Pharmaceutical Sciences
September 2019, 25(3), 262-267
doi: 10.15171/PS.2019.30
https://ps.tbzmed.ac.ir/

Research Article

Spectrofluorimetric Determination of Atenolol and Carvedilol in Pharmaceutical Preparations after Optimization of Parameters Using Response Surface Methodology

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Article Info

Article History:
Received: 3 November 2018
Revised: 2 January 2019
Accepted: 23 January 2019
ePublished: 30 September 2019

Keywords:
-Atenolol
-Carvedilol
-Spectrofluorimetry
-Experimental design
-Pharmaceutical preparations

ABSTRACT

Background: The present work is aimed to study the effect of different parameters on the fluorescence intensity of atenolol (ATE) and carvedilol (CAR) and optimization by response surface methodology (RSM) to provide a simple analytical method for quantification of ATE and CAR in their pharmaceutical formulations.
Methods: Various parameters affecting the fluorescence intensity, i.e., sodium dodecyl sulfate (SDS) concentration, pH, volume fraction of solvents were optimized using RSM. Then, the optimized parameters were applied to the validation of a method for fluorimetric determination of β-blockers in their pharmaceutical preparations.
Results: It is obtained that under the optimum conditions for determination of ATE, the method provided a linear range between 130 to 750 ng/mL with a coefficient of correlation (r) of 0.9996. Also, the limit of detection and limit of quantification (LOD and LOQ) were 40 ng/mL and 130 ng/mL, respectively. Moreover, it is observed that, the linearity of method for determination of CAR was between 0.37 to 4.0 ng/mL and LOD and LOQ of method were 0.11 ng/mL and 0.37 ng/mL, respectively.
Conclusion: An accurate, sensitive and reliable spectrofluorimetric method was developed anf successfully used to determine the (ATE) and carvedilol (CAR) in their pharmaceutical preparations.

Introduction

Hypertension is a growing medical concern worldwide. ATE is (RS)-2-{4-[2-hydroxy-3-(propan-2-ylamino) propoxy] phenyl} acetamide (Figure 1a). ATE is a selective β1 receptor antagonist, a drug belonging to the group of beta blockers (β-blockers), used primarily in cardiovascular diseases.[1]

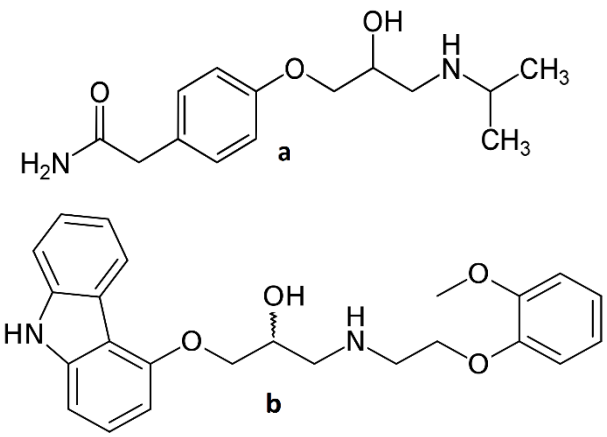

Figure 1. Chemical structure of ATE (a) and CAR (b).

CAR ((2RS)-1-(9H-carbazol-4-yloxy)-3-[[2-(2-methoxy-phenoxy)ethyl]amino]propan-2-ol) (Figure 1b) is a potent non-selective β-blocker and is widely used to treat a variety of cardiovascular ailments, including hypertension, heart failure and left ventricular dysfunction following myocardial infarction.[2,3] ATE and CAR have been determined in pure and pharmaceutical preparations using techniques such as high performance liquid chromatography (HPLC),[3-6] spectrophotometry,[6-9] electrochemical,[10-14] resonance Rayleigh scattering[15] chemiluminescence[16] and capillary electrophoresis methods.[17] Fluorescence spectrometry due to its low cost as well as great sensitivity and selectivity is widely used in quantitative analysis of different materials such as drugs,[18-22] thus several spectrofluorimetric methods have been proposed for the determination of ATE and CAR in their pharmaceutical preparation.[22-29]

In practice, usually trial and error or simple optimization such as one parameter at a time is used to find the best analytical conditions, but these are time-consuming procedures. Multivariate experimental design strategies are useful methods for the optimization of a response, i.e., the experimental conditions that produce the best results.

