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SPECTROPHOTOMETRIC DETERMINATION OF ATORVASTATIN CALCIUM AND VALSARTAN IN BULK AND TABLET DOSAGE FORMS USING 1,2-NAPHTHOQUINON-4-SULPHONATE

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Abstract: A simple and sensitive spectrophotometric method is described for determination of atorvastatin calcium and valsartan in bulk and tablet forms. The method depends onthe formation of colored chromogen between atorvastatin calcium or valsartan and 1,2-naphthoquinon-4-sulphonate (NQS) and the reaction mixture exhibits maximum absorbance at λ max 464 and 466 nm for atorvastatin calcium and valsartan, respectively. Under the indicated conditions, this method was linear over the concentration range of 2-14 µg/mL and 10-120 µg/mL for atorvastatin calcium and valsartan, respectively. The results were statistically processed to evaluate the method for determining the drugs in both bulk and tablet forms. Results were compared with reference methods, and no significant difference was obtained. The proposed method was validated in terms of linearity, accuracy, precision and limits of detection and quantitation according to ICH.

Keywords: atorvastatin calcium, valsartan, NQS, chromogen, sodium hydroxide

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Introduction

Chemically, Atorvastatin Calcium is [(3R, 5R)-7-[3-(phenyl carbamoyl)-5-(4-fluorophenyl)-2-isopropyl 4-phenyl-1H-pyrrol-1-yl]-3, 5-dihydroxyheptanoic acid, calcium salt] (Figure 1). It has a molecular formula of $C_{66}H_{68}CaF_2N_4O_{10}$ and a molecular weight of 1155.34 g/mol.[1] It acts by inhibiting the enzyme 3-hydroxy-3-methyl glutaryl coenzyme A (HMG-co A) reductase.[2] Several analytical methods, including spectrophotometric methods,[3-7] spectrofluorometric,[8] chromatographic methods[9-16] and potentiometric method[17] have already been reported for its determination, either alone or in combination with other drugs. Valsartan is chemically, (2*S*)-3-methyl-2-[pentanoyl[[2́-(1H-tetrazol-5-yl) biphenyl-4-yl] methyl]amino] butanoic acid (Figure 1). It has a molecular formula of $C_{24}H_{29}N_5O_3$ and a molecular weight of 435.5 g/mol.[18] It is an orally active angiotensin II subtype 1 receptor blocker effective in lowering blood pressure in hypertensive patients.[19] Valsartan, when administered in combination with a neprilysin inhibitor (sacubitril), is indicated for the treatment of symptomatic chronic heart failure in adults with reduced ejection fraction.[20] Several analytical methods, including spectrophotometric methods,[21-24] miscellaneous methods[25-27] and chromatographic methods[28-31] have already been reported for its determination, either alone or in combination with other drugs. 1,2-naphthoquinon-4-sulphonate has been utilized as a chromogenic reagent for the spectrophotometric determination of a few compounds of pharmaceutical interest such as amikacin,[32] stavudine[33] and cinacalcet hydrochloride.[34] This study aimed to establish and validate a sensitive spectrophotometric method based on NQS derivatization for the quantitative determination of atorvastatin calcium and valsartan in bulk materials and tablet dosage forms, in accordance with ICH guidelines.

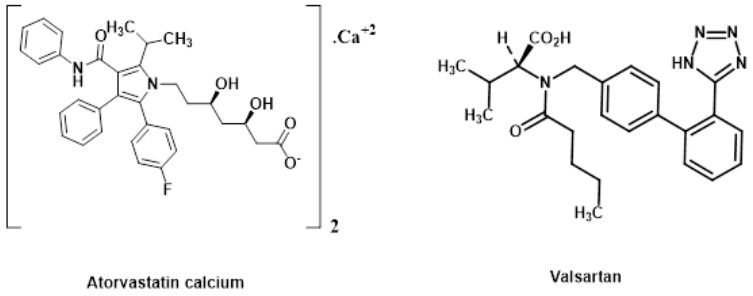


Figure 1. Chemical structures of the cited drugs.

Experimental

Apparatus

Labomed® Spectro UV-VIS Double Beam (UVD-2950**)** spectrophotometer with matched 1 cm quartz cells connected to Windows compatible computer using UV Win 5 Software v5.0.5.

A four-digit analytical balance (Shimadzu AUW220D, readability 0.0001 g) was used for all weighing procedures.

