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Spectrophotometric determination of Atorvastatin Calcium in pharmaceutical by charge transfer complexation

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Abstract: Rapid useful and easy spectrophotometric method for the quantitative analysis of (Atorvastatin calcium) (ATV) in raw material and tablets pharmaceutical formulation has been described. This method is based on the formation of yellow ion-pair complex between Atorvastatin calcium and Bromocresol purple (BCP) in Dichloromethane medium .

Different parameters affecting the reaction such as: effect of solvents, stability, reagent concentration, correlation ratio, etc. were optimized. The absorbance of the formed compound was measured by visible spectrum at absorption maximum 405 nm. The range of linearity was 3.02 – 42.33 µg/mL, , regression analysis had a good correlation coefficient $R^2 = 0.9994$. The limit of detection (LOD) and limit of quantification (LOQ) were to be 0.463 µg/mL and 1.403 µg/mL respectively. The (average percent recovery) was found to be (99.26 – 99.85) % for (Atorvastatin Calcium). This study was applied on Syrian pharmaceutical products: (Atoraz 20 mg & Atoraz 40 mg). The method was successfully applied for the determination of Atorvastatin calcium in tablets pharmaceutical formulation.

The proposed method is simple, direct, sensitive doesn't require any (extraction) process. Thus, the method could be ready to apply in routine analysis and quality control.

Keywords: (Atorvastatin calcium) (ATV), (Bromocresol purple) (BCP), (Spectrophotometric).

التحديد الطيفي لأتورفاستاتين الكالسيوم في المستحضرات الصيدلانية عن طريق معقدات نقل الشحنة

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المستخلص: تم وصف طريقة طيفية بسيطة وسريعة للتحليل الكمي (لاتورفاستاتين الكالسيوم) (ATV) في المواد الخام والأشكال الصيدلانية للأقراص. تعتمد الطريقة على تكوين معقد زوج شاردي أصفر اللون بين (الاتورفاستاتين الكالسيوم) و(البروموكريزول الأرجواني) في وسط من (ثنائي كلورومتان). تم تحسين العوامل المختلفة التي تؤثر على التفاعل مثل: تأثير المذيبات، الوقت، تركيز الكاشف، نسبة الارتباط، إلخ. تم قياس امتصاصية المعقد المتشكل بالطيف المرئي عند طول موجة امتصاص أعظمي عند 405 نانومتر. كان المجال الخطي واقعاً بين 3.02 – 42.33 ميكروغرام / مل، وأظهر تحليل الانحدار معامل ارتباط جيد $R^2 = 0.9994$. كان حد الكشف

(LOD) وحد القياس الكمي (LOQ) هو 0.463 ميكروغرام / مل و1.403 ميكروغرام / مل على التسلسل. نسبة متوسط الاسترداد بلغت (99.26 – 99.85) ٪ للاتورفاستاتين الكالسيوم.

تم تطبيق هذه الدراسة على العلامة التجارية الصيدلانية السورية: (اتوراز 20ملغ و اتوراز 40 ملغ). تم تطبيق الطريقة بنجاح لتحديد الاتورفاستاتين الكالسيوم في المستحضر الصيدلاني على شكل أقراص. الطريقة المطبقة بسيطة ومباشرة وحساسة ولا تتطلب لاي عملية استخلاص الطريقة قابلة للتطبيق في التحليل الروتيني وضبط الجودة.

الكلمات المفتاحية: اتورفاستاتين الكالسيوم، طريقة طيفية، بروموكريزول أرجواني.

1. INTRODUCTION

Atorvastatin is a synthetic hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase inhibitor. Atorvastatin reduces total cholesterol, low-density lipoprotein (LDL)-cholesterol and triglycerides, very-low-density lipoprotein (VLDL)-cholesterol and increases high-density lipoprotein (HDL)-cholesterol in patients with hyperlipidemia..[1,2]

Atorvastatin calcium {[R-(R, R*)]-2-(4-flurophenyl)-β,δ-dihydroxy5(1-methylethyl)-3-phenyl-4-[phenylamino) carbonyl]-1H-pyrrole1-heptanoic acid, calcium salt (2:1) trihydrate} is the most commonly occurring drug in commercially available pharmaceutical formulations used for the clinical treatment of hypercholesterolemia[3].