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These methods are considered to obtain the optimized response for an analytical performance or an extraction method and development of a formulation in pharmaceutical sciences.[30-32] Response surface methodology (RSM) is the common statistical approach based on the fit of a polynomial equation (in order to find the critical point) to the experimental data, which can be expressed by the following equation:

$$Y = \beta_0 + \sum_{i=1} \beta_i x_i + \sum_{i=1} \beta ii x_i^2 + \sum_{i=1} \sum_{j=1} \beta_{ij} x_i x_j \quad \text{Eq. (1)}$$

where, Y is the response function, x_i and x_j are the independent variables, β_0 is the intercept and βi, βij and βii are the linear, interaction, and quadratic parameters of the model, respectively.[30-32]

The objective of the study is to investigate the effect of different factors on the fluorescence of β-blockers by using experimental design methods and providing an analysis method based on the optimal conditions for the determination of ATE and CAR.

Materials and Methods

Apparatus

Fluorescence intensity measurements were performed using a Shimadzu RF-5301 PC spectrofluorophotometer, equipped with a 150 W Xenon lamp in 1-cm path length quartz cell. The excitation and emission slits were fixed at 5 nm. pH was adjusted using a Metrohm Model 744 pH meter (Herisau, Switzerland).

Reagents

ATE and CAR were obtained as gifts from Pars Darou Co. (Tehran, Iran) and Salehan Chimi Co. (Tehran, Iran), respectively. Disodium hydrogen phosphate, sodium dodecyl sulfate (SDS), sodium hydroxide (NaOH), hydrochloric acid (HCl) and solvents such as methanol, ethanol, acetonitrile, aceton and sulfuric acid were obtained from E. Merck (Darmstadt, Germany). All of the other applied materials in this study were analytical grade. A stock standard solution of ATE and CAR at a concentration of 1000 µg/mL was prepared by dissolving an appropriate amount of each drug in 10 mL of water and methanol, respectively, and diluting to 25 mL with double distilled water. These solutions were stored under dark conditions in refrigerator when not in use for three months. These stock solutions were diluted consecutively for daily preparation of working standard solutions. SDS (1.0 mol/L) was prepared by dissolving an appropriate amount of this compound in 10 mL deionized water and diluting to 25 mL with this water.

For the preparing of the standard buffer solution (0.1 M), 1.65 g of disodium hydrogen phosphate (Reidel-de Hean, Berlin, Germany) was transferred to 100 mL beaker and dissolved in deionized water up to 100 mL. Then buffers with pHs in the range of 2 and 12 were prepared by transferring of an appropriate volume of this solution to another beaker and adjusting to pHs of 2, 4, 7, 10 and 12 by adding 1 mol/L solutions of HCl or NaOH.

Recommended procedure for calibration

An aliquot of the sample solution containing ATE in the range of 0.13-0.75 µg/mL or CAR in the range of 0.37-4.0 ng/mL were transferred into 15-mL calibrated centrifuge tubes. After addition of other reagents, e.g. 70 and 130 mM of SDS, 75% and 25% v/v of ethanol and methanol and adjusting of pH to 4.7 and 4.0 (with phosphate buffer), in the case of ATE and CAR, respectively, the content of each tube was mixed well and diluted to 10 mL with deionized water. The fluorescence intensity of resultant solutions was measured at 302±3 and 340±3 nm while excited at 274±3 and 286±3 nm, respectively, against reagent's blank prepared in similar way.

