Sensitive Simultaneous Estimation of Atorvastatin. Ca in Pure and Dosage Forms Via Developed CFIA Using 1,2 Naphthoquinone-4-Sulfonate as a Suitable Organic Agent

Bushra B. Qassim[1], Asmaa A. Zydan[2]

[1]Department of Chemistry, College of Science, University of Baghdad, Iraq

Abstract

Objectives: A sensitive visible spectrophotometric method and FIA/merging zones technique was developed for the determination of atorvastatin calcium in pure material and tablet dosage form.

Method: Atorvastatin calcium has a free carboxylic moiety in its structure, which when being deprotonated in basic medium facilitates associated the reagent with the drug. This method was based on the formation of red colored chromogen of drug with 1,2-Naphthaquinone-4-sulfonate(NQS) in basic medium (NaOH). The absorbance of the chromogens was measured at their respective wavelengths of maximum absorbance against the corresponding reagent blank

Results: The red colored product is directly completed in basic medium and exhibits maximum absorption at 525 nm. Different factors affecting the formation of the product and optimized in order to obtain the best conditions for the experiment and its stability were studied. Method validation was done over a concentration range of 2-10 and 1-20 µg/mL for batch and FIA method respectively.

Keywords: *Atorvastatin calcium; 1,2-Naphthoquinone-4-sulfonate, sodium hydroxide; Pharmaceutical formulation; CFIA/merging zones technique.*

Introduction

ATRV.Ca {[R-(R, R*)]-2-(4-flurophenyl)-β,δ-dihydroxy- 5(1-methylethyl)-3-phenyl-4-[phenylamino) carbonyl]-1H-pyrrole-1-heptanoic acid, calcium salt (2:1)} is the most commonly occurring drug in commercially available pharmaceutical formulations used for the clinical treatment of hypercholesterolemia [(1)]. Several methods have been described for the determination of ATRV.Ca HPTLC [(2)], (HPLC) in different pharmaceutical preparations, either alone [(3-8)] or with other active ingredients [(9-17)], electrochemical [(18,19)], spectrofluorimetric [(20)] and capillary electrophoresis [(21)] methods have been developed for the analysis of ATRV.Ca in pharmaceutical preparations. Various spectrophotometric methods have been reported for the determination of ATRV [(9,15,22-26)] from its individual and combined formulations with other active ingredients. The official procedures in pharmaceutical preparations utilize non-aqueous titration method [(27)]. Kinetic methods have certain advantages in pharmaceutical analysis regarding selectivity and elimination of additive interferences, which affect direct spectrophotometric methods. Some specific advantages that the spectrophotometric FIA methods possess are as follows [(28)].

- High selectivity since they involve the measurement of the absorbance as a function of reaction time instead of measuring the concrete absorbance value.

- Simple and fast methods because some experimental steps such as filtration, extraction, etc.

-Other active compounds present in the commercial dosage forms may not interfere if they are resisting the chemical reaction conditions established for the proposed method.

- Colored and/or turbid sample background may possibly not interfere with the determination process.

Corresponding author:
Asmaa A. Zydan
asmaahmed930@yahoo.com

Materials and reagent

A standard solution of ATRV.Ca ($C_{66}H_{68}CaF_2N_4O_{10}$= 1155.34 g mol^{-1}, Sigma Aldrich). A 0.05 g of pure ATOR was dissolved in 100 mL methanol to prepare 500 µg/mL of standard ATRV.Ca. A standard stock solution of NQS ($C_6H_4COCOCH:CSO_3$ Na = 260.20 g mol^{-1}, Fluke) A 0.05 M of Reagent was prepared by weighing a 1.3 g of reagent and dissolving in distilled water and made up to 100 mL with it. A stock solution of NaOH (40 g mol^{-1}, BDH) A NaOH 1M was prepared by weighing a 4g of oxidant and dissolving in distilled water and made up to 100 mL with it.

