

1-1-2013

## Spectrophotometric determination of 3-hydroxy-3-methylglutaryl coenzyme-A reductase inhibitors in pharmaceutical preparations

GAMZE ERGİN

SENA ÇAĞLAR

ARMAĞAN ÖNAL

SIDIKA ERTÜRK TOKER

Follow this and additional works at: <https://journals.tubitak.gov.tr/chem>

 Part of the [Chemistry Commons](#)

---

### Recommended Citation

ERGİN, GAMZE; ÇAĞLAR, SENA; ÖNAL, ARMAĞAN; and TOKER, SIDIKA ERTÜRK (2013)

"Spectrophotometric determination of 3-hydroxy-3-methylglutaryl coenzyme-A reductase inhibitors in pharmaceutical preparations," *Turkish Journal of Chemistry*. Vol. 37: No. 2, Article 1. <https://doi.org/10.3906/kim-1106-58>

Available at: <https://journals.tubitak.gov.tr/chem/vol37/iss2/1>

This Article is brought to you for free and open access by TÜBİTAK Academic Journals. It has been accepted for inclusion in Turkish Journal of Chemistry by an authorized editor of TÜBİTAK Academic Journals. For more information, please contact [academic.publications@tubitak.gov.tr](mailto:academic.publications@tubitak.gov.tr).

## Spectrophotometric determination of 3-hydroxy-3-methylglutaryl coenzyme-A reductase inhibitors in pharmaceutical preparations

Gamze ERGİN, Sena ÇAĞLAR, Armağan ÖNAL, Sıdıka ERTÜRK TOKER\*

Department of Analytical Chemistry, Faculty of Pharmacy, İstanbul University,  
34116, Beyazıt, İstanbul, Turkey

Received: 27.06.2011 • Accepted: 27.02.2012 • Published Online: 17.04.2013 • Printed: 13.05.2013

**Abstract:** Simple and accurate spectrophotometric methods are presented for the determination of five 3-hydroxy-3-methylglutaryl coenzyme-A (HMG-CoA) reductase inhibitors (statins), namely atorvastatin, fluvastatin, pitavastatin, rosuvastatin, and simvastatin, in pharmaceutical preparations. The methods are based on the reaction of drugs as n-electron donor with 7,7,8,8-tetracyanoquinodimethane as  $\pi$ -acceptors to give highly colored complex species. All variables were studied in order to optimize the reaction conditions. Beer's law was obeyed in the concentration ranges 4–20  $\mu\text{g mL}^{-1}$ , 4–12  $\mu\text{g mL}^{-1}$ , 0.8–2.4  $\mu\text{g mL}^{-1}$ , 4–14  $\mu\text{g mL}^{-1}$ , and 2.5–20  $\mu\text{g mL}^{-1}$  for atorvastatin, fluvastatin, pitavastatin, rosuvastatin, and simvastatin, respectively. The proposed methods were successfully applied to the pharmaceutical preparations without any interference from excipients.

**Key words:** HMG-CoA reductase inhibitors, spectrophotometric determination, charge-transfer reaction, TCNQ, pharmaceutical preparations

### 1. Introduction

The discovery of 3-hydroxy-3-methylglutaryl coenzyme-A (HMG-CoA) reductase inhibitors, called statins, was a breakthrough in the prevention of hypercholesterolemia and related diseases. Statins specifically inhibit HMG-CoA reductase by competition, the enzyme that catalyzes the conversion of HMG-CoA to mevalonate, which is an early rate-limiting step in cholesterol biosynthesis in the body. These agents are highly effective in reducing total cholesterol and the low-density lipoprotein levels in several forms of hypercholesterolemia.<sup>1–4</sup>

Statins have been available in the market for the past 30 years.<sup>5,6</sup> Lovastatin is a natural product; simvastatin and pravastatin are semisynthetic products; and atorvastatin, fluvastatin, rosuvastatin, and pitavastatin are completely synthetic compounds.