Several methods have been reported in the literature for the analysis of atorvastatin by (High-Performance Liquid Chromatography) (HPLC) in different pharmaceutical preparations, either alone [4,5,6,7,8] or with other active ingredients [9,10,11,12]. High performance thin layer chromatography (HPTLC)[13]. Reversed-Phase High-Performance Thin-Layer Chromatography (RP-HPTLC)[14]. Electrochemical[15]. Capillary electrophoresis[16]. Liquid chromatography-tandem mass spectrometry (LC-MS/MS)[17]. Ultra performance liquid chromatography (UPLC)[18]. UV spectrophotometriy [19,20,21,22] Spectrophotometric method[23-25]. First-order derivative spectrophotometry[26].

Bromocresol purple (BCP) is a brominated acid dye of the sulfone phthalein series derived from ortho-cresol indicator commonly used as indicator and spectrophotometric reagent.

2. MATERIALS AND METHODS

2.1 Apparatus

All spectral measurements were carried out using a Spector Scan (Jasco V-630 UV–VIS) spectrophotometer (Japan) with 1 cm quartz cells. Ultrasonic bath Daihan (China), and stirrer Velp Scientifica (Europe), Sartorius balance, sensitivity 10^{-5} g.

2.2 Chemical regents

Atorvastatin calcium (ATV): $C_{66}H_{68}CaF_2N_4O_{10}.\ 3H_2O$, Mw = 1209.4 g/mol from (INDIA) (Dr. Reddy's Laboratories Ltd), its purity 99.5 %. Bromocresol purple (BCP): $C_{21}H_{16}Br_2O_5S$, Mw = 540.22 g/mol from Merck (Germany). Methanol from Merck (Germany). Dichloromethane from Chem-Lab (Belgium).

3. STANDARD PREPARATION

3.1 Atorvastatin calcium stock solution

Stock solution 5×10^{-4} M of Atorvastatin calcium (Mw = 1209.4 g/mol) was prepared by dissolving 6.077 mg of raw material equivalent to 6.047 mg (by taken the purity in consideration) in volumetric flask 10 mL with 2 mL methanol and completed to volume with Dichloromethane to give concentration 5×10^{-4} M equivalent to 604.7 μg/mL. Prepared working standard solutions of Atorvastatin calcium by appropriate dilutions among (50 - 700) μL of 604.7 μg/mL solution in volumetric flasks 10 mL and added to each one of BCP 5×10^{-3} M equals to ten times of Atorvastatin calcium concentration, then completed to volume with Dichloromethane to give concentrations between (3.02 – 42.33) μg/mL of Atorvastatin calcium.

3.2 Reagent stock solution

Bromocresol purple 5×10^{-3} M was prepared by dissolving 135.06 mg of BCP (Mw = 540.22 g/mol) in volumetric flask 50 mL and completing to volume with Dichloromethane.

3.3 Calibration Curve

To construct the calibration curve, for each concentration five standard solutions were prepared and measured the absorbance five times for each solution.

3.4 Sample preparation

One Syrian product with two concentration were studied :

- Twenty tablets from Atoraz 20 mg was weighed and finely powdered and an accurate weight equivalent to 20 mg (ATV) was accurately weighed, dissolved in volumetric flask 10 ml of Methanol, then has been taken 1 mL of the solution to volumetric flask 10 mL and diluted to volume with Dichloromethane. 0.5 mL of the last solution we took to 10 ml volumetric flask and added 0.7 mL of Bromocresol purple 5×10^{-3} M, then diluted to volume with Dichloromethane, to obtained theoretically equivalent to 10.0 μg/mL of (ATV).
- Atoraz 40 mg. the same procedure were repeated for Atoraz 40 mg to prepare a solution equivalent to 20.0 µg/ml of ATV.