Instrumentation

A Optima, Photomech 301-D^+, UV-Visible Spectrophotometer single beam recording spectrophotometer (Japan) was used for performed all absorbance and spectral measurements of FIA procedures, for the absorbance measurements as peak height through Kompensograph C1032, Siemens or absorbance with digital multimeter (DT9205A, China). Inside the detection unit, there is a flow cell (quartz silica (QS), 1 cm) with 80 µL internal volume. A Shimadzu UV-1800 (Japan) double-beam spectrophotometer were used for batch procedure, and quartz cuvette with an optical path length of 1 cm. A one channel manifold was employed for the FIA/merging zones system. A peristaltic pump of four channels (Shenchen, LabM1) used for pumping the distilled water as a carrier stream of through the valve (homemade, six-three injection valve (merging zone version)), which moves at 90° and three Teflon loops were loaded with the sample solutions and reagent. Mixing coil that was manufactured from glass with 2 mm (I.D). A single channel manifold system in FIA was shown in Figure.1.

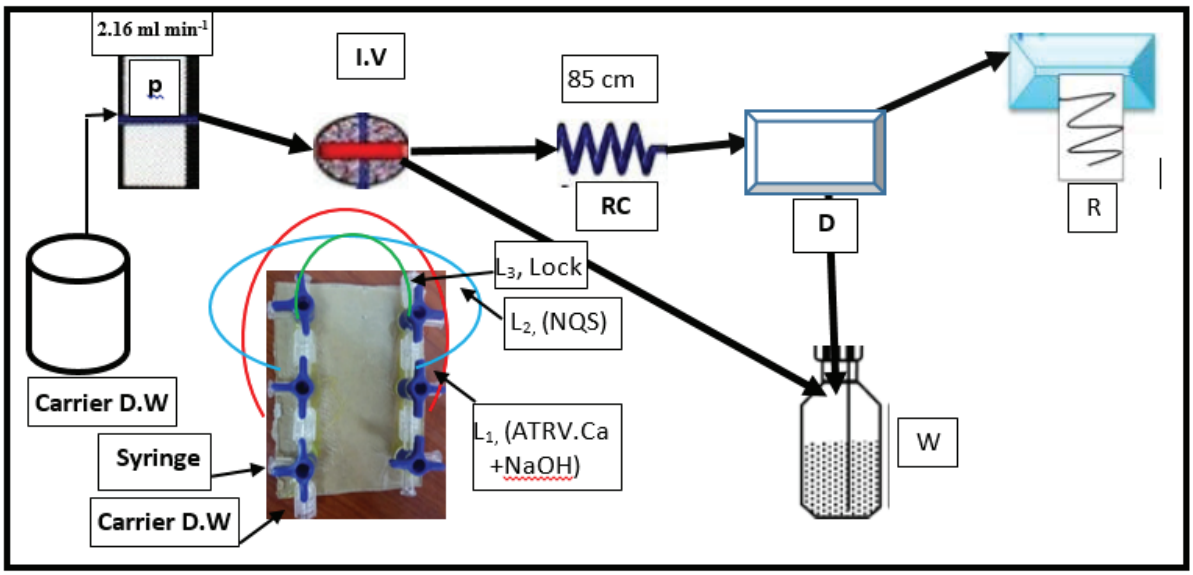


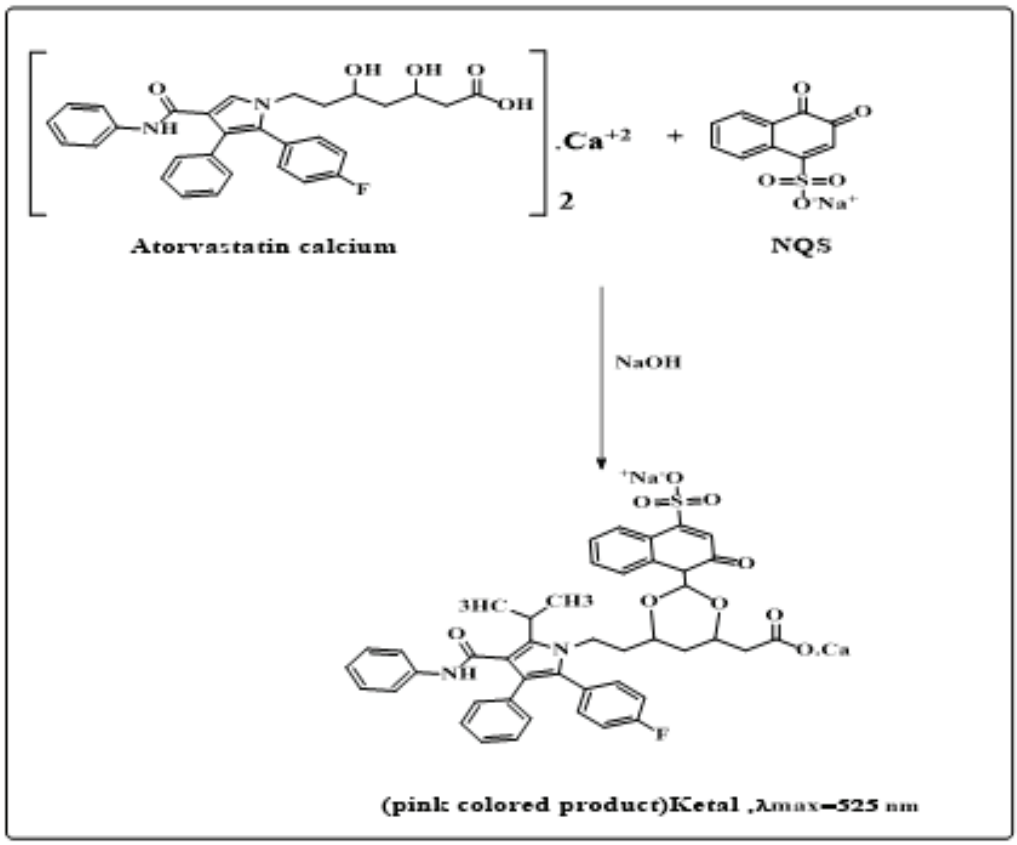
Figure.1. Manifold employed for FIA-Spectrophotometric determination of ATRV.Ca, where: I.V, injection valve; R.C, reaction coil; P, peristaltic pump; D, detector; R, Recorder; W, waste.

Assay procedure for tablets