Since lovastatin is not a major cholesterol-lowering drug used therapeutically in the treatment of hypercholesterolemia and pravastatin is not currently used in Turkey, these drugs were not included in this study.

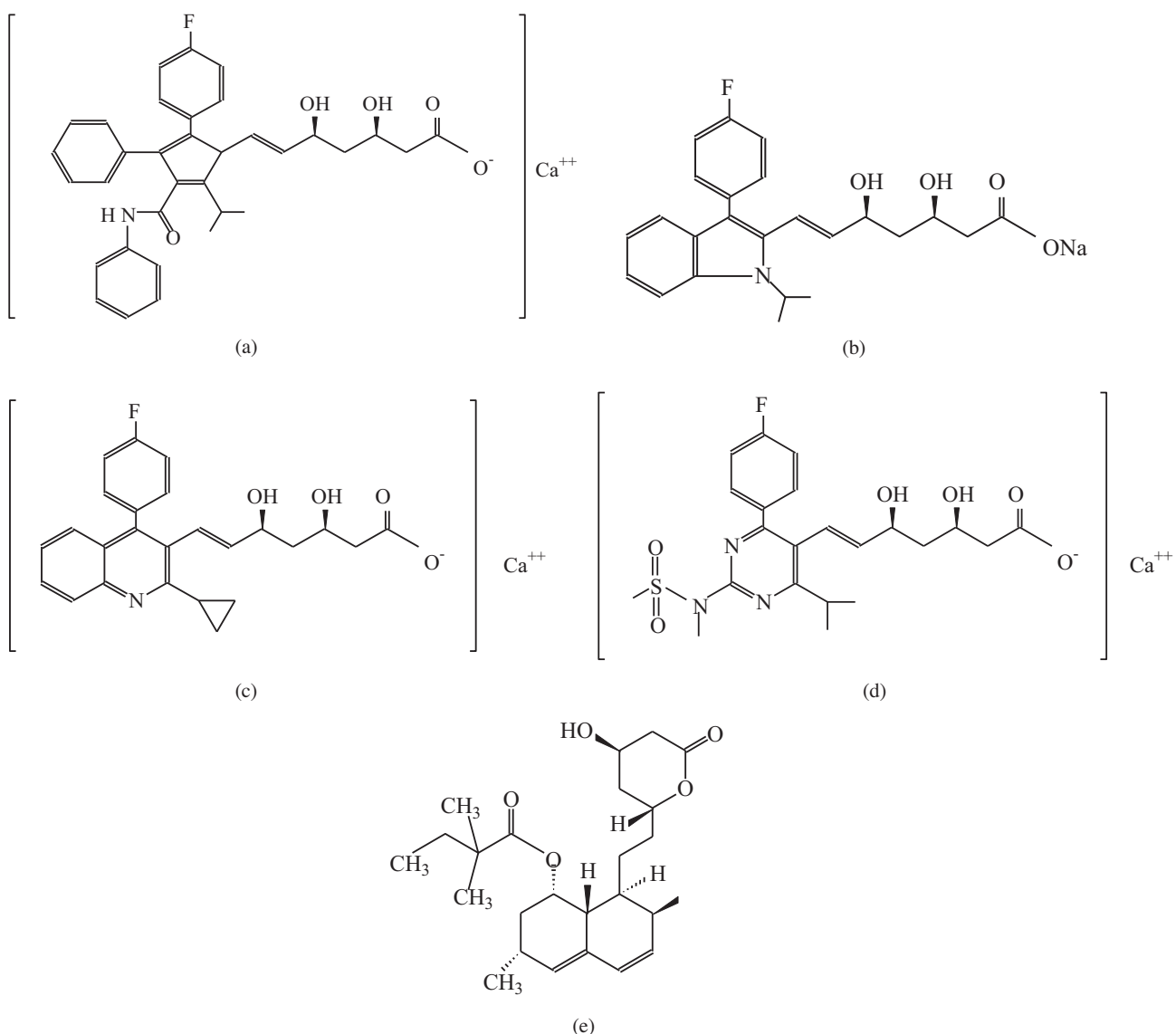
Atorvastatin calcium (ATV) [R-(R, R\*)]-2-(4-fluorophenyl)- $\beta,\delta$ -dihydroxy-5(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1H-pyrrole-1-heptanoic acid, calcium salt, fluvastatin sodium (FLV) [R\*,S\*-(E)]-( $\pm$ )-7-[3-(4-fluorophenyl)-1-(1-methylethyl)-1H-indol-2-yl]-3,5-dihydroxy-6-heptenoic acid, monosodium salt, pitavastatin calcium (PTV), monocalcium bis (3R,5S,6E)-7-[2-cyclopropyl-4-(4-fluorophenyl)-3-quinolyl]-3,5-dihydroxy-6-heptenoate, rosuvastatin calcium (RSV), bis[(E)-7[4-(4-fluorophenyl)-6-isopropyl-2-[methyl (methyl-sulphonyl]

