 1000 μg mL^{-1} was dissolved in methanol and sonicated for 10 min. Working solutions in the range of 5-120 and 2-100 μg mL^{-1} were prepared by diluting different aliquots using the mobile phase.

2.3.3. Preparation of CRV and PVS laboratory mixture
The proposed RP-HPLC-DAD method for separation and determination of the investigated drugs CRV and PVS was applied to detect both drugs in their laboratory prepared mixtures. Ten mixtures of CRV: PRV (5:5, 5:10, 25:25, 10:50, 20:30, 40:20, 20:100, 60:90, 100:120, and 30:80 v/v) were prepared and subjected to analysis using the proposed method.

2.4. General chromatographic procedure

The chromatographic separation was carried out using a mobile phase of acetonitrile: 0.04 mol L^{-1} orthophosphoric acid: triethylamine (40:60:0.15 v/v/v) which was filtered prior to use by a 0.22 μm pore size filter. The applied flow rate was 1.0 mL min^{-1} and a volume injection of 20 μL. Diode array detector was used for monitoring the eluent of the selected drugs at 238 and 240 nm for CRV and PVS, respectively. All measurements were applied at ambient temperature 25°C. Under optimum chromatographic conditions each concentration was injected three times and the chromatograms were recorded. Then, the calibration graphs were constructed by plotting the peak areas vs. the corresponding concentrations.

3. Results and Discussion

3.1. Optimization of chromatographic conditions

Isocratic chromatographic separation and determination of CRV and PVS by diode array detection was described to improve suitable, simple and reliable analytical process for detection of both drugs in their intact drugs, commercial tablets and laboratory-prepared mixtures. The optimization of the chromatographic separation should be based on the most important target which is the achievement of good resolution and symmetric peaks in acceptable time.

To select the suitable stationary phase, various reversed phase columns (Poroshell 120 EC. C18 (4.6×250 mm, 2.7 μm particle size), Zorbax Eclipse XDB-C18 (4.6×150 mm, 5 μm particle size), Raptor ^{TM} C18 Column (150×4.6 mm, 5 μm particle size), and Waters Symmetry-C18 (3.9×150 mm, μm particle size) were examined. Several trials revealed that all the tested columns could resolve the analytes under analysis, however Poroshell 120 EC. C18 (4.6×250 mm, 2.7 μm particle size) displayed better resolution peaks within short time. Therefore, it was selected as suitable stationary phase for further studies.

To optimize the applied mobile phase, several mobile phases were tried in order to separate the selected drugs CRV and PVS including aqueous and organic modifiers. Different ratios of acetonitrile: methanol, acetonitrile: phosphoric acid: triethylamine and acetonitrile: water (v/v) were tried and it was found that the best separation with reasonable retention time, high resolution and symmetric peak areas was obtained by using a combination of acetonitrile: 0.04 mol L^{-1} phosphoric acid: triethylamine (40:60:0.15 v/v/v) as a mobile phase. However, when using methanol or water we found that the retention time was increased with poor peak shape in some obtained chromatograms.

Isocratic elution of different combined proportions of acetonitrile: 0.04 mol L^{-1} phosphoric acid: triethylamine (v/v/v) was tested and it displayed a satisfactory separation with good resolution between the successive CRV and PVS peaks at reasonable retention times. Therefore, no need for applying gradient program and the isocratic elution of mobile phase was selected in all measurements.

The use of multiple wavelength detectors such as diode array offers many advantages including the ability to measure each analyte in its maximum wavelength, improves the sensitivity and enhances the HPLC power. Thus diode array was applied for detection of CRV and PVS at 238 and 240 for the previously mentioned drugs, respectively. The

aforementioned optimized conditions were applied for detection of the investigated drugs and Figure 2, displayed a typical chromatogram for separation of the two drugs with good resolution (R_s) which evaluated by measuring the
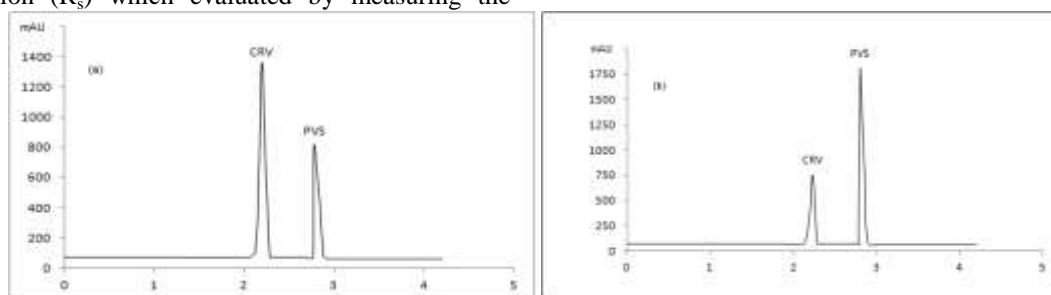
degree of separation between two adjacent peaks. Table 1, listed the obtained data for resolution, selectivity, retention times, system suitability and other parameters.