The solutions of pharmaceutical preparations by appropriate amount equivalent 0.02 g of the each sample was weighting that be equal to 200 µg mL^{-1} of resulting powder were dissolved in 100 ml volumetric flask with 25 mL of methanol for and then shaken and filtered into a volumetric flask of 100 mL. The residue was washed and diluted to volume with distilled water to gain 200 µg/mL of statin drugs.

Mechanism of the Reaction

The suggested mechanism of this reaction of ATRV. Ca with (NQS) in basic medium to form a red complex directly as shown in scheme (I). The stoichiometry of the reaction between ATRV.Ca and NQS was investigated [22].



Scheme I: The suggested mechanism of the reaction between ATRV.Ca with (NQS) complex

Result and Discussion

Batch spectrophotometric determination: In the subsequent experiments, 4 µg mL^{-1} of ATRV.Ca was taken in 10 mL final volume and performed by changed one factors at a time and keeping the other parameters fixed and observing the effects of the product on the absorbance.

Concentration of NQS:

The effect of various concentration of NQS was investigated using different concentration ranging from (0.001-0.01 M). A concentration of 0.005 M reagent gave the highest absorbance and was chosen for further experiments.

Concentration of sodium hydroxide: The effect of concentration of sodium hydroxide was investigated by carrying out the reaction using different volumes of NaOH ranging (0.005-0.2 M). The maximum absorbance was obtained upon 0.05 M.

