Simultaneous Determination of Atenolol and Nifedipine by Using Spectrophotometric Method with Multivariate Calibration and HPLC Method Implementing “Design of Experiment”

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Abstract

Objectives: The aim of the present work is to develop rapid and simple methods for the simultaneous determination of atenolol and nifedipine. Multivariate calibration using preprocessing methods to enhance results in case of presence of any interference in samples and RP-HPLC method for achieving a good separation with accepted system suitability parameters with the use of design of experiment for robustness testing according to Plackett-Burman design.

Methods: The spectrophotometric method is based on the measurement of the mixture in the range of 200-400 nm then applies the multivariate calibration methods for resolution of the binary mixture mainly using partial least squares (PLS) and principal component regression (PCR). The proposed RP-HPLC method utilizes an YMC-pack pro C₁₈ column (250 mm x 4.6 mm, 5 μm). Optimum chromatographic conditions were attained by application of Design of experiment (DOE). Both spectrophotometric and chromatographic methods were applied on the determination of both drugs in marketed capsules. Also, dissolution testing of these capsules was investigated.

Results: The recovery percentage for nifedipine and atenolol in capsules dosage form were found to be in PLS method (100.50 ± 0.850, 100.78 ± 1.07), PCR method (100.60 ± 0.960, 100.72 ± 1.09) and RP-HPLC method (99.77 ± 0.560, 100.90 ± 1.23); respectively.

Conclusion: The methods were validated according to ICH guidelines. All the results obtained were found to be within the acceptable limits. The methods were successful to estimate nifedipine and atenolol in bulk powder and pharmaceutical preparation.

Keywords: Nifedipine; Atenolol; Spectrophotometry; Chromatography

Introduction

Nifedipine [3, 5-dimethyl 2, 6-dimethyl-4-(2-nitrophenyl) - 1, 4-dihydropyridine-3, 5-dicarboxylate] [1]. It is a calcium channel blocker that inhibits the trans membrane influx of calcium ions into vascular smooth muscle and cardiac muscle [2]. Atenolol [(RS)-2-[4-[2-hydroxy-3-(propan-2-ylamino) propoxy] phenyl] acetamide] [1]. It is a Beta blocker that interferes with the binding to the receptor of epinephrine and other stress hormones, and weakens the effects of these hormones. Beta blockers are particularly used for the management of cardiac arrhythmias, protecting the heart from a second heart attack (myocardial infarction) after a first heart attack (secondary prevention), and hypertension [3]. The combination dosage form of nifedipine and atenolol are available in the market for the treatment of hypertension. Nifedipine and atenolol chemical structures are shown in Figure 1.

According to the international chemometrics society (ICS), chemometrics is defined as the science of relating measurements

made on a chemical system or process to the state of the system via application of mathematical or statistical methods [4]. In this study two chemometric techniques were used, the first technique is the multivariate calibration in spectrophotometry where the absorption spectra of nifedipine and atenolol have a severe overlap that could not be resolved by the simple univariate methods as shown in Figure 2. While the second one is the “Design of experiment” which is used in developing and optimizing a robust chromatographic method.

Quantitative determination using spectrophotometric methods has been greatly improved by the use of multivariate methods. The methods used were principal component regression (PCR) and partial least squares regression (PLS), both are the most popular chemometric tools used for quantitative modeling of the multidimensional spectroscopic data [5]. The method involves calibration; which is the relation between spectra and component concentrations. The validation set and the unknown set can be predicted from a set of reference samples.

Figure 1: Chemical structure of: (A) nifedipine and (B) atenolol.

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Figure 2: Absorption spectrum of nifedipine and atenolol showing the spectra overlapping.

Design of experiments (DOE) remains a core area of study in chemometrics and it is a well-established statistical method [6,7]. There are levels of design which can be applied: these range from the simplest fractional factorial (which includes experiments to identify which factors are most critical), followed by full factorial (which enables identification of significant interactions between factors), and the more complex surface area design (which facilitates optimization of factors). Accordingly, we applied the simplest design which is two-level Plackett-Burman design which is a class from a fractional factorial design to identify the most critical factors to examine the method robustness [8]. The design allows examining N-1 factors in N experiments, which save time while optimizing experimental conditions. Factorial principles were also used in designing the calibration and validation sets in multivariate calibration.