4. RESULTS

Atorvastatin calcium forms with Bromocresol purple at 25 ± 5 ºC yellow ion-pair complex. We scanned between the range of wavelengths 300 - 550 nm against a blank of BCP solved in Dichloromethane, and then measured the absorbance at maximum wavelength 405 nm. the parameters were studied of the colored result solutions to obtain the optimal conditions.

5. DISCUSSION

This method depends on study a color ion-pair complex between the Atorvastatin calcium and Bromocresol purple for the first time. It gives the lowest linearity range comparing with others spectrophotometric studies for color complexes, so we can consider it sensitive method. The results showed good results for percentage of recovery, accuracy and precision.

Fig. 1 shows the complex spectrum between Atorvastatin calcium and Bromocresol purple in Dichloromethane medium.

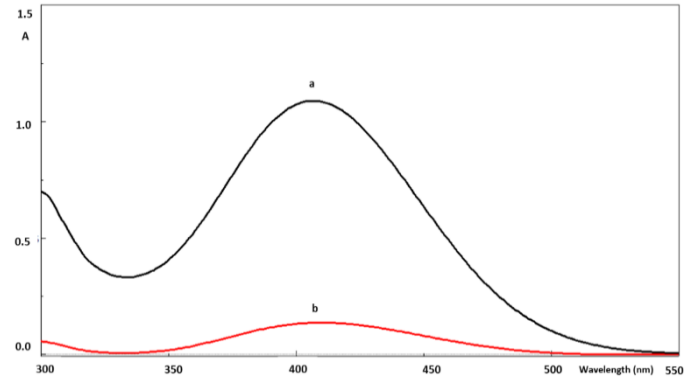

Fig. 1: a- Spectrum of complex ATV-BCP in Dichloromethane medium,[ATV] = 2×10^{-5} M,
b- Spectrum of BCP in Dichloromethane medium, [BCP] = 2×10^{-4} M.

5.1 Stability of stock solution

Time effect on stability standard stock solution of Atorvastatin calcium in Methanol was studied in three different concentrations 0.62×10^{-5}, 1.25×10^{-5} and 1.87×10^{-5} M. We did not notice any significant absorption changes within one month.

5.2 Effect of reagent concentration

To study the effect of reagent concentration on the colored complex solution, has been made a series of 10 mL of separated Volumetric Flasks, by adding 0.300 mL of Atorvastatin calcium 5×10^{-4} M equivalent to 15 µM and added between (0.015 – 0.360 mL) of (BCP) 5×10^{-3} M, equivalent to (7.5 - 180 µM) after completing the volume to 10 mL by Dichloromethane. The absorbance at 405 nm for each one (BCP) reagent was measured against the blank of Dichloromethane. It was found that the completed

colored complex formation in the best condition was 150 µM of (BCP) equivalent to 0.300 mL of (BCP) which equal to ten times of Atorvastatin calcium concentration, as it is shown in Fig. 2.

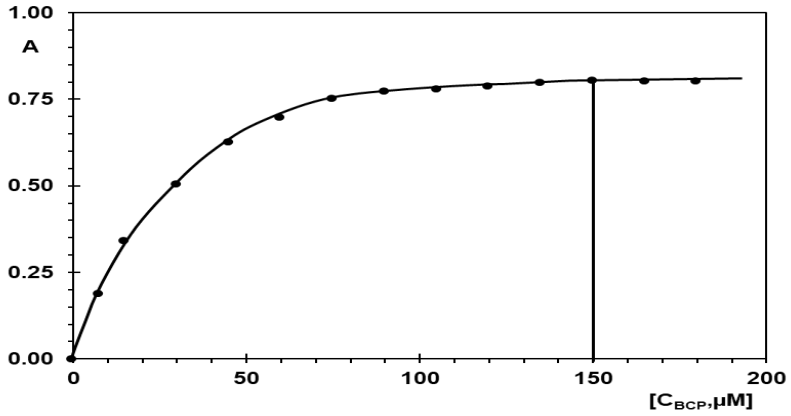

Fig. 2: Effect of reagent concentration.
Atorvastatin Calcium concentration 15 µM.