Materials and Methods

All chemicals used are of analytical reagent grade: Atorvastatin calcium (Eipico Company, Egypt; 99.06 % purity); Valsartan (Global Napi Pharmaceutical, Egypt; 99.8 % purity); Ator® tablets labeled to contain 10 mg of atorvastatin. Batch No. 1302776 (Eipico, Egypt); Disartan® tablets labeled to contain 80 mg of valsartan. Batch No. 77072 (Global Napi Pharmaceutical); 1,2-naphthoquinon-4-sulphonate (Fisher Scientific U.K. Limited, U.K.): 0.2 g of NQS was accurately weighed and transferred into a 100 mL calibrated flask, dissolved in 20 mL bidistilled water, and make up the volume up to the mark with bidistilled water to obtain a solution of 0.2 % (w/v). The solution was freshly prepared and protected from light during the use; Sodium hydroxide solution (9×10^{-2} M) (Elgomhouria Chemicals

Company, Egypt): 0.36 g of sodium hydroxide was accurately weighed and transferred into 100 mL volumetric flask and completed to the mark with bidistilled water.

Atorvastatin calcium standard solution equivalent to 0.2 $mg \cdot mL^{-1}$ of atorvastatin calcium was prepared by dissolving 20 mg of pure drug in 20 mL methanol and diluting to 100 mL in calibrated flask with the same solvent. Valsartan standard solution equivalent to 1 $mg \cdot mL^{-1}$ of valsartan was prepared by dissolving 100 mg of pure drug in 20 mL methanol and diluting to 100 mL in calibrated flask with the same solvent.

General procedures

To a series of 10 mL calibrated flasks, an increasing volume covering the concentration range (2 - 14) $\mu g \cdot mL^{-1}$ of atorvastatin calcium solution and (10 - 120) $\mu g \cdot mL^{-1}$ of valsartan solution were transferred, followed by addition of 1.2 mL of 0.2 % NQS and 1.8, 1.6 mL of 0.09 M NaOH for atorvastatin calcium and valsartan, respectively with occasional shaking and left the solutions 15 min, 25 min for atorvastatin calcium and valsartan, respectively at room temperature, finally the volume was brought up to the mark with bi distilled water. The absorbance was measured at 464 nm, and 466 nm versus reagent blank for atorvastatin calcium and valsartan, respectively. A calibration graph was prepared by plotting the measured absorbance versus concentration. The concentration of the unknown samples was determined by substituting the measured absorbance values into the regression equation of the calibration curve ($Y = a + bX$) and calculating the corresponding concentration.

Pharmaceutical preparations

For Ator® tablets

Twenty tablets of Ator® tablets were weighed and powdered. An accurately amount of the powder equivalent to 20 mg of atorvastatin

calcium were dissolved in 30 mL of methanol, shaken for about 10 min, filtered through Whatman filter paper to remove insoluble matter. The residue was washed with 10 mL portions of methanol three times, collected and completed with methanol to 100 mL in a volumetric flask to give a concentration of 0.2 mg mL^{-1}. The procedures were completed as for sample solution and as described under general procedure for atorvastatin. Aliquots from this solution equivalent to those in authentic samples were used for the application of the proposed methods applying standard addition techniques.

For Disartan® tablets

Twenty tablets of Disartan® tablets were weighed and powdered. An accurately amount of the powder equivalent to 100 mg of valsartan were dissolved in 30 mL of methanol, shaken for about 10 min, filtered through Whatman filter paper to remove insoluble matter. The residue was washed with 10 mL portions of methanol three times, collected and completed with methanol to 100 mL in a volumetric flask to give a concentration of 1 mg·mL^{-1}. The procedures were completed as for sample solution and as described under general procedure for Valsartan. Aliquots from this solution equivalent to those in authentic samples were used for the application of the proposed methods applying standard addition techniques.

Results and discussion

The NQS reagent reacts with atorvastatin calcium containing of alcohol groups and form ketal molecules[38] and through Knoevenagel condensation reaction with valsartan. It is an organic reaction used to convert an aldehyde or ketone and an activated methylene to a substituted olefin using an amine base as a catalyst. The reaction begins by deprotonation of the activated methylene by the base to give a resonance

stabilized enolate. The amine catalyst also reacts with the aldehyde or ketone to form an iminium ion intermediate, which then gets attacked by the enolate. The intermediate compound formed gets deprotonated by the base to give another enolate while the amine of the intermediate gets protonated. A rearrangement then ensues which releases the amine base, regenerates the catalyst, and yields the final olefin product.[39] The proposed mechanisms for the two drugs are presented in schemes 1 and 2.