Several analytical methods have been developed for nifedipine and atenolol quantification in pharmaceuticals. The most recent methods include UV-VIS spectrophotometry [9-14] and HPLC [15-19]. The

scientific novelty of the present work is that the methods used are simple, rapid, selective, and less expensive and less time consuming compared with the other published methods. So, the aim of this work was to develop simple, sensitive and validated chemometric and chromatographic methods for the determination of both nifedipine and atenolol in powdered forms and in pharmaceutical formulation. The applied methods are partial least squares (PLS) and principal component regression (PCR) as chemometric methods and reversed phase chromatographic method with the application of design of experiments that lead to fast development and optimization of the experimental conditions.

Materials and Methods

Chemicals and reagents

Nifedipine and Atenolol were supplied by E.I.P.I.Co. (Egyptian International Pharmaceutical Industries Company). Methanol was supplied from (Sigma-Aldrich, Germany) (HPLC grade). Sodium dihydrogen phosphate and orthophosphoric acid were supplied from (Adwic, Egypt). Tenolate S.R capsules (B.N. 12746) were manufactured by Tiba pharmaceutical industries. It is labeled to contain 20 mg of nifedipine and 50 mg of atenolol and was obtained from the local market.

Instruments

A double beam UV-Visible spectrophotometer (SHIMADZU, Kyoto, Japan) model UV-1601 PC. The bundled software was UV-PC personal spectroscopy software version 2.10 (Shimadzu). The spectral bandwidth was 0.1 nm and wavelength-scanning speed 1800 nm/min. The absorption spectra of the standard were recorded in 1 cm quartz cells over the range of 200-400 nm at room temperature. Data handling was done using PLS toolbox, Solo version 7.0.3.

An HPLC system equipped with an isocratic pump UV detector Agilent 1100. Chromatographic signals were acquired processed by Agilent LC Chemstation software 1100. YMC-pack pro C₁₈ ODSA (250 mm x 4.6 mm, 5 µm) column was used for separation.

Preparation of solutions

Stock solutions of 0.1 and 0.25 mg/mL of nifedipine and atenolol; respectively were prepared in methanol. Working solutions were prepared by diluting the stock solution with methanol as appropriate. The stock solutions were found to be stable for one week if kept in the refrigerator and protected from light.

Standard solution for multivariate calibration

A multilevel multifactor design was used for the construction of the calibration and validation set. A training set of 25 synthetic mixtures with different concentration ratios of nifedipine and atenolol was prepared by mixing different aliquots of working standard solutions in 10 mL volumetric flasks then the volumes were completed with methanol. The resulting concentrations of nifedipine and atenolol were in the ranges 5-15 µg/mL and 20-30 µg/mL; respectively. One third of the samples (Eight samples) were randomly chosen and used for external validation (validation set) and the rest of the samples were used for construction of the regression model (calibration set). Table 1 shows the concentrations of the prepared mixtures.

Chromatographic conditions for HPLC method

Different chromatographic conditions were tested by changing different factors (acetonitrile ratio, pH of phosphate buffer, flow rate

Sample No.	Calibration set		Validation set	
	Nifedipine concentration (µg/mL)	Atenolol concentration (µg/mL)	Nifedipine concentration (µg/mL)	Atenolol concentration (µg/mL)
1	5	25	10	25
2	5	20	7.5	22.5
3	15	20	12.5	30
4	7.5	30	10	27.5
5	15	22.5	15	25
6	10	30	12.5	20
7	7.5	25	10	20
8	12.5	22.5	12.5	27.5
9	15	27.5	-	-
10	15	30	-	-
11	5	30	-	-
12	5	27.5	-	-
13	12.5	25	-	-
14	7.5	27.5	-	-
15	5	22.5	-	-
16	7.5	20	-	-
17	10	22.5	-	-

Table 1: The five level two factor experimental design of the calibration and validation set mixtures.