Figure 2: Typical RP-HPLC-Chromatogram of 20 µL injection of a mixture containing 50 µg mL^{-1} of each CRV and PVS at 238 and 240 nm, respectively

Table 1: System suitability parameters for the RP-HPLC-DAD detection of a mixture of CRV and PVS

Parameter	CRV	PVS
$t_R \pm SD$ (min)	2.19±0.015	2.82±0.022
Capacity factor (k')	1.73	2.52
Theoretical plates (N)	7674	15708
Selectivity (α)	1.46	---
Resolution (R_s)	1.65	---
Peak asymmetry (As)	1.02	1

3.2. Method validation

3.2.1. Linear concentration ranges

The proposed RP-HPLC-DAD method was applied to detect a series of different concentrations of each drug to evaluate the linear concentration range. Least square treatment of the obtained calibrated data was used to calculate the linearity. Table 2, presented the performance characteristics of the analytical separation of CRV and PVS. The calculated regression equations were Y = 12.16C + 376.60 (r = 0.9997) and Y = 12.26C + 118.41, (r = 0.9995) for CRV and PVS, respectively.

Table 2: Analytical data obtained from the determination of CRV and PVS mixture using RP-HPLC-DAD method

Parameter	CRV	PVS
Wavelength (nm)	238	240
Linear concentration range (µg mL^{-1})	5-120	2-100
Intercept (a)	376.6	118.41
$S_a{}^a$	5.79	6.84
Slope (b)	12.16	12.26

$S_b{}^b$	0.13	0.91
%RSD of the slope (S_b %)	1.07	0.25
Correlation coefficients (r)	0.9997	0.9995
$S_{y/x}{}^c$	30.91	42.71
LOD^d (µg mL^{-1})	2.59	0.16
LOQ^e (µg mL^{-1})	3.86	0.98

^{a}Standard deviation of intercept, ^{b}Standard deviation of slope, ^{c}Standard deviation of residuals, ^{d}Limit of detection and ^{e}Limit of quantification

3.2.2. Limits of detection and quantification

According to ICH guidelines [24], S/N = 3 and S/N = 10 were used to calculate LOD and LOQ, respectively. As indicated in Table 2, the obtained values were 2.59 and 0.16 for lower limit of detection, while, for lower limit of quantification were 3.86 and 0.98 for CRV and PVS, respectively.

3.2.3. Accuracy and precision

Intra-day and inter-day precision and accuracy of the developed RP-HPLC-DAD method for detection of CRV and PVS were evaluated using 3 concentrations of each drug under 3 replicate measurements within the day and between three successive days. The summarized results in Table 3 showed the percentage recoveries which were calculated from the regression equations. The percentage of relative standard deviation (%RSD) and percentage relative error (% E_r) were found to be less than 2% indicating high repeatability and good accuracy of the developed chromatographic separation method.

Table 3: Analytical data of accuracy and precision for the determination of CRV and PVS in bulk form using RP-HPLC-DAD method

Drug	Taken µg mL^{-1}	Intra-day			Inter-day		
		Found±SD^a µg mL^{-1}	% RSD^b	% $E_r{}^c$	Found±SD^a µg mL^{-1}	% RSD^b	% $E_r{}^c$
CRV	5	4.99±0.23	0.23	-0.20	5.09±0.84	0.84	1.80
	50	49.88±0.14	0.18	-0.24	49.98±1.29	1.30	-0.04
	100	99.74±0.67	0.67	-0.26	100.25±0.83	0.83	0.25
PVS	10	10.02±0.18	0.18	0.20	9.99±0.96	0.96	-0.10
	50	49.99±0.72	0.72	-0.02	50.05±0.11	0.11	0.10
	100	100.21±0.09	0.09	0.21	99.87±0.06	0.06	-0.13

^{a}Mean± SD of three determinations ^{b}% Relative standard deviation ^{c}% Relative error

3.2.4. Specificity and interference study

To evaluate the specificity of the developed RP-HPLC-DAD method for detection of CRV and PVS, several

pharmaceutical compounds with different pharmacological actions were investigated with respect to the resolution and retention time in the presence of the two selected drugs. The