5.3 Correlation ratios by molecular ratio

We have prepared a series of complex solutions ATV-BCP in Dichloromethane medium. The concentration of the (BCP) reagent changes within the ratio (0.2×10^{-5} – 5.6×10^{-5}) M while the concentration of Atorvastatin calcium was constant in each solution and equal to 2×10^{-5} M. We **have** measured the absorbance values of these solutions at the wavelength of the maximum absorbance 405 nm (using Dichloromethane as a blank). The absorption changes of the molecular ratio of the reagent to the Atorvastatin calcium permitted to measure correlation ratio. We obtained the curve A = f ([BCP]/ [ATV]) shown in Fig. 3 where the correlation ratios are (1:1 & 2:1).

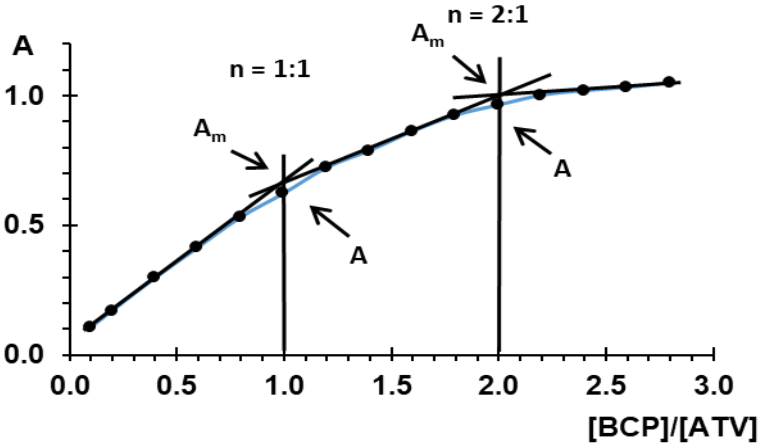

Fig. 3: Correlation molecular ratios (1:1 & 2:1).

5.4 Correlation ratios by continuous variation

We have prepared a series of complex solutions ATV-BCP in the medium of the Dichloromethane. The concentration of the reagent and the concentration of Atorvastatin calcium changes in solutions between (0.5 –5) 10^{-5} M where the sum of both concentrations remains constant and equal to 5×10^{-5} M.

We measured the absorbance values of these solutions at the wavelength of the maximum absorbance 405 nm according to the used reagent percentage of the formed complex in terms of molecular fraction of Atorvastatin calcium.

We obtained the curve A = f ([BCP] / {[BCP] + [ATV]}), shown in fig. 4, where the correlation ratios are also (1:1 & 2:1).

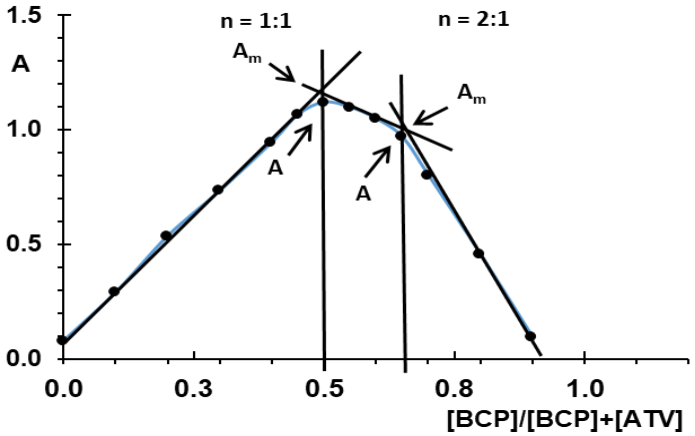

Fig. 4: Correlation ratio by continuous variation (1:1 & 2:1).

5.5 Calculation of formation constant for the (ATV: BCP) complex

We calculated the conditional stability constants (K_f) of the ion-pair complexes from molecular ratio and the continuous variation curves.