Calibration curve of classical method:

The impact of using different concentration of ATRV.Ca (1,2, 2.3, 2.5, 3, 4, 5, 6, 7, 8,10,12) µg mL^{-1} were examined with stabilized the other parameters. Transfer set of volumetric (10 ml) contain 2.5 mL of (NQS) (0.02 M) followed by 1 mL of NaOH (0.5 M) then an increasing volumes from standard solutions (100 $\mu g.ml^{-1}$). The solutions had been diluted to the marked using distilled water. The reaction mixture measured the maximum absorption of the colored product at 525 nm. The standard curve was constructed and linear range (2-8) $\mu g.mL^{-1}$ for the determination of ATRV.Ca, as shown in Figure (2).

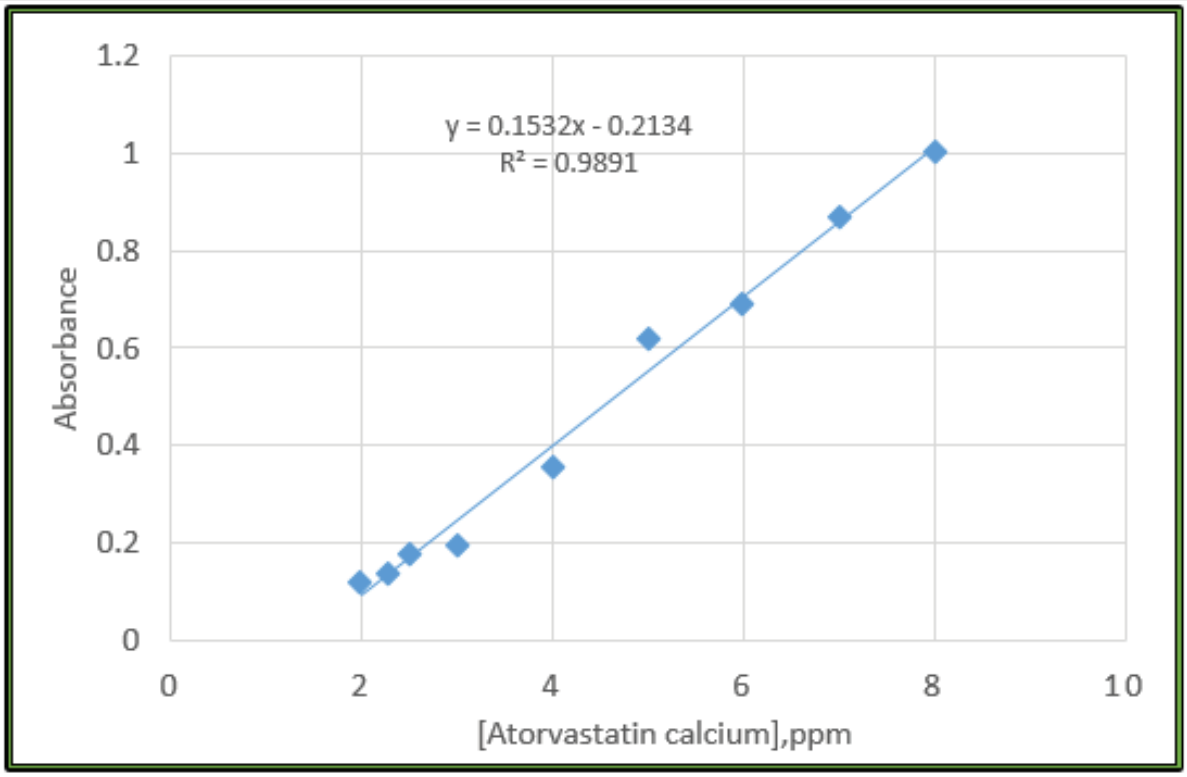


Figure.2. Calibration curve of reaction between ATRV.Ca and NQS in basic medium

Calculations of stability constant:

$K = 1-\alpha/\alpha^3C^2$ …….. (1), (α) (degree of dissociation) can be written as follows:

α = Am - As /Am…….. (2), Am; As are the values of absorbance of the aqueous solution including a more than enough and stoichiometric amount of the reagent.

Optimization of the FIA system conditions

Initial studies were directed towards the optimization of the experimental conditions for FIA system.