Factors	DOE I	DOE II	DOE III	DOE IV
pH	-1 = 3 ,0 = 4 ,+1 = 5	-1 = 4 ,0 = 5 ,+1 = 6	-1 = 3.9 ,0 = 4 ,+1 = 4.1	-1 = 3.9 ,0 = 4 ,+1 = 4.1
Flow rate	-1 = 0.8 ,0 = 1 ,+1 = 1.2	-1 = 1 ,0 = 1.2 ,+1 = 1.4	-1 = 1.2 ,0 = 1.4 ,+1 = 1.6	-1 = 1.1 ,0 = 1.2 ,+1 = 1.3
Acetonitrile	-1 = 40 ,0 = 50 ,+1 = 60	-1 = 55 ,0 = 60 ,+1 = 65	-1 = 62.5 ,0 = 65 ,+1 = 67.5	-1 = 60.5 ,0 = 62.5 ,+1 = 64.5
Wavelength	-1 = 220 ,0 = 230 ,+1 = 240	-1 = 210 ,0 = 220 ,+1 = 230	-1 = 229 ,0 = 230 ,+1 = 231	-1 = 229 ,0 = 230 ,+1 = 231

Table 2: Experimental design (DOE I, II, III and IV) to develop and optimize the chromatographic conditions for nifedipine and atenolol mixture.

and wavelength of detection) using experimental design till reaching optimum chromatographic conditions for good resolution of both drugs as shown in Table 2. The optimum conditions that was achieved: (acetonitrile: 0.02 M phosphate buffer, pH= 4.0) (62.5: 37.5, v/v). The phosphate buffer was prepared by adding 10 mL of triethylamine to the prepared buffer (0.01 M). The pH adjusted using orthophosphoric acid. The mobile phase was prepared, filtered through 0.45 µm membrane filter and degassed before use, at flow rate 1.2 mL/min and detection wavelength at 230 nm. Validation of the chromatographic method was done according to ICH guidelines [20].

Linearity

Different aliquots (0.5-3.5 mL) of nifedipine and (3.5-6.5 mL) of atenolol standard solutions (100 µg/mL) were transferred into two series of 10 mL volumetric flasks; each was diluted with the mobile phase to reach the required concentration. Solutions were injected in triplicate with 20 µL injection volume.

Accuracy

The accuracy of the method was demonstrated by analyzing different concentrations covering the points in the calibration range (10, 15, 20, 25, 30 µg/mL) for nifedipine and (40, 45, 50, 55, 60 µg/mL) for atenolol. All concentrations were injected in triplicate.

Precision

Repeatability: Repeatability was demonstrated by assaying three freshly prepared solutions in triplicates on the same day at concentrations (18, 20, 22 µg/mL) for nifedipine and (45, 50, 55 µg/mL) for atenolol.

Intermediate precision: Intermediate precision was studied by

assaying the previously mentioned concentration under repeatability within different days.

Sensitivity

Limit of detection (LOD): The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The Limit of detection was calculated according to the following equation: $LOD = 3.3 \sigma/S$ where, σ = the standard deviation of the response and S = the slope of the calibration curve.

Limit of quantitation (LOQ): The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products.

The limit of quantitation was calculated according to the following equation: $LOQ = 10 \sigma/S$ where σ = the standard deviation of the response S = the slope of the calibration curve.

Application to Pharmaceutical Preparation

The method was applied on Tenolate® S.R capsules (labeled to contain 20 mg nifedipine and 50 mg atenolol per capsule). Twenty capsules were emptied, weighed and finely grinded, an amount of the powder equivalent to 10 mg nifedipine and 25 mg of atenolol were placed in a 100 mL volumetric flask and dissolved in 50 mL of methanol and the solution was sonicated for 45 minutes. The volume was completed with the same solvent and the solution was then filtered through a filter paper to a concentration of 100 and 250 µg/mL for nifedipine and atenolol; respectively. An aliquot of (2.5 mL) was diluted with methanol

in a 10 mL volumetric flask to reach a final concentration of 10 and 25 µg/mL for both nifedipine and atenolol; respectively.

Dissolution and release studies

Dissolution study was done on Tenolate® S.R. capsule (20 mg nifedipine/50 mg atenolol). The release studies of a delayed drug delivery system (DDS) as Tenolate® S.R capsules which releases nifedipine after a programmable period of time, which is intended for the therapy of diseases that depend on circadian rhythms. While, atenolol is usually given as a single oral dose effective antihypertensive agent providing 24 - hour reduction of blood pressure [21].

USP Dissolution testing of Tenolate® S.R. capsules

The release studies of nifedipine and atenolol were determined using USP dissolution testing apparatus I (Basket apparatus) [22] where it is preferable for floating dosage forms as capsules. The dissolution testing was performed using 900 mL phosphate buffer of pH 6.8 at 37 ± 0.5°C and speed 100 rpm. An aliquot of 9 mL was withdrawn at regular interval of time (10, 20, 30, 60, 90, 120, 180, 210, 240, 300 and 360 min.) and replaced with fresh medium to maintain sink conditions. These aliquots were filtered and diluted to a 10 mL volumetric flask with the mobile phase to give a final concentration of (50 µg/mL atenolol and 20 µg/mL nifedipine) then analyzed using the developed HPLC method. For calibration purposes, the linearity of both drugs were used as a secondary standard of known content and quantity of nifedipine and atenolol dissolved in the medium were calculated.