\*Correspondence: serturk@yahoo.com

amino]pyrimidin-5-yl](3R,5S)-3,5-dihydroxyhept-6-enoic acid] calcium salt, and simvastatin (SMV), 2, 2-dimethyl-1, 2, 3, 7, 8, 8a-hexahydro-3, 7-dimethyl-8-[2-(tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl)-ethyl]-1-naphthalenyl ester (Figure 1) are the most commonly used statins in the treatment of hyperlipidemia.<sup>5-8</sup> Several analytical methods such as spectrophotometric,<sup>9-19</sup> high performance liquid chromatographic (HPLC),<sup>20-30</sup> high performance thin layer chromatography (HPTLC)<sup>31,32</sup> and electroanalytical techniques<sup>33,34</sup> are reported for the determination of these drugs simultaneously or alone in bulk drug and formulations.

To the best of our knowledge, there is no report available on the spectrophotometric determination of these drugs based on a charge transfer reaction with 7,7,8,8-tetracyanoquinodimethane (TCNQ).

A significant advantage of spectrophotometric methods, unlike other analytical methods, is the simplicity and low cost of the instrument. The sensitivity in terms of molar absorptivity and the precision of the methods



**Figure 1.** Chemical structure of statins, (A): ATV, (B): FLV, (C): PTV, (D): RSV, (E): SMV.

are very suitable for the determination of drugs in pure and dosage forms. Therefore, we decided to develop visible spectrophotometric determination of these drugs using TCNQ reagent. TCNQ has been widely used as the reagent for visible spectrophotometric methods of a number of n-electron donor drugs.<sup>35-44</sup> These methods are based on the blue colored TCNQ<sup>•-</sup> radical anion formed by interaction of the drugs (as base) with the reagent in acetonitrile at room temperature. Because of the wide applicability of the proposed method, it is suggested for routine quality control assay of the drugs in pure form and its pharmaceutical preparations.

## 2. Experimental

### 2.1. Apparatus

Spectrophotometric measurements were carried out using a Shimadzu UV-160 A spectrophotometer with 1-cm glass cells.

### 2.2. Reagents and solutions

ATV and its pharmaceutical preparation Ator film tablet<sup>®</sup>, containing 20 mg of atorvastatin calcium per tablet, were kindly supplied by Sanovel Pharmaceuticals (İstanbul, Turkey). FLV and its pharmaceutical preparation Lescol capsule<sup>®</sup>, containing 40 mg of fluvastatin per capsule, were kindly supplied by Novartis Pharmaceuticals (İstanbul, Turkey). PTV and its pharmaceutical preparation Livalo film tablet<sup>®</sup>, containing 2 mg of PTV per tablet, were kindly supplied by Kowa Pharmaceuticals (Japan). RSV and its pharmaceutical preparation Crestor film tablet<sup>®</sup>, containing 20 mg of RSV per tablet, were kindly supplied by Astra Zeneca Pharmaceuticals (İstanbul, Turkey). SMV and its pharmaceutical preparation Zocor film tablet<sup>®</sup>, containing 20 mg of SMV per tablet, were kindly supplied by Nobel Pharmaceuticals (İstanbul, Turkey). TCNQ was obtained from Merck (Darmstadt, Germany). All chemicals and reagents were of analytical-reagent grade.