Effect of reagent and basic medium: Optimum concentration of the reagent was studied by injecting different concentrations (0.005-0.08) M using IV. The results indicated that the 0.05 M gave the good repeatability with highest value of absorbance.

NaOH found to be a useful basic medium for this reaction, different concentrations of NaOH were also studied in the range of 0.01 to 0.08 M. The result referred to increase the value of absorbance with increasing the concentrations of basic medium up to 0.02 M and after this concentration the value of absorbance decreased. As a result, 0.02 M was chosen for the subsequent experiments.

Effect of physical parameters

Effect of optimum total flow rate

Optimum flow rate was studied using a range changed flow rates (1.2-2.6) $mLmin^{-1}$. The result demonstrates that a flow rate of 2.16 $mLmin^{-1}$ gave the highest absorbance value.

Effect reaction coil length and injection volume

Optimum length of reaction coil was studied in range of 85-250 cm. A best absorbance with acceptable repeatability was gained from the length of 85 cm. Absorbance decreased upon using a coil length of more than 85 cm.

Various volumes of injector loop were tested in this study. Effect of injected sample volume (L_1) was changed (58.875, 68.687, 88.312 and 127.562) µL and the volume of injection reagent (L_2) also studies was in deferent volume (68.687-127.562) µL. a 58.875, 68.687

µL for L_1, L_2 respectively was used in the next experiments.

Method validation

The linearity of the calibration graph for FIA method was obtained by injecting a series of solutions of ATRV. Ca (1-20 µg mL^{-1}) prepared from stock solution (100 µg mL^{-1}) with 0.02 M of basic medium as shown in figure (3). A portion of NQS (0.005 M) was injected as summarized in Table 1.These small points were referred to high reproducibility and repeatability of the developed FIA contrasted with the batch procedure.

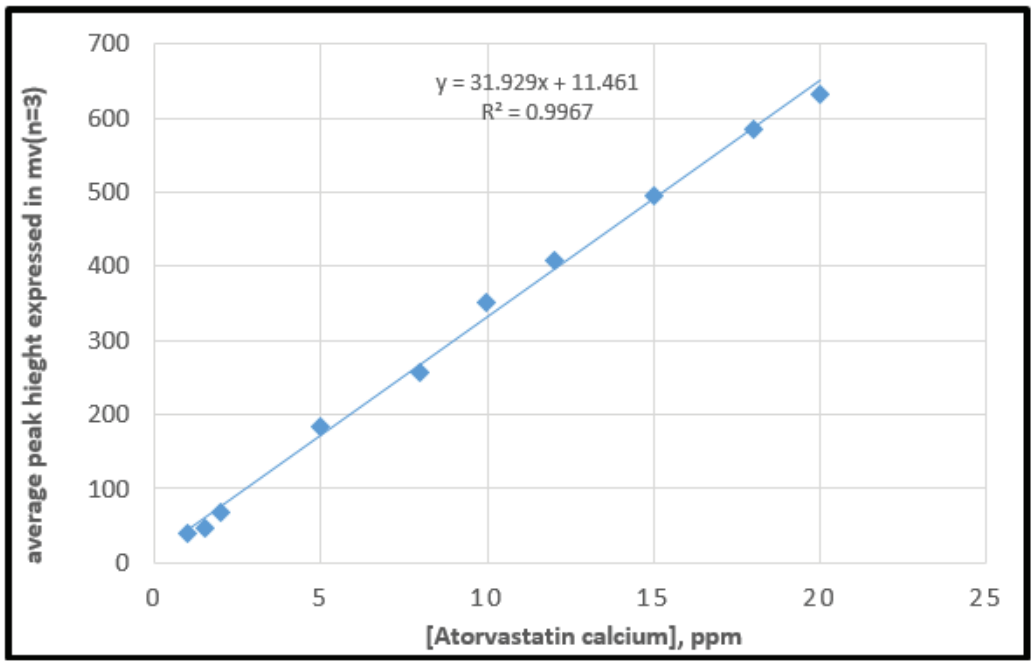


Figure .3. Linear calibration curve for determination of atorvastatin calcium with NQS using the developed FIA system.