Under the optimized experimental conditions, both drugs reacted instantaneously with NQS to form an orange-colored product exhibiting λ_max at 464 nm for atorvastatin calcium and 466 nm for valsartan (Figure 2). Linearity was evaluated according to ICH Q2(R1) guidelines by preparing a series of standard solutions covering the proposed concentration ranges. The absorbance values were plotted against concentration, and least-squares linear regression analysis was performed to determine the slope, intercept, and coefficient of determination (R^2). The residuals were examined to check for deviations from linearity. Based on these analyses, the absorbance was found to obey Beer's law over the ranges of 2 – 14 μg·mL^{-1} for atorvastatin calcium and 10 – 120 μg·mL^{-1} for valsartan.

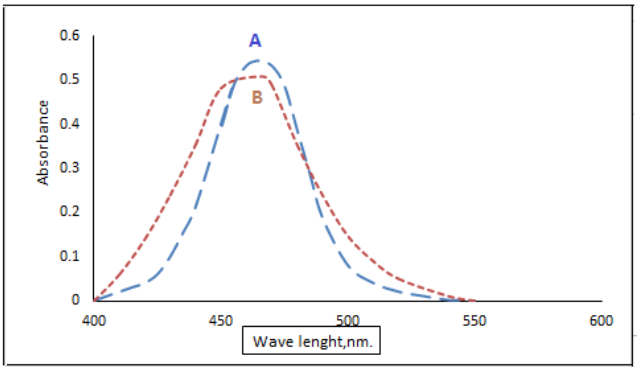
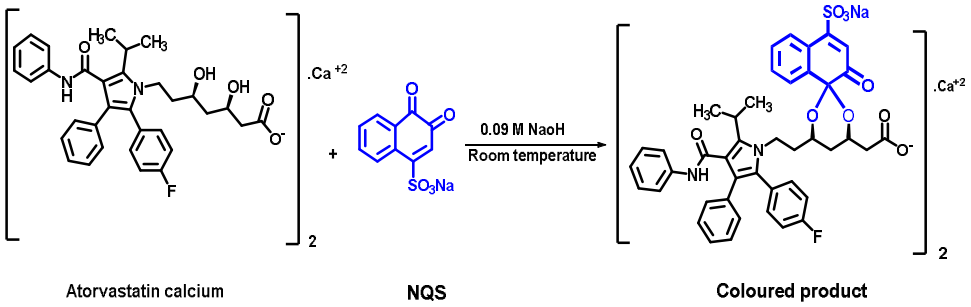
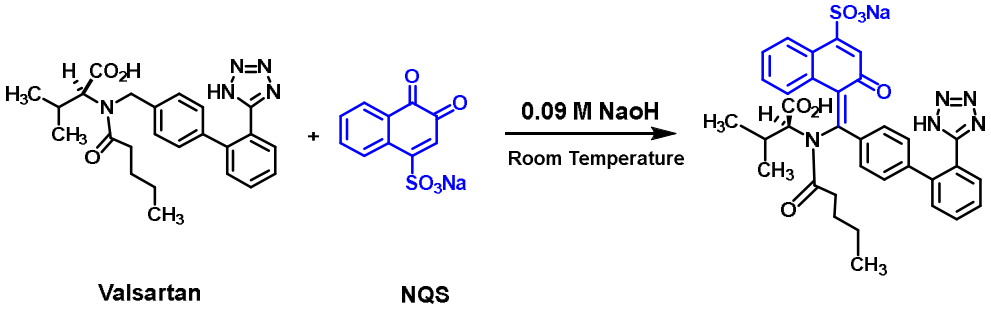


Figure 2. Absorption spectra for the reaction between NQS with (A) Atorvastatin calcium (10μg·mL^{-1}) at λ_{max} 464 nm and (B) Valsartan (80 μg·mL^{-1}) at λ_{max} 466 nm.

The suggested mechanisms are explained below:



Scheme 1. Proposed mechanism for the reaction between atorvastatin calcium and NQS.



Scheme 2. Proposed mechanism for the reaction between valsartan and NQS.