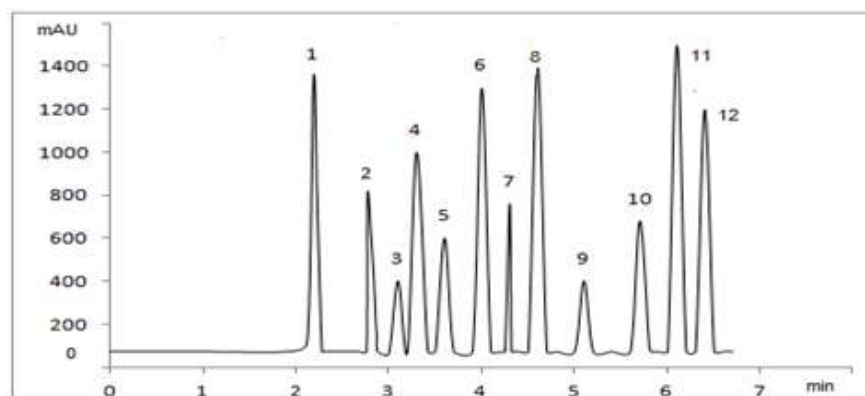chromatogram of ten compounds was recorded under the same chromatographic conditions as indicated in Figure 3.

Figure 3: RP-HPLC-chromatogram of 20 µL injection of a mixture of (1) CRV, (2) PVS, (3) propranolol, (4) atenolol, (5) paracetamol, (6) simvastatin, (7) amlodipine, (8) ezetimibe (9) candesartan, (10) nifedipine, (11) valsartan, (12) telmisartan

Moreover, the retention time for each compound was reported in Table 4. The obtained results revealed the suitability of the proposed RP-HPLC-DAD method for monitoring study of various pharmaceutical compounds.

3.2.5. Robustness
The robustness of the proposed method was examined by introducing small but deliberated changes in method parameters such as the injection volume ± 0.1 mL, the wavelengths ±1 nm, concentration of the phosphoric acid solution 0.04 ± 0.005 mol L^{-1} and the flow rate ±0.1 mL min^{-1}. Under these changes of method parameters we can deduce that there is no significant influence on the chromatographic resolution, measured peak areas and retention time. Furthermore, the % RSD under such conditions was found to be less than 2%.

Table 4: The retention times of the interference compounds using RP-HPLC-DAD method, the detection carried out at 360 nm

Interference compound	Retention time (min)
Propranolol	2.85
Atenolol	3.32
Paracetamol	3.61
Simvastatin	4
Amlodipine	4.32
Ezetimibe	4.69
Candesartan	5.11
Nifedipine	5.78
Valsartan	6.2
Telmisartan	6.43

3.2.6. Stability of solution
The stability of the stock solution of both drugs in methanol and the working solutions of them were investigated with respect to the retention time, resolution and the peak areas within two days. It was observed that no changes in recorded chromatograms at room temperature. The stock solutions of CRV and PVS were stored stable in 4 ºC in refrigerator for two weeks.

3.2.7. Analysis of laboratory-prepared mixtures

The proposed RP-HPLC-DAD method was applied for detection of the selected drugs CVD and PVS in their binary mixture. Ten laboratory prepared mixtures of different ratios were prepared and subjected to analysis under the previously mentioned chromatographic procedure. The prepared ratios were (5:5, 5:10, 25:25, 10:50, 20:30, 40:20, 20:100, 60:90, 100:120, 30:80) for CRV: PVS. The mean recoveries percentage, the % RSD and % E_r were calculated and the obtained results were addressed in Table 5. Excellent data were obtained indicating satisfactory ability to resolve and detect the selected drugs in different ratios.

Table 5: Detection of CRV and PVS laboratory prepared mixtures using the proposed RP-HPLC-DAD method

Taken (µg mL^{-1})	Found ± SD, (µg mL^{-1})		%RSD		% E_r	
CRV:PVS	CRV	PVS	CRV	PRV	CRV	PVS
5:05	4.99±0.52	5.05±0.16	0.52	0.16	-0.2	1
5:10	4.98±1.20	9.99±0.84	1.2	0.84	-0.4	-0.1
25:25:00	25.03±0.29	24.97±1.32	0.29	1.32	0.12	-0.12
40:20:00	40.95±0.35	20.04±0.68	0.34	0.65	2.37	0.2
20:30	19.86±0.66	29.69±0.16	0.66	0.16	-0.7	-1.03
10:50	9.89±0.08	49.42±0.25	0.08	0.25	-1.1	-0.01
0.465277778	10.07±0.96	69.89±0.57	0.96	0.57	0.7	-0.16
60:80	60.05±0.18	80.01±0.76	0.18	0.76	0.08	0.01
25:80	24.97±0.54	79.96±0.26	0.54	0.26	-1.2	-0.05
25:80	25.02±0.15	79.99±0.34	0.15	0.34	0.08	-0.01