Data using the following equation [27-29].

$$K_f = \frac{\mathrm{A}/\mathrm{A_m}}{\left[1 - \frac{A}{A_m}\right]^{n+2} C_M (n)^n} \quad (1).$$

Where A and A_m are the absorbance value and the observed maximum absorbance value when all the Atorvastatin calcium is completely associated with Bromocresol purple, respectively. C_M is the mole concentration of Atorvastatin calcium at the maximum absorbance and n is the stoichiometry, which dye ion associates with Atorvastatin calcium. The log K_f values for ATV-BCP ion-pair, associated at correlation ratio (1:1 & 2:1) by molecular ratio were 7.93 and 10.50 respectively, and by continuous

variation were 7.83 and 10.22 respectively, so log K_f average are 7.88 and 10.36 at correlation ratio (1:1 & 2:1) respectively.

6. Method's validation

The validity and suitability of the proposed method was assessed by linearity (evaluated by regression equation), (Limit of Detection) (LOD), (Limit of Quantification) (LOQ), accuracy (reported as percent %), precision (reported as RSD %), robustness, and Sandall's sensitivity.

6.1 Linearity

We studied the linearity of Atorvastatin calcium concentrations at the optimal conditions we made a series of 10 mL of separated volumetric flasks , each one contains concentration of BCP equals to ten times of Atorvastatin calcium concentration, where the variable concentrations of ATV stock solution 5 ×10-4 M and the concentration of BCP stock solution 5 ×10-3 M, then the volumetric flasks completed to 10 mL with Dichloromethane, finally we measured the absorbance at 405 nm for each concentration

against the blank of BCP in Dichloromethane. Fig. 5 presents the complex of Atorvastatin calcium with BCP spectra. The range of linearity obeyed to (Beer's Law) in concentration (3.02 – 42.32) µg/mL and the linearity curve is presented in Fig. 6.

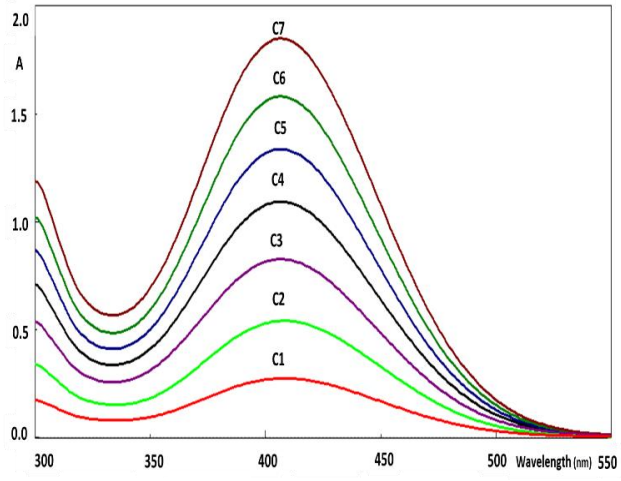

Fig. 5: spectra of (ATV-BCP) complex for different concentration of (ATV) :
C_1: 6.04 µg/mL, C_2: 12.09 µg/mL,
C_3: 18.14 µg/mL, C_4: 24.18 µg/mL,
C_5: 30.23 µg/mL, C_6: 36.28 µg/mL,
C_7: 42.33 µg/mL,
n = 5 for each concentration.

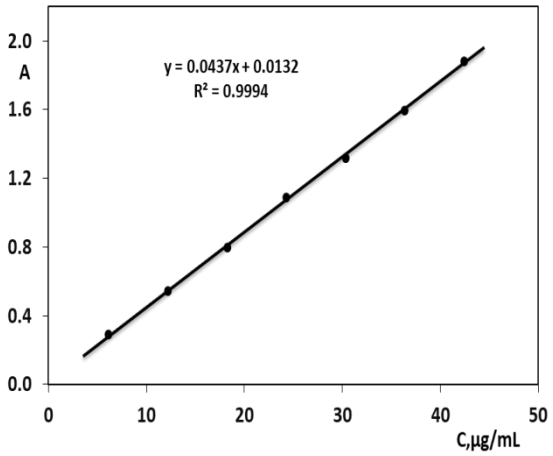

Fig. 6: Calibration curve for (ATV-BCP) complex for different concentration of (ATV) :
C_1: 6.04 µg/mL, C_2: 12.09 µg/mL،
C_3: 18.14 µg/mL, C_4: 24.18 µg/mL
C_5: 30.23 µg/mL, C_6: 36.28 µg/mL
C_7: 42.33 µg/mL,
n = 5 for each concentration..