Study of the experimental parameters

(i) Effect of reagent concentration

The effect of NQS on the absorbance of solutions containing fixed concentrations of atorvastatin calcium and valsartan was studied. Different volumes of 0.2 % (w/v) NQS stock solution were tested to determine the volume that produces maximum absorbance. The final concentration of NQS in the reaction mixture was calculated based on the volume added relative to the total reaction volume. Maximum absorbance was observed at a final NQS concentration of 2.79×10^{-3} mol·L^{-1}, corresponding to the addition of 1.2 mL of the stock solution. Further increases in NQS concentration did not

significantly enhance absorbance. Therefore, this concentration was selected as optimal for subsequent experiments (Figure 3).

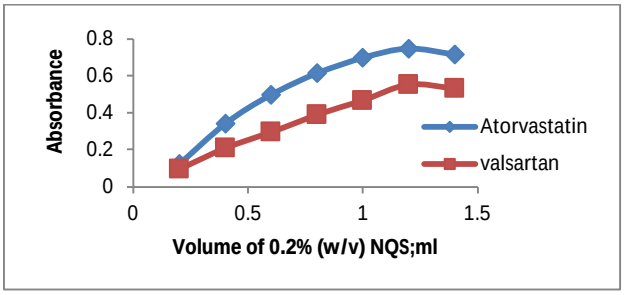


Figure 3. Effect of volume of 0.2% (w/v) NQS on the absorbance of (14 and 80 µg. mL^{-1}) Atorvastatin calcium and Valsartan, respectively.

(ii) Effect of base

The effect of alkalinity of the reaction medium was studied by testing a range of different NaOH molarity from 0.02 to 0.09 $mol \cdot L^{-1}$ to determine the optimum concentration that produces maximum absorbance (Figure 4). Additionally, the effect of the volume of NaOH added was examined to identify the volume giving the highest absorbance (Figure 5). The maximum absorbance was obtained at final NaOH concentrations of 0.032 $mol \cdot L^{-1}$ and 0.029 $mol \cdot L^{-1}$ for atorvastatin calcium and valsartan, respectively. Higher concentrations resulted in decreased absorbance. Therefore, these concentrations were selected as the optimum conditions for subsequent experiments.

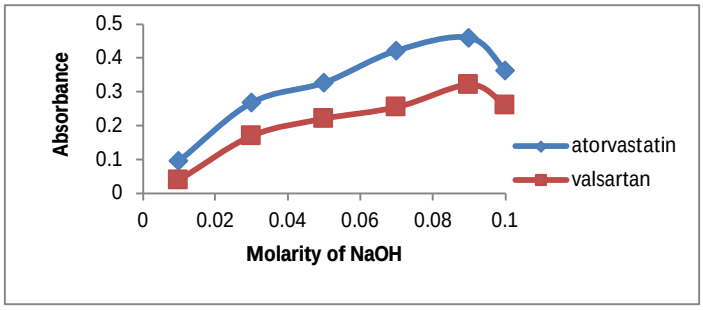


Figure 4. Effect of molarity of sodium hydroxide on the absorbance of (8 and 40 $\mu g \cdot ml^{-1}$) Atorvastatin calcium and Valsartan with NQS, respectively.

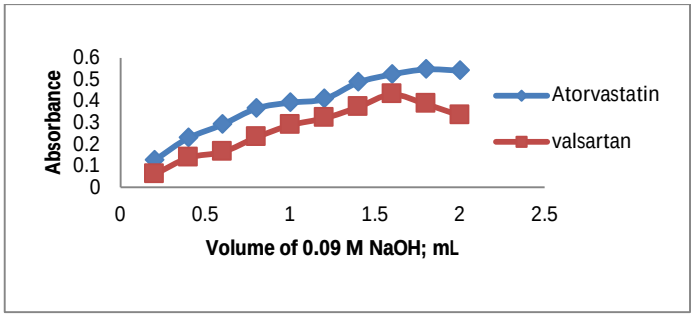


Figure 5. Effect of sodium hydroxide volume on the absorbance of (10 and 60 µg·ml^{-1}) Atorvastatin calcium and Valsartan with NQS, respectively.