Table 1. Summary of optical characteristics

Parameters	Batch method	FIA method
Linear range (µg mL-1)	2-8	1-20
Regression equation	y = 0.1532x + 0.2134	y = 31.929x +11.461
Correlation coefficient (r)/ r2	0.9945	0.9983
Linearity (r2 %)	98.91	99.67
Relative standard deviation (RSD %)	0.21 (at 5 ppm)	0.3 (at 10 ppm)
Slope (b); (mL.µg-1)	0.1532	31.929
Intercept (a); (a = y– b x)	0.2134	11.461
Standard deviation of intercept (Sa)	7.07 × 10-5	4.47 × 10-4
Confidence limit of intercept (a) = a ± tSa	0.2134 ± 0.0007	11.461± 0.163
Standard deviation of slope (Sb)	8.49 × 10-4	5.74 × 10-4
Confidence limit of slope (b) = b ± tSb	0.1532 ± 0.0002	31.929 ± 0.0049
Standard deviation of the residuals;	0.38 × 10-4	0.015
Average of recovery (%)	99.66	100.4
Limit of detection (LOD)	0.06	0.002
Limit of quantification (LOQ)	0.2	0.006
Sample through put (h-1)	10	68

Application of the proposed method using pharmaceutical:

The proposed batch and FIA method was successfully applied for estimation ATRV.Ca in tablets by the analysis of three types in two different concentrations of ATRV.Ca tablets and the results are listed in Table 2. In the direction of assessing the proficiency of the method. The statistical comparison between proposed and official methods using the student t- and F-test [27] indicated that the calculated values for F-test were (2.57) and (1.22), t-test values were (2.08) and (1.13) for the FIA and batch methods, respectively, were less than the theoretical one of F-test = 6.388 (n1 + n2 – 2 = 6) and t-test = 2.31.

Table 2. Application of the proposed batch and FIA and official methods for estimation of ATRV.Ca in tablets.

Dosage form	Proposed methods						Official method-od recovery (%)
	Batch			FIA-merging zones			
	Present conc. ($\mu g\ mL^{-1}$)	Rec (%)	RSD (%)	Present conc. ($\mu g\ mL^{-1}$)	Rec (%)	RSD (%)	
AVAS Tablets (10 mg/tablet) 3 100.30 0.41 MICRO LABS LIMITED 5 99.92 0.09				10 99.80 0.28 15 101.10 0.14			100.60
AVAS Tablets (20 mg /tablet) 3 101.30 0.20 MICRO LABS LIMITED 5 101.00 0.19				10 100.70 0.15 15 99.50 0.20			99.20
LIPODAR Tablets (10mg /tablet) 3 99.00 0.21 Dar Al Dawa, Na,ur - Jordan 5 99.40 0.18				10 98.20 0.22 15 100.13 0.10			100.50
LIPODAR Tablets (20mg /tablet) 3 99.67 0.52 Dar Al Dawa, Na,ur – Jordan 5 101.20 0.089				10 101.00 0.30 15 100.93 0.09			101.00
ATEROZ Tablets (20mg /tablet) 3 98.67 0.93 bilim 5 100.60 0.04				10 100.50 0.45 15 99.80 0.04			99.90

Conclusion

The developed methods were selective, rapid, simple and inexpensive and exhibits a fair degree of accuracy and precision . The method does not involve any critical reaction conditions. The proposed method can serve as an alternative method for the routine analysis of ATRV. Ca in pure drug and in pharmaceutical formulations. The methods is based on formation of a red condensation adduct upon reaction of ATRV.Ca and NQS in (NaOH). The method has low detection limit and high sample throughput. The proposed methods that followed Beer's law and give a good application for the pharmaceutical preparation. The wide applicability of the FIA method for daily quality control is well proven by analyzing the assay of ATRV.Ca at effect concentration level in dosage forms

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

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