Preparation of pharmaceutical formulations

Ten ATE (Raha, Isfahan, Iran) and CAR (Jalinous, Tehran, Iran) tablets, each containing 50 and 6.25 mg ATE and CAR, respectively, were accurately weighed individually and finely powdered. Powdered sample containing 5 mg ATE and 6.25 mg CAR were weighed and placed into a 25-mL beaker, dissolved with the use of stirring (for 10 min) in 25-mL deionized water and methanol, respectively. The solution was then filtered and transferred into a 50-mL volumetric flask. The residue was washed in enough water or methanol and the solution was finally made up to the mark with double distilled water or methanol, respectively. Thus, a 100 µg/mL solution of ATE and 125 µg/mL solution of CAR were obtained, respectively. These solutions were diluted to obtain 10 and 0.04 µg/mL solutions of ATE and CAR, respectively, then 100 and 200 µL portions of these diluted solutions used for the analysis of ATE and CAR or recovery experiments, respectively.

Optimization of parameters using RSM

The RSM method identifies the relationships between independent and dependent variables (based on the fit of a polynomial equation to the experimental data) and indicates the way to obtain an optimal response). In addition, the secondary goal of RSM is to extract the maximum amount of information with the minimum expenditure of resources. The range of optimized parameters was selected according to preliminary studies. The experimental design in this study was performed by RSM where the experiments were designed by central composite approach using Minitab 17 software. The optimized condition by experimental design was used to prepare ATE and CAR solutions with different concentrations to obtain the calibration curve. Moreover, the accuracy and precision were checked.

Results and Discussion

Optimization of parameters by RSM

The range of optimized parameters and the best organic solvent was selected according to previous studies for CAR[26] and preliminary studies for ATE. The following ranges were selected: pH 4-10 for both drugs, 15 to 50 mM and 3 to 10 mM of SDS for ATE and CAR,

respectively, and 25-70% (v/v) of organic solvent (ethanol for ATE and methanol for CAR).

Table 1. Twenty experiments designed by central composite design and the corresponding fluorescence intensity for 300 ng/mL of ATE.

Code	pH	SDS(Mm)	Ethanol (v/v %)	F
1	12.0	32.5	47.5	375
2	7.0	32.5	47.5	460
3	7.0	32.5	47.5	438
4	4.0	50.0	25.0	470
5	7.0	3.1	47.5	382
6	7.0	32.5	47.5	455
7	7.0	32.5	47.5	501
8	10.0	50.0	25.0	424
9	7.0	32.5	47.5	491
10	2.0	32.5	47.5	476
11	7.0	32.5	85.3	587
12	10.0	15.0	70.0	440
13	4.0	50.0	70.0	568
14	4.0	15.0	70.0	469
15	7.0	32.5	47.5	401
16	10.0	50.0	70.0	523
17	4.0	15.0	25.0	337
18	7.0	32.5	9.7	378
19	10.0	15.0	25.0	283
20	7.0	61.9	47.5	521

Table 2. Twenty experiments designed by central composite design and the corresponding fluorescence intensity for 1 ng/mL of CAR

No.	pH	SDS	Methanol (v/v %)	F
1	4.0	10.0	70	821
2	4.0	3.0	70	863
3	4.0	3.0	25	571
4	12.0	6.5	47.5	588
5	7.0	6.5	85.3	912
6	7.0	6.5	47.5	824
7	7.0	6.5	47.5	687
8	7.0	6.5	9.66	652
9	7.0	6.5	47.5	771
10	4.0	10.0	25	919
11	7.0	6.5	47.5	762
12	7.0	6.5	47.5	747
13	7.0	0.6	47.5	585
14	7.0	6.5	47.5	728
15	7.0	12.4	47.5	852
16	2.0	6.5	47.5	757
17	10.0	10.0	70	821
18	10.0	10.0	25	852
19	10.0	3.0	70	750
20	10.0	3.0	25	501

Three independent variables, including pH, SDS concentration, and volume of organic solvent, were studied at three levels for 0.3 µg/mL and 1 ng/mL of ATE and CAR solution, respectively. Three parameters at three levels include 20 experiments that should be performed for the central composite design. After conducting the experiments, according to the values of variables and response, a second order polynomial was constructed. The central composite design matrix, with three independent parameters, is listed in Table 1 and 2, respectively.