(iii) Effect of time and temperature

The reaction time was determined by following the color development at room temperature and thermostatically controlled water-bath at different temperatures. It was observed that the absorbance reached maximum when the solution, left for 15 min or 25 min respectively at room temperature in case atorvastatin calcium and valsartan. This temperature and reaction time were chosen for color development. It was found that the absorbance of the chromogen remained stable for at least 40 minutes (Figure 6).

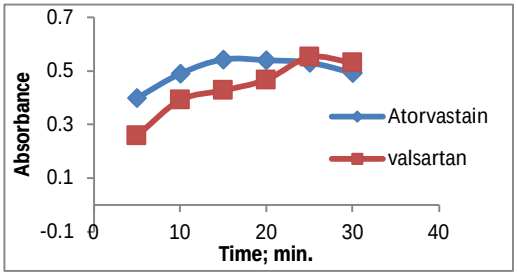


Figure 6. Effect of time on the reaction of NQS with (10 and 80 µg ml^{-1}) of Atorvastatin and Valsartan, respectively.

Validation of the proposed methods

The validity of the proposed methods was tested regarding linearity, range, limits of detection, limits of quantification, accuracy, precision, robustness and specificity according to ICH recommendations.[35]

Linearity and range

The calibration graphs obtained by plotting the values of the absorbance versus the final concentrations (µg/ml) were found to be rectilinear over the concentration ranges cited in table 1 and figure 7.

Table 1. Analytical parameters for spectrophotometric determination of Atorvastatin calcium and Valsartan through the proposed NQS method.

Parameters		Atorvastatin calcium	Valsartan
λmax, nm		464	466
Volume of 0.09M NaOH (ml)		1.8	1.6
Final NaOH Conc. mol·L^{-1}		0.032	0.029
Volume of 0.2% (w/v) NQS (ml)		1.2	1.2
Final NQS Conc. mol·L^{-1}		2.79×10^{-3}	2.79×10^{-3}
Temperature (^{o}C)		25±5°C	25±5°C
Reaction time (minutes)		15	25
Diluting solvent		bidistilled water	bidistilled water
Beer's law limits (µg·ml^{-1})		2-14	10-120
Regression equation*	Slope (b)	0.0496	0.0057
	Intercept (a)	0.0533	0.0954
Coefficient of determination		0.9998	0.9999

*A = a + bC where A is absorbance, C is the concentration of the drug in µg·mL^{-1}

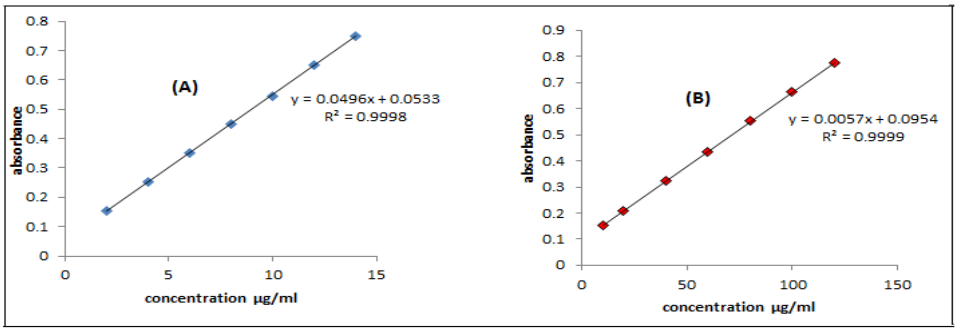


Figure 7. Calibration curve of the colored product formed by the reaction between NQS with Atorvastatin calcium (A) and Valsartan (B) at λmax 464 nm and 466 nm, respectively.

Limits of detection and limits of quantification

Limits of detection (LOD) were determined by evaluating the lowest concentrations of the analyte that can be detected according to the following equation:

$$LOD = 3.3\ S/K$$

Limits of quantification (LOQ) were determined also by establishing the lowest concentrations that can be quantitated according to the following equation:

$$LOQ = 10\ S/K$$

In the LOD and LOQ equations, S represents the standard deviation of three replicate blank determinations measured under the same experimental conditions and expressed in concentration units after conversion using the calibration curve. In Table 2, SD denotes the standard deviation of replicate measurements of the analyte concentration and is also expressed in concentration units. and K is the sensitivity, namely, the slope of calibration graph. The data presented in Table 2 correspond to the analysis of standard solutions of the studied analyte at different concentration levels prepared under the optimized experimental conditions. The taken concentration refers to the nominal concentration of the prepared standard solution, while the found concentration represents the experimentally determined concentration calculated from the calibration curve. The statistical parameters reported in Table 2 were calculated from seven replicate measurements at each concentration level, while three replicate blank determinations were used exclusively for LOD and LOQ calculations.