6.2 Limit of detection (LOD) and limit of quantification (LOQ)

In spite of the measurement LOD and LOQ, five concentrations calculated in five replicates.

LOD and LOQ for Atorvastatin Calcium calculated by using the following equations:

(2). $$LOD = \frac{3.3 \times SD}{m}, LOQ = \frac{10 \times SD}{m}$$

Where SD: is the (Standard Deviation) of (y) intercepts of regression lines and (m) is the slope of the calibration curve. The (Limit of Detection) (LOD) and (Limit of Quantification) (LOQ) were to be 0.463 μg/mL and 1.403 μg/mL respectively.

6.3 Accuracy

To determine the accuracy and precision of the proposed method, five replicates determinations has been carried out on five different concentrations of standards (ATV).

The validation results was presented in table 1.

Table 1: Precision and accuracy for determination of Atorvastatin calcium.

Sample	Theoretical concentration (µg/mL)	$\bar{x}$ Observed concentration (µg/mL)	SD (µg/mL)	Precision RSD (%)	Accuracy (%)	LC = $\bar{x}$ ± [t .SD/(n)½] (µg/mL)
Atorvastatinn calcium	3.02	3.07	0.108	3.52	101.66	3.07 ± 0.134
	6.05	6.13	0.247	4.03	101.32	6.13 ± 0.307
	12.09	11.93	0.084	0.70	98.68	11.93 ± 0.104
	24.19	24.52	0.170	0.69	101.36	24.52 ± 0.211
	36.28	36.20	0.335	0.93	99.78	36.20 ± 0.416

$\bar{x}$: mean of five replicated determinations,

Accuracy (%) = (observed concentration/theoretical concentration) x 100,

Precision (RSD %) = (standard deviation/mean concentration) x 100.

LC: Limit of confidence at 95 %; t = 2.78.

6.4 Precision

In order to demonstrate the precision of the proposed method, (Intra-day) and (Inter-day) variability studies performed at three different concentrations (12.09, 24.19, and 36.28) μg/mL for Atorvastatin Calcium at the same day in two hours' time interval and at three days. Method efficiency was tested in terms of RSD % for both intra-day and inter-day precisions.

Accuracy confirmed by making five replications of standard Atorvastatin calcium under study and the mean was calculated. The results were showed in Table 2. The RSD % results didn't more than 3.61 % during the determination in one day or three days, where the method is considered precise.

Table 2: Intra-day and inter-day precision for determination of Atorvastatin calcium.

Intra-day							
Sample	Concentration µg/mL	Found concentration µg/mL					
		* Time I	Precision RSD%	* Time II	Precision RSD %	* Time III	Precision RSD%
Atorvastatin calcium	12.09	12.03	0.68	12.14	1.08	12.15	1.18
	24.19	24.52	0.78	24.62	1.32	24.44	1.96
	36.28	36.16	1.03	35.94	2.00	36.46	2.82
Inter-day							
Sample	concentration µg/mL	Found Concentration µg/mL					
		* Day I	Precision RSD%	*Day II	Precision RSD %	*Day III	Precision RSD%
Atorvastatin Calcium	12.09	12.11	0.98	12.15	1.24	12.07	3.61
	24.19	24.53	1.35	24.85	1.03	24.49	3.15
	36.28	36.18	1.95	36.93	2.17	36.01	2.34

*n = 5.

6.5 Robustness

The robustness of an analytical procedure is a test of its ability to maintain the integrity of unaffected results through a very small variance in some parameters and is an indicator of its reliability during normal analysis. Variables of the studied variables were slit scan speed and wavelength, which were performed at a concentration (24.19 µg/mL) of atorvastatin calcium. Table 3.

Table 3: Robustness test.

Initial conditions	Measured deviation	* µg/mL	SD µg/mL	RSD %	Percent (%)
Step size	0.5 nm	24.36	0.73	3.00	100.70
1 nm	2 nm	24.31	0.44	1.81	100.50
Scan speed	Fast	24.32	0.36	1.48	100.54
medium	Slow	24.28	0.41	1.69	100.37
Wavelength	2 nm +	24.13	0.45	1.86	99.75
405 nm	- 2 nm	24.14	0.48	1.99	99.79

*n = 5.