The mean R^2 explained by the model obtained for the developed models for ATE and CAR were acceptable (R^2 = 0.90 and 0.92, respectively). Furthermore, the level of the significance of the results was checked, and nonsignificant parameters were excluded from the equation ($p > 0.1$). The following equations were obtained for ATE (Eq. 2) and CAR (Eq. 3):

$$F=216.7+13.9pH+2.866C_{SDS}+2.722\times V_{Ethanol}-1.592pH\times pH \quad \text{Eq. (2)}$$

$$F=57.4 - 1.69\times pH - 0.254\times V_{Methanol} + 7.21\times C_{SDS} + 0.02200\times V_{Methanol} \times V_{Methanol} \quad \text{Eq. (3)}$$

where F is fluorescence intensity; C_{SDS}= SDS concentration; $V_{ethanol}$ = volume fraction of ethanol and $V_{methanol}$ = volume fraction of methanol. The three applied independent parameters were significant. However, the interaction parameters and the quadratic parameters were nonsignificant, except for pH and methanol for ATE and CAR, respectively.

For ATE, a linear relation exists between SDS concentration and the volume fraction of ethanol (Figure 2b). However, Figure 2a and 2c show a nonlinear relation between pH and SDS concentration and pH and volume fraction of ethanol, respectively. Counter plots for fluorescence of CAR are illustrated in Figure 3 and show that lower pHs give the best results. Increasing the SDS and volume fraction of organic solvent can enhance the fluorescence intensity.

Based on Eq. 1 and 2 and the results from the Minitab 17 software; pH 4.7, ethanol volume fraction of 75% and SDS concentration of 70 mM for ATE and pH 4.0, methanol volume fraction of 25% and SDS concentration of 130 mM for CAR provide the maximum fluorescence intensity.

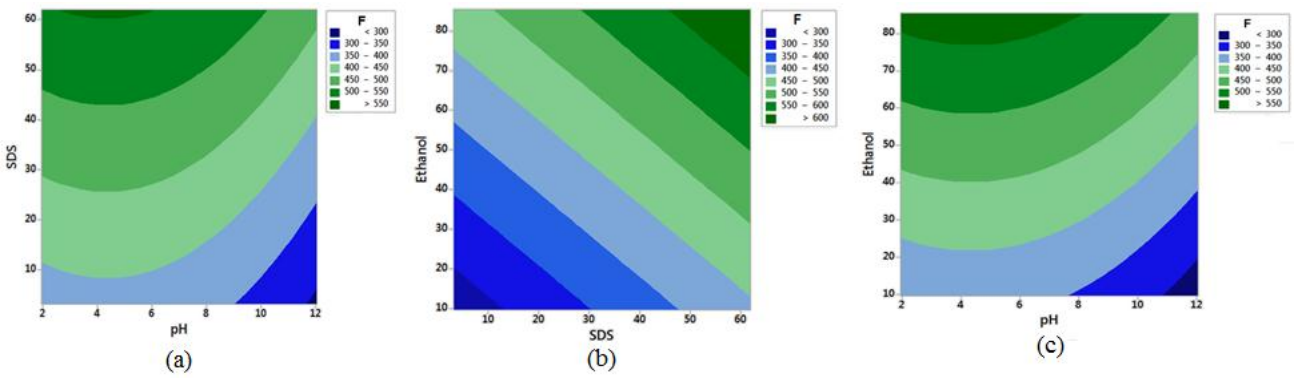

Figure 2. Effect of SDS (mM) and pH (a), ethanol (volume fraction) and SDS (mM) concentration (b) and ethanol (volume fraction) concentration and pH (c) on the fluorescence intensity of ATE.