Table 2. Results of analysis for determination of Atorvastatin calcium and Valsartan with the proposed NQS method.

Parameters	Atorvastatin calcium			Valsartan		
	Conc. taken µg/mL	Conc. found µg/mL	Recovery %	Conc. taken µg/mL	Conc. found µg/mL	Recovery %
	2	2.01	100.50	10	9.92	99.29
	4	4.04	101.15	20	19.75	98.77
	6	5.96	99.36	40	40.10	100.26
	8	8.01	100.22	60	59.40	99.00
	10	9.89	98.93	80	80.28	100.35
	12	12.07	100.58	100	99.75	99.75
	14	14.02	100.18	120	119.22	99.35
Mean*			100.13			99.54
N			7			7
S.D.			0.75			0.60
R.S.D.			0.75			0.60
S.E.			0.28			0.22
V			0.57			0.36
LOD, $\mu g \cdot mL^{-1}$			0.44			2.73
LOQ, $\mu g \cdot mL^{-1}$			1.49			9.11
Sandell's sensitivity ($\mu g \cdot ml^{-1}$ per 0.001A)			0.00			0.06
Apparent Molar absorptivity**			68788.42			3791.59

* Mean recovery calculated from seven replicate measurements ($N = 7$).

**Calculated in the basis of molecular weight of the drug.

NOTE Conc. taken refers to the nominal concentration of the standard solutions, while *Conc. found* represents the experimentally determined concentration calculated from the calibration curve. *N* is the number of replicate measurements ($N = 7$) used for statistical evaluation of recovery and precision. *SD* is the standard deviation, *RSD* (%) is the relative standard deviation, *SE* is the standard error of the mean, and *V* is the variance (SD^2), all calculated from the found concentrations and expressed in concentration units ($\mu g mL^{-1}$), except RSD which is expressed as %. The standard deviation (*S*) used for LOD and LOQ calculation represents the standard deviation of three replicate blank determinations

Accuracy and precision

Accuracy was evaluated as percentage relative error of the measured concentration for atorvastatin calcium and valsartan. The accuracy of the proposed methods was checked by performing recovery experiments through standard addition techniques. The results are shown in Table 3, show that the accuracy is good. The precision of the method was calculated in term of intermediate precision (intraday and interday). Three different concentration five times of atorvastatin and valsartan were analyzed during the same day (intra-day precision) and five consecutive days (inter-day precision). The standard analytical errors, relative standard deviations (RSD) and recoveries obtained by the proposed method were found to be acceptable. The results are summarized in Table 4.

Table 3. Application of standard addition technique for the determination of Ator® (Atorvastatin calcium) and Disartan® (Valsartan) tablets using the proposed NQS method.

	NQS							
	Ator® tablets				Disartan®tablet			
Items	Conc. added from pure drug (µg/ml)	Conc. taken from tablet (µg/ml)	Conc. found (µg/ml)	Recovery* %	Conc. added from pure drug (µg/ml)	Conc. taken from tablet (µg/ml)	Conc. found (µg/ml)	Recovery* %
	4.0	0	3.94	98.66	20	0	19.75	98.75
	4.0	2.0	6.01	100.19	20	20	39.94	99.85
	4.0	4.0	8.07	100.95	20	40	59.55	99.26
	4.0	6.0	10.02	100.26	20	60	80.13	100.16
	4.0	8.0	11.93	99.49	20	80	99.17	99.17
Mean*				99.91				99.44
N				5				5
S.D.				0.87				0.56
R.S.D.				0.87				0.56
V				0.75				0.32
S.E.				0.35				0.25

*Mean of three different experiments.

Table 4. Results of the intraday and interday precision for the determination of Atorvastatin calcium and Valsartan with NQS.