Dissolution of Tenolate® S.R. capsules using a surfactant

The release studies of nifedipine and atenolol were determined using USP dissolution testing apparatus I (Basket apparatus). The dissolution testing was performed using 900 mL phosphate buffer of pH 6.8 with the addition of 1% sodium lauryl sulfate (SLS) at 37 ± 0.5°C and speed 100 rpm. An aliquot of 9 mL was withdrawn at regular interval of time (5, 10, 20, 30, 45, 60, 75, 90, 120, 180, 240, 270, 300 and 330 min.) and replaced with fresh medium to maintain sink conditions. These aliquots were filtered and diluted to a 10 mL volumetric flask with the mobile phase to give a final concentration of (50 µg/mL atenolol and 20 µg/mL nifedipine) and analyzed using the developed HPLC method.

Results and Discussion

Multivariate calibration

In PCR and PLS methods, data were mean centered as a preprocessing step and leave one out was applied as a cross validation method [23]. To select the optimum number of PCs and LVs, F statistics was used in which the root mean square error of cross validation (RMSECV) values were compared and the selected model was that with the smallest number of factors such that RMSECV for that model was not significantly greater than RMSECV from the model with an additional factor (LV) [24]. If we decided to retain more factors than we should, we would be retaining some factors that can only add more noise to our data. On the other hand, if we don't keep enough factors, we will be discarding potentially meaningful information that could be necessary for a successful calibration. It was found that the optimum number of PCs and LVs were two as shown in Figure 3.

To validate the suggested models, several diagnostic tools were used for predicting a mixture containing different ratios of both drugs. The predicted validation set were shown with their recoveries, standard deviation and relative standard deviations values using PLS and PCR models are summarized in Table 3.

Figure 3: Plot of RMSECV versus (a) the number of principal components using PCR model and (b) the number of Latent variable using PLS model.

Sample No.	PCR (%Recovery)		PLS (%Recovery)	
	Nifedipine	Atenolol	Nifedipine	Atenolol
1	99.90	100.80	99.90	100.72
2	101.33	99.77	101.33	99.77
3	99.28	101.46	99.44	101.36
4	98.50	101.89	98.50	101.89
5	101.66	100.24	101.60	100.24
6	100.00	102.75	100.08	102.70
7	97.80	99.50	97.80	99.50
8	100.72	101.78	100.72	101.78
Mean	99.89	101.02	99.92	100.99
% RSD	1.34	1.35	1.32	1.10

Table 3: Percent recoveries of nifedipine and atenolol in the validation set by PCR and PLS regression models.

In order to assess the predictive ability of the developed models, each model was applied for determination of nifedipine and atenolol in the validation set. The expected concentration versus the predicted concentration for each compound is shown in Figures 4 and 5. Root mean squared error of cross-validation (RMSECV), of calibration (RMSEC) and prediction (RMSEP), together with the statistical

Figure 4: Plot of expected concentrations ($\mu\text{g/mL}$) versus predicted concentrations ($\mu\text{g/mL}$) for nifedipine using (a) PCR, (b) PLS models.

Figure 5: Plot of expected concentrations ($\mu\text{g/mL}$) versus predicted concentrations ($\mu\text{g/mL}$) for atenolol using (a) PCR, (b) PLS models.

parameters of the linear relationship between the predicted and the expected concentration of nifedipine and atenolol in the validation set are presented in Table 4.

Chromatographic method

An HPLC method was developed and validated for quantitative determination of nifedipine and atenolol in bulk powder and in pharmaceutical preparation. The chromatograms of both drugs are shown in Figure 6.

The method was developed using the concept of DOE after the

application of four design of experiments (DOE) by testing 4 factors (pH, Acetonitrile ratio, flow rate and wavelength of detection). Non-Significant experimental factors were attained and hence a robust method.

The method was validated for Linearity, Precision (repeatability and intermediate precision), LOD (Limit of detection), LOQ (Limit of quantitation) and accuracy according to the ICH guidelines [20]. The robustness study was carried out statistically by the design of experiments using MODDE 9.0 (trial version).