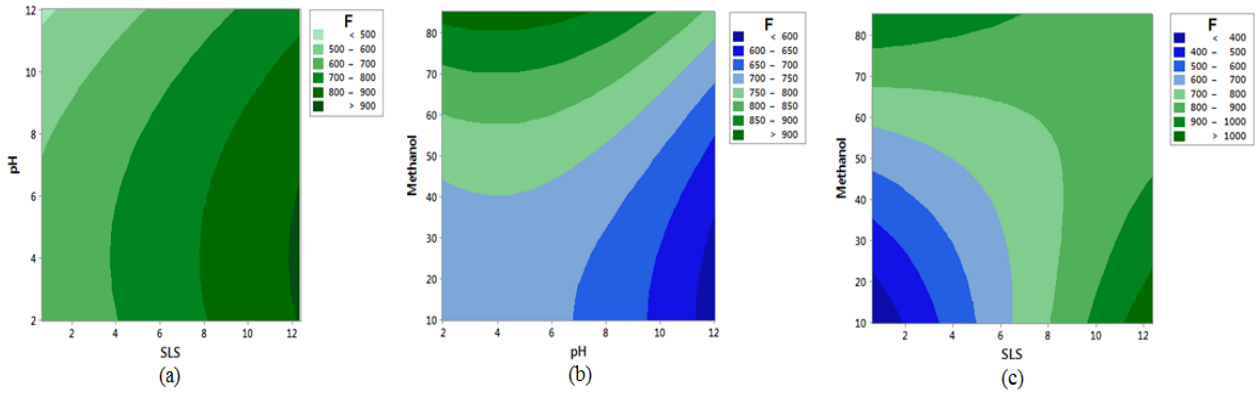

Figure 3. The effect of pH and SDS (mM) (a), methanol (volume fraction) and pH (b) and methanol (volume fraction) and SDS (mM) (c) on the fluorescence intensity of CAR.

Analytical characteristics

The calibration curves were obtained by measuring the fluorescence intensity of standard solutions of each drug. Linear relations between fluorescence intensity and concentration of each drug was found in the range of 130-750 and 0.37-4.0 ng/mL of ATE and CAR, respectively.

Table 3. Analytical characteristics of the proposed method for ATE & CAR.

Analyte	LR (ng/mL)	r	Calibration equation	LOD (ng/mL)	LOQ (ng/mL)
ATE	130-750	0.999	396.0C+13.19	40	130
CAR	0.37-4.0	0.996	111.7C+25.48	0.11	0.37

LR = linear range

The LODs and LOQs were calculated as three and ten times the standard deviation of the blank signals (S_b) and based on $3S_b/m$ and $10S_b/m$ equations, respectively, where m is the slope of the calibration curve. The characteristics of the proposed method are summarized in Table 3. The precision at each concentration level from the nominal concentration was expected to be not greater than 15% and the accuracy to be within ±15% as reported in the guidelines.[33] In order to do this, quality control (QC) samples were prepared at three concentration ranges (*e.g.* low, medium and high) and analyzed by 3 replicates on the same day.

Precision was expressed as the percentage relative standard deviations (RSD, %) and accuracy was expressed as the percentage efficiency. As can be seen in Table 4, good precisions were achieved with RSD values lower than 9% and the accuracy was better than 13.0%. These results indicated that the method met the requirements of a assay. Also, Table 5 compares the characteristic data of the present method with other similar methods used for the determination of ATE and CAR. The significant feature of the proposed method is the very low obtained LOD for CAR but the results for ATE are somewhat higher. It is also evident that the dynamic linear range, precision and recoveries achieved using the proposed method are better or comparable to those achieved using other fluorimetric methods.

Table 4. Repeatability and accuracies for the determination of ATE and CAR.