Item	conc·ug/mL	Intraday		Interday	
		mean ± SD	RSD	mean± SD	RSD
Atorvastatin calcium	4 µg/ml	99.48 ± 1.268	1.275	99.44 ± 1.160	1.167
	8 µg/ml	99.471 ± 0.909	0.913	99.82 ± 1.135	1.137
	12 µg/ml	100.08 ± 0.504	0.504	100.42 ± 0.682	0.679
Valsartan	20 µg/ml	100.23 ± 1.339	1.337	99.65 ± 1.387	1.392
	60 µg/ml	100.47 ± 1.462	1.455	100.41 ± 1.158	1.154
	100 µg/ml	99.29 ± 0.441	0.445	100.281 ± 0.985	0.982

Robustness

Robustness was tested by making small incremental change in the volume of NQS (± 0.05 mL), change in volume of sodium hydroxide (± 0.05 mL) and reaction time (± 2 min) the effect of these changes and percent recovery of drugs were calculated in Table 5. The minor changes that may take place during the experiment didn't affect the absorbance of the reaction products.

Table 5. Results of the robustness for the determination of Atorvastatin calcium and Valsartan using NQS method.

Robustness		
% of recovery ± SD		
Item	Atorvastatin calcium	Valsartan
NQS+0.05ml	99.96±0.824	100.29±0.881
NQS-0.05	100.159± 0.667	99.139± 0.755
NaOH+0.05	100.12±0.688	99.30±0.674
NaOH-0.05	99.99± 0.670	100.58± 0.984
Time+2 min.	100.098± 0.668	98.98 ± 0.854
Time-2 min.	100.076± 0.733	99.705± 0.602

Stoichiometry of the reaction

The molar ratio of the reagent and the two drugs in the reaction was studied by using the continuous variation method (Job's method). The molar ratio was found to be 1:2 (drug: reagent) in case of atorvastatin calcium and 1:1 in case of valsartan, figure 8.

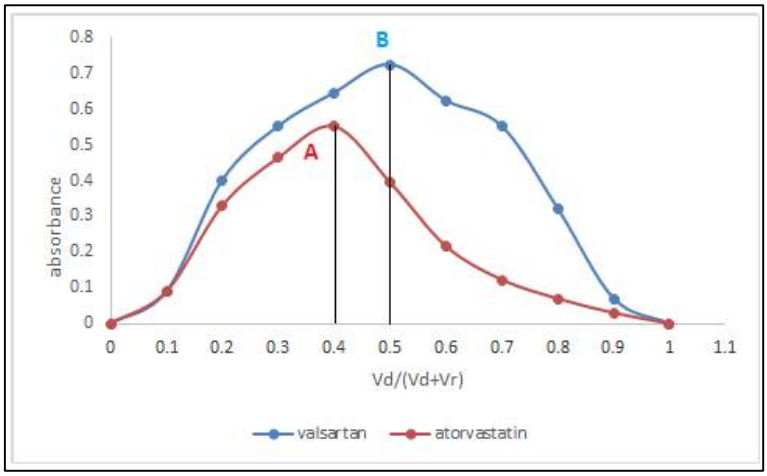


Figure 8. Continuous variation plots for the reaction between: (A) 5×10-3 M of NQS and 5×10-3 M of Atorvastatin calcium, (B) 1×10-2 M of NQS and 1×10-2 M of Valsartan.

Analysis of pharmaceutical preparations

The proposed method was applied to the analysis of the drugs in dosage forms, and the results were statistically compared with reference methods [36,37] by calculating Student's *t*- and F-values. The evaluated *t*-and F-values were less than the tabulated values at the 95% confidence level. The results are listed in table 6.

Table 6. Statistical data for the determination of pharmaceutical tablets of Ator® and Disartan® through the proposed methods compared with the reference methods.

Statistics	Ator® tablet		Disartan ®tablet	
	Reference method[36]	proposed method	Reference method[37]	Proposed method
Mean recovery*± SD	99.82±1.05	99.913±0.871	99.62±0.704	99.442±0.566
N	5	5	5	5
Variance	1.1025	0.759	0.496	0.3205
t-value		0.152 (2.306)[a]		0.490(2.306)[a]
F-ratio		1.452(6.3882)[b]		1.547(6.3882)[b]

* Average of three experiments.

a and b are Theoretical Student *t-values and* F- ratio at p=0.05.

Conclusion

The proposed method is accurate and precise as indicated by good recoveries of the drugs and low RSD values. The recovery % obtained by the proposed method is between 98.12% and 101.8%, within the acceptance level of 95 % to 105%. The proposed method could be applied for routine analysis and in quality control laboratories for quantitative determination of the cited drugs in the pure and dosage forms.

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