Linearity

The chromatographic method was found to be linear in the range of (5-35 µg/mL) for nifedipine and (35-65 µg/mL) for atenolol. Regression equations were as follows:

$$Y = 62.83 X + 24.06 \text{ (} r = 0.9999 \text{) (Nifedipine) and } Y = 35.21 X - 31 \text{ (} r = 0.9998 \text{) (Atenolol)}$$

Accuracy

The average percentage recovery at each concentration level was determined, the mean percentage recovery and RSD values were calculated as shown in Table 5.

Precision

Repeatability: Relative standard deviation values were 1.58 and 1.32 for nifedipine and atenolol; respectively.

Intermediate precision: Relative standard deviation values were 0.549 and 0.706 for nifedipine and atenolol; respectively.

Sensitivity

Limit of detection (LOD): The Limit of detection values were found to be 1.08 and 2.18 µg/mL for nifedipine and atenolol; respectively.

Limit of quantitation (LOQ): The limit of quantitation values

Parameters	PCR(2) ^a		PLS(2) ^b	
	Nifedipine	Atenolol	Nifedipine	Atenolol
Root mean square errors				
RMSEC	0.13	0.71	0.13	0.70
RMSECV	0.15	0.93	0.15	0.92
RMSEP	0.13	0.36	0.13	0.36
Regression parameters				
Slope	1.026	1.035	1.027	1.033
Intercept	-0.305	-0.601	-0.312	-0.577
Correlation Coefficient (r)	0.9983	0.9980	0.9984	0.9980

^aThe number of PCR factors (PCs) are represented in parentheses.

^bThe number of PLS factors (LVs) are represented in parentheses.

Table 4: Root mean squared error of cross-validation (RMSECV), of calibration (RMSEC) and prediction (RMSEP), together with linear regression parameters of the linear relationship between the expected and the predicted values of nifedipine and atenolol in the validation set by the proposed chemometric methods

Figure 6: HPLC chromatogram of a resolved mixture of atenolol (50 µg/mL) ($t_R = 1.77$) and nifedipine (20 µg/mL) ($t_R = 5.13$).

Sample No.	Taken (µg/mL)		Found* (µg/mL)		% Recovery	
	Nifedipine	Atenolol	Atenolol	Nifedipine	Atenolol	Nifedipine
1	10	40	9.98	39.93	99.84	99.83
2	15	45	15.12	45.34	100.86	100.76
3	20	50	20.07	50.08	100.37	100.17
4	25	55	24.97	54.39	99.90	98.90
5	30	60	30.00	59.96	100.00	99.94
Mean ± SD					100.19 ± 0.380	99.92 ± 0.662
% RSD					0.379	0.660

*Average of 3 determinations.

Table 5: Accuracy expressed as % recovery of nifedipine and atenolol in their standard solutions by the proposed HPLC method.

were found to be 3.29 and 6.62 µg/mL for nifedipine and atenolol; respectively.

The summary of regression and validation parameters for determination of nifedipine and atenolol are shown in Table 6.

Suitability parameters: System suitability parameters [25] were calculated for each chromatographic run for both drugs as shown in Table 7.

Statistically Designed Robustness Experiments: The concept

Parameters	Nifedipine	Atenolol
Linearity (Range) (µg/mL)	5-35	35-65
Intercept coefficient	24.05	-31
Slope coefficient	62.834	35.21
Correlation coefficient (r)	0.9999	0.9998
LOD (µg/mL)	1.08	2.18
LOQ (µg/mL)	3.29	6.62
Precision	Repeatability	1.582
	Intermediate precision	0.706

Table 6: Assay parameters and method validation for the determination of nifedipine and atenolol by the proposed HPLC method.

of DOE was used for determining the effect of 4 factors in only 11 experiments. The design of experiment (DOE) used was two - level placket - Burman design, the effect of each factor was computed by MODDE 9.0 trial version with respect to resolution, symmetry and selectivity. All factors were found to be non-significant ($P < 0.05$) as shown in the coefficient plot as presented in Figure 7.

Application to pharmaceutical preparation: Application of both spectrophotometric and chromatographic methods on pharmaceutical dosage form were demonstrated by preparing six replicate sample solutions of Tenolate® SR capsules (20 mg nifedipine/50 mg atenolol) with good recoveries as shown in Table 8.