6.6 Sensitivity Sandell's and molar absorptivity ε:

Sensitivity of the proposed method for Atorvastatin Calcium was determined by calculating Sandell's sensitivity (SS), it was to be SS = 0.044 µg/cm2. The mean molar absorptivity ε was found equal to 53908.37 L/mol.cm.

6.7 RECOVERY

The recovery studied by three addition standards (80 %, 100 %, and 120 %) for every doses.

Table 4 presents the recoveries results for the two Syrian pharmaceutical products (Atoraz 20 mg and Atoraz 40 mg).

Table 4: Recoveries of Atorvastatin Calcium in Atoraz 20 and 40 mg.

Product	Pharmaceutical dosage	Sample µg/mL	Added µg/mL	Total Found $\bar{x}$ µg/mL	Recovery Average %	SD µg/mL	RSD%	Recovery Average %
Atoraz 20	Atorvastatin Calcium 20 mg/tab.	10.16	8.0	18.08	99.00	2.87	2.90	**99.26**
			10.0	20.13	99.70	3.18	3.19	
			12.0	22.05	99.08	1.66	1.68	
Atoraz 40	Atorvastatin Calcium 40 mg/tab.	20.15	16.0	36.08	99.56	2.08	2.09	**99.85**
			20.0	40.04	99.45	3.15	3.17	
			24.0	44.28	100.54	0.85	0.85	

$\bar{x}$ Mean for five replicates.

7. APPLICATIONS

Estimation of Atorvastatin Calcium in Atoraz 20 mg 40 mg doses

The method was applied for quantitative determination and identification Atorvastatin Calcium in one Syrian pharmaceutical product (Atoraz 20 mg and 40 mg doses) for three different batches for each one. The samples were prepared as mention before in the in the section of samples preparation and analyzed. Quantitative analysis was done by using calibration curve. The obtained results are summarized in table 5. In general, the concentrations of the detected Atorvastatin Calcium compounds in the one products were within the allowed limits under USP legislation [30], The tablets must contain not less than 90.00 % and not more than 110.00 % of labeled amount. So the obtained results are conformed to USP legislation [30]. The relative standard deviations RSD % (n = 5) of the quantitative results were in the range of 1.68 – 3.19 % for Atoraz 20 and 0.85 – 3.17 % for Atoraz 40.

Table 5: Results of Atorvastatin Calcium in (Atoraz 20 mg and 40 doses) tablets.

Product	Atoraz 20 mg/tab.		
Number of batch	1	2	3
Concentration	20.32	19.82	19.89

Product	Atoraz 20 mg/tab.		
$\bar{x}$mg/tab.			
Range mg/tab.	19.82 – 20.32		
SD mg/tab.	0.35	0.27	0.28
RSD %	1.72	1.36	1.41
Range RSD %	1.36 – 1.72		
Per %	101.60	99.05	99.45
Range Per %	99.05 – 101.60		
Product	Atoraz 40 mg/tab.		
Concentration $\bar{x}$mg/tab.	40.73	40.31	40.51
Range mg/tab.	40.31 – 40.73		
SD mg/vial	0.74	0.24	0.45
RSD %	1.82	0.60	1.11
Range RSD %	0.60 – 1.82		
Per %	101.83	100.78	101.28
Range Per %	100.78 – 101.83		

* $\bar{x}$Mean for five replicates.

8. CONCLUSION

We developed a new spectrophotometric method, which is suitable for the quantification and identification of Atorvastatin Calcium in raw material and tablets formulation. This method can be simply and successfully used in routine analyses. The proposed method is simple, sensitive, rapid, specific, a little cost. It could has been applied for quality control of Atorvastatin Calcium in pharmaceutical factories. The levels of Atorvastatin Calcium compounds in the analyzed preparations were found to be within the permissible limits set by the USP legislation[30].

9. ACKNOWLEDGEMENT

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