Stock solutions were prepared by dissolving 100 mg of ATV, FLV, PTV, RSV, and SMV (equivalent to 100 mg of these drugs' bases) in acetonitrile (RSV, SMV), methanol (FLV), acetonitrile-methanol mixture (4:1) (ATV), and methanol-water mixture (4:1) (PTV) in a 100-mL volumetric flask to give a concentration of 1.0 mg mL<sup>-1</sup> of drugs.

The drugs' base solutions were prepared. For this purpose, appropriate volumes of the stock solutions were transferred to a stoppered tube and 1 mL of 5 N NaOH (for ATV and RSV) and 0.1 M acetate buffer solution pH 4 (for PTV and SMV) were added. Two 5-mL portions of chloroform (for ATV, RSV) and two 5-mL portions of methyl tertiary buthyl ether:ethyl acetate (1:1) mixtures (for PTV, SMV) were added for the extraction. Extracts were dried using anhydrous Na<sub>2</sub>SO<sub>4</sub> and evaporated to dryness under nitrogen with mild heating. The residues were dissolved with acetonitrile in 20-mL volumetric flasks using an ultrasonic bath. Acetonitrile was added to the mark (100 μg mL<sup>-1</sup> as the base). FLV's base solution was not prepared since it gave the reaction directly with TCNQ reagent.

In acetonitrile 0.2% TCNQ solution was prepared. The solution was stable for 1 week at 4 °C.

### 2.3. Choice of solvent

Different solvents, namely chloroform, acetonitrile, acetone, ethanol, 1,4-dioxan, methanol, and methylene chloride, were investigated in order to select the most suitable one.

#### 2.4. Reagent concentration

The effect of TCNQ concentration (% w/v) on its reaction with the statins was investigated. For this purpose, various concentrations (% w/v) of TCNQ solution were added to a fixed concentration of statins.

#### 2.5. Reaction time and temperature

The optimum reaction time was determined by following the color development at room temperature and 60–80 °C.

#### 2.6. Stoichiometry of the reaction

The molar ratio of TCNQ to given drugs in the reaction mixture was studied according to Job's method of continuous variation.<sup>45</sup>

#### 2.7. General procedure

Aliquots of 0.02–0.240 mL of the stock (for FLV) or drugs' base solutions were pipetted into a series of 5.0-mL volumetric flasks, 1.0 mL of TCNQ solution was added, and acetonitrile was added to make them up to the volume. The reaction mixture was allowed to stand for 5 min at room temperature and then the absorbance of the resulting solutions was measured at 843 nm against a reagent blank treated similarly.

#### 2.8. Assay procedure for pharmaceutical preparations

Ten capsules or tablets were weighed and powdered using a pestle and mortar. An accurately weighed portion of the powder, equivalent to 1 tablet weight for each drug, was transferred into a 100-mL volumetric flask. Then a 50-mL portion of acetonitrile (for RSV and SMV), methanol (for FLV), acetonitrile–methanol mixture (4:1) (for ATV), methanol–water mixture (4:1) (for PTV) was added into the flask containing powdered drug substance. The mixture was shaken mechanically for 5 min, sonicated in an ultrasonic bath for 30 min, diluted to the volume with solvent above, mixed, and filtered through a filter. Drug base solutions were prepared (except SIM) as described in the Reagents and Solutions section with an appropriate aliquot of the filtrate and assayed as described in the General Procedure section.

#### 2.9. Method validation

Validation studies were performed according to International Conference on Harmonization guidelines.<sup>46</sup>

Selectivity of the method was studied with a mixture of commonly tablet excipients such as starch, magnesium stearate, lactose, glucose, fructose, sucrose, talc, cellulose, and titanium dioxide.