USP Dissolution testing of Tenolate® S.R. capsules: It was clear from the dissolution profiles of both nifedipine and atenolol that atenolol is immediately released. While, nifedipine is sustainably released after 7 hours as shown in Figure 8. To accelerate the release of the nifedipine, sodium lauryl sulphate (SLS) with a concentration of 1% was added to the medium with same USP dissolution conditions. As a result, nifedipine released faster till it was completely released after 6 hours while atenolol was not affected as shown in Figure 9.

The results obtained by applying the proposed spectrophotometric

Parameters	Nifedipine	Atenolol	Reference value*
Resolution (Rs)	-	18.32	≥ 2
Relative retention time	1.78	4.84	> 1
Tailing factor (t)	0.85	1.01	0.5-2
Capacity factor (K')	1.78	1.70	> 2
Height equivalent to theoretical plates (H=L/N)	0.006	0.0008	The smaller the value, the more efficient separation
Number of theoretical plates (N)	2234	10929	The higher the value, the more efficient separation

*FDA regulations [25]

Table 7: System suitability parameters of analysis of nifedipine and atenolol by the proposed HPLC method.

Figure 7: The Coefficient plot of DOE IV (robustness) of nifedipine and atenolol that shows the factors influencing the chromatographic separation of the critical pair.

	Methods	Nifedipine (Mean $\pm$ SD)*	Atenolol (Mean $\pm$ SD)*
Tenolate® SR Capsules Claimed to contain 20 mg Nifedipine and 50 mg Atenolol Batch No. 12746	PLS	100.50 $\pm$ 0.85	100.78 $\pm$ 1.07
	PCR	100.60 $\pm$ 0.96	100.72 $\pm$ 1.09
	HPLC	99.77 $\pm$ 0.56	100.90 $\pm$ 1.23

*Average of 3 determinations.

Table 8: Application of the proposed methods to Tenolate SR® capsules (20 mg Nifedipine/50 mg Atenolol).

Figure 8: Dissolution of (A) nifedipine and (B) atenolol using USP apparatus method.

Figure 9: Dissolution of (A) nifedipine and (B) atenolol using USP apparatus method with 1 % SLS.

and chromatographic methods were statistically compared to those of the reported HPLC method by Vidyadhara et al. [18] used for both nifedipine and atenolol analysis. It is conducted that; 95% confidence, there is no significant difference between them since the calculated t - and F-values are less than the theoretical values; as presented in (Table 9).

Conclusion

In this work simple, sensitive, accurate, precise, reproducible, repeatable, specific and robust chemometric and chromatographic methods were established for the determination of nifedipine and atenolol in bulk powder and pharmaceutical preparations. The results

Parameters	PCR		PLS		HPLC		Reported HPLC method	
	NIF	ATN	NIF	ATN	NIF	ATN	NIF	ATN
Mean	100.60	100.75	100.50	100.78	100.40	99.74	100.75	100.43
SD	0.96	0.42	0.85	1.07	0.36	0.81	0.42	0.51
N	5	5	5	5	5	5	5	5
Variance	0.92	0.18	0.72	1.14	0.12	0.65	0.18	0.26
t-test (2.306) ^a	0.33	0.52	0.59	0.65	1.39	1.58	-	-
F-test (6.388) ^b	5.11	4.58	4.04	4.38	1.32	2.54	-	-

^aThe values in parentheses are the corresponding tabulated values at P<0.05.

^bHPLC method (C₁₈ Agilent ODS column, (5µm, 250 mm×4.6mm, id. column), using methanol: acetonitrile: 0.025 M sodium dihydrogen phosphate 60:20:20 v/v/v and adjusted to pH 3 with phosphoric acid, flow rate 1 mL/ min, 235 nm)

Table 9: Statistical comparison of the results obtained by the proposed spectrophotometric and RP-HPLC methods and with the reported HPLC method [18] for the analysis of nifedipine and atenolol in Tenolate® SR capsules.

obtained from both methods were compared to those of the reported HPLC method and a good coincidence was observed as there was no significant difference between both methods and the reported HPLC method. However, the chemometric method is less expensive and don't require sophisticated instrumentation nor any prior separation step. The multivariate methods are characterized by being fast, easy, and simple. While, the RP-HPLC was developed using the DOE concept which lead to a highly robust method of high specificity and good resolution. The proposed methods are quite sensitive for quantitative determination of nifedipine and atenolol in their pharmaceutical preparations and also used for their dissolution and release studies and hence used for routine analysis and quality control.

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