ATE Nominal C (µg/mL)	ATE determined	RSD (%)	Recovery (%)	CAR Nominal C (ng/mL)	CAR determined	RSD (%)	Recovery (%)
0.15	0.16 ± 0.002	1.25	106.7	1.0	1.09 ± 0.100	9.17	109.0
0.30	0.29 ± 0.005	1.72	96.7	2.0	2.26 ± 0.075	3.32	113.0
0.45	0.46 ± 0.005	1.09	102.2	3.0	3.24 ± 0.042	1.30	108.0

Repeatability expressed as RSD% and for 3 replicate determinations.

Table 5. Analytical characteristics of different methods used for the determination of ATE and CAR.

Method	Analyte	Concentration range (µg/mL)	r	RSD%	LOD (µg/mL)	Mean R (%)	Ref.
S	CAR	1.0-10.0	0.9999	0.89-1.57	0.20-0.33	99.5-100.0	8
S	CAR	1.0-8.0, 2.0-20.0	0.9997-0.9999	0.46-1.24	0.41-1.00	99.9-100.1	9
F	CAR	0.10-2.25	0.9998	0.54-1.30	0.024	101.0-101.6	23
F	CAR	0.01-0.25	0.9990	3.80	0.002	-	24
F & S	CAR	7.0-60.0	0.9998	0.98	0.055	98.2-100.5	25
F	CAR	0.04- 0.41	-	-	0.014	-	26
F	CAR	0.37-4.0($\times10^{-3}$)	0.9960	3.32-6.79	0.11($\times10^{-3}$)	108.0-113.0	This work
F	ATE	0.025-0.45	0.9940	0.52-0.71	0.008	98.6-113.2	22
F	ATE	0.05-4.0	0.9998	<2.97	0.015	99.3	27
F	ATE	0.01-0.40	0.9999	2.50	-	96.8-110.0	28
F	ATE	1.0-11.0	0.9999	-	0.20	100.7	29
F	ATE	0.13-0.75	0.9996	1.09-1.72	0.04	96.7-106.7	This work

S = spectrophotometry; F = spectrofluorimetry; R = recovery

The recovery experiments

Aliquot volumes of each prepared pharmaceutical preparation spiked with drug at the three test concentrations and then analyzed following the optimized procedure. For each concentration level, three repeated experiments were made and the mean values were taken. The obtained recoveries ranged from 90.0% to 110.0% and 96.0-109.5% in the case of ATE and CAR, respectively, which seem to be satisfactory (see Table 6).

Table 6. Results of recoveries of spiked samples.

Sample	Added (µg/mL)	Found ± SD (n = 3), µg/mL	R %
ATE tablet	-	0.10 ± 0.01	-
	0.05	0.15 ± 0.01	100
	0.20	0.32 ± 0.03	110
	0.50	0.55 ± 0.04	90
CAR tablet	-	2.02 ± 0.01	-
	0.50	2.50 ± 0.01	96
	1.00	3.02 ± 0.03	100
	2.00	4.21 ± 0.04	110

The application of the method

The recommended methodology was successfully applied to the determination of ATE and CAR in their pharmaceutical preparations and the results are showed in Table 7.

Table 7. Determination of the drugs in their pharmaceutical formulations.

Sample (Tablet)	Labeled amount (mg)	Found amount ± SD (mg)*	Experimental *t*-values	R%
ATE	50	50.0 ± 0.78	0.00	100
CAR	6.25	6.31 ± 0.55	0.19	101

Tabulated *t*-test at P=0.05, $t = 4.3$ (n = 3)
*Three successive determinations in the case of ATE and CAR have been done.

Conclusion

In this study application of experimental design methodologies to optimize the effective parameters on the quantification of ATE and CAR were studied. It was concluded that, the experimental design methodologies can be used to optimize the effective parameters on the quantification of ATE and CAR, especially when the parameters have effects on each other. Also, optimized method was applied to determine ATE and CAR in their pharmaceutical preparations with good accuracy and precision. Moreover, these results showed that the developed method was simple, low-cost and suitable analytical approach for the quantification of ATE and CAR in their pharmaceutical preparations.

Conflict of interests

The authors claim that there is no conflict of interest.

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