The calibration graph was constructed by considering the absorbance measured at 5 concentration levels of each of statins (5 determinations for each level).

The limits of detection (LOD) and limits of quantitation (LOQ) were determined using the formula: LOD or LOQ =  $\kappa$  SDa/b, where  $\kappa$  = 3 for LOD and 10 for LOQ, SDa is the standard deviation of the intercept, and b is the slope.

The inter- and intra-day precision were examined by analysis of standards for the same day and 5 consecutive days (each  $n$  = 5).

To check the accuracy of the proposed methods, the standard addition technique was applied. A different amount of pure sample solution was added to 3 different concentrations of the standard drug solutions and

assayed. The percent recovery of the added standard to the assay samples was calculated from:

$$\text{Recovery \%} = [(C_t - C_u)/C_a] \times 100$$

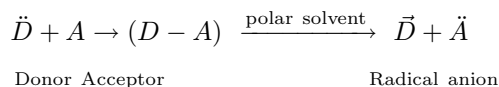
where  $C_t$  is the total concentration of the analyte found,  $C_u$  is the concentration of the analyte present in the formulation, and  $C_a$  is the concentration of the pure analyte added to the formulation.

The robustness of the proposed method was examined by evaluating the influence of small variations in the procedure variables, such as time of the reaction ( $5 \pm 0.5$  min) and added reagent volume ( $1.0 \pm 0.05$  mL).

The applicability of the proposed method was tested by the determination of drugs in their pharmaceutical preparations.

### 3. Results and discussion

ATV, FLV, PTV, RSV, and SMV are the most common statins used in hyperlipidemia. To the best of our knowledge, visible spectrophotometric determination of these drugs in tablets based on a charge transfer reaction with TCNQ has not been described. Therefore, visible spectrophotometric analyses using TCNQ reagent were developed for the determination of these drugs in tablets. The developed methods are based on the reaction of these statins as n-electron donors with TCNQ as a  $\pi$ -acceptor to give highly colored complex species.  $\pi$ -Acceptors are known to yield charge transfer complexes and radical anions with a variety of electron donors.<sup>34–44</sup> The drug–TCNQ charge transfer complexes in polar solvent are given in the Scheme.



#### Scheme

The interaction of statins with TCNQ in acetonitrile yielded a bluish-green colored chromogen, which absorbs maximally at wavelength 843 nm (Figure 2).

The influence of different parameters on color development was studied to determine optimum conditions.

#### 3.1. Choice of solvent

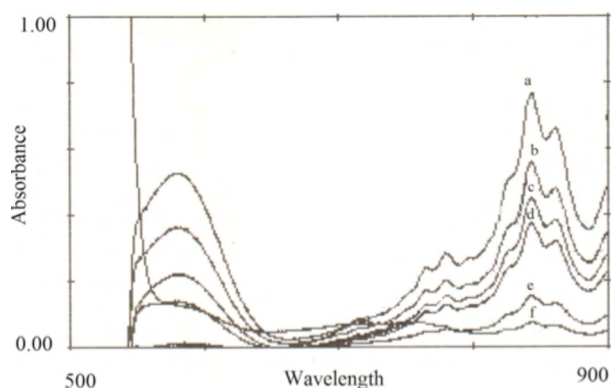
Acetonitrile is considered an ideal solvent for the color reaction as it offers solvent capacity and gives the highest yield of the radical as indicated by high  $\epsilon$  values.