3.2.8. Analysis of CRV and PVS in commercial dosage forms
The binary mixture of CRV and PVS in their tablet solution was tested using the proposed RP-HPLC-DAD method. Figure 4, represented the chromatograms recorded at the specific retention time of both drugs. It was clear that no interferent peaks observed from the coformulated inactive ingredients. The data summarized in Table 6, was statistically treated and compared with a spectrophotometric method based on detection of CRV in acetonitrile: water (60:40 v/v) at 332 nm [3], however, for PVS the detection was carried out in presence of distilled water at 240 nm [18].
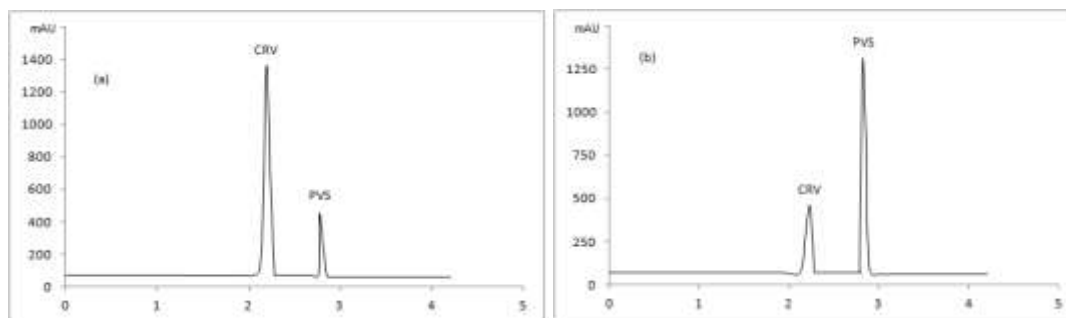
Figure 4: RP-HPLC-DAD chromatogram of 20 µL injection of mixture containing 50:50 µg mL^{-1} of CRV and PVS tablets, the detection carried out at 238 and 240 nm, respectively

Table 6: Application of the proposed RP- HPLC-DAD method for the analysis of a mixture of CRV: PVS in commercial tablets

Tablet Mixture	Taken µg mL^{-1}	Proposed method CRV		Proposed method PVS		Reference method [3] CRV			Reference method [18] PVS		
		Found µg mL^{-1}	% Recovery	Found µg mL^{-1}	% Recovery	Taken µg mL^{-1}	Found µg mL^{-1}	% Recovery	Taken µg mL^{-1}	Found µg mL^{-1}	% Recovery
CRV:PVS	5:2	4.98	99.6	2.00	100.0	5	5.00	100.0	2	1.97	98.50
	10:10	9.99	99.9	9.78	97.8	10	9.95	99.5	8	7.99	99.87
	30:30	30.01	100.3	29.99	99.9	20	19.98	99.9	10	9.96	99.60
	50:50	49.96	99.9	49.85	99.7	30	29.84	99.5	12	11.85	98.75
	80:80	79.88	99.8	79.68	99.6	50	49.88	99.8	14	14.00	100.00
	120:100	120.0	100.0	98.84	98.8	80	79.84	99.8	18	17.95	99.72
Mean±SD		99.92±0.23		99.30±0.85		99.85±0.20			99.41±0.63		
n		6		6		6			6		
Variance		0.05		0.72		0.04			0.39		
%SE**		0.09		0.35		0.08			0.26		
%RSD		0.23		0.85		0.20			0.63		
t-test		0.581(2.228)*		0.252(2.228)*							
F-test		1.25(5.05)*		1.85(5.05)*							

* Figures in parentheses are the tabulated values of t-and F- testes at 95% confidence limit [25]   **%SE= SD/√n

4. Conclusion

This study, introduced a new RP-HPLC-DAD method for separation and determination of CRV and PVS in their binary mixture and commercial tablets. The chromatographic method based on the development of simple, rapid and reproducible procedure for quantification of the investigated drugs with high sensitivity and selectivity. The obtained results proved that the developed method is suitable to resolve and quantify both selected drugs in their laboratory-prepared mixtures with good resolution and acceptable short retention time. Furthermore, the recorded results indicated high precision and accuracy.

Acknowledgment

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