#### 3.2. Reagent concentration

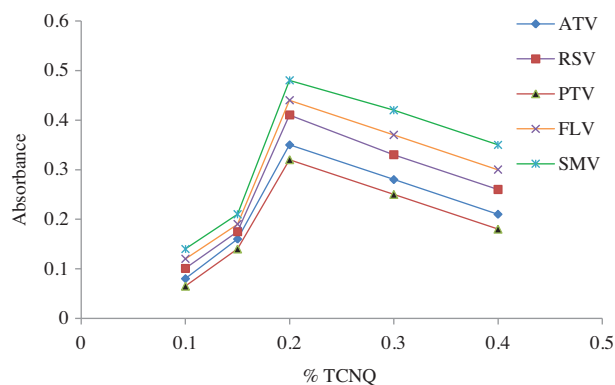
It is found that when various concentrations (by volume) of TCNQ solution were added to a fixed concentration of statins 1.0 mL of 0.2% (w/v) TCNQ was sufficient for quantitative determination of statins (Figure 3).

#### 3.3. Reaction time and temperature

Complete color development was attained after 5 min at room temperature (Figure 4). The resultant complexes were stable up to 24 h at room temperature in the dark.



**Figure 2.** Absorption spectrum of charge transfer complex between drugs and TCNQ reagent: a: ATV;  $16 \mu\text{g mL}^{-1}$ , b: FLV;  $10 \mu\text{g mL}^{-1}$ , c: RSV;  $10 \mu\text{g mL}^{-1}$ , d: PTV;  $1.2 \mu\text{g mL}^{-1}$ , e: SMV;  $5 \mu\text{g mL}^{-1}$ , f: reagent blank.



**Figure 3.** Effect of % TCNQ (w/v) on the development of the reaction product of drugs.

### 3.4. Stoichiometry of the reaction

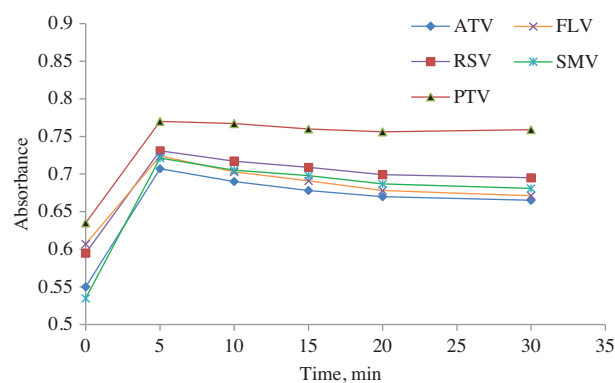
Utilizing equimolar solution of drugs and TCNQ, the reaction stoichiometry was found to be close to 1:1 ratio (drug to reagent), confirming that 1 molecule of these drugs reacts with 1 molecule of TCNQ (Figure 5).

### 3.5. Method validation

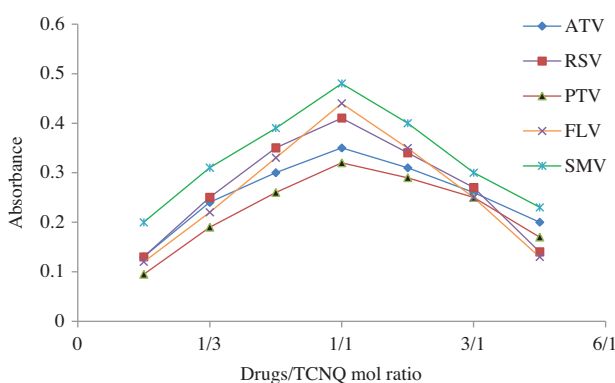
Experiments showed that there was no interference from the additions and excipients, e.g., lactose, glucose, fructose, magnesium stearate, and starch.

A linear relationship was found with between the absorbance at  $\lambda_{\text{max}}$  and the concentration of the drug in the ranges  $4\text{--}20 \mu\text{g mL}^{-1}$ ,  $4\text{--}12 \mu\text{g mL}^{-1}$ ,  $0.8\text{--}2.4 \mu\text{g mL}^{-1}$ ,  $4\text{--}14 \mu\text{g mL}^{-1}$ , and  $2.5\text{--}20 \mu\text{g mL}^{-1}$  for ATV, FLV, PTV, RSV, and SMV, respectively. Regression equations of the developed methods are given in Table 1.

While LOD values were  $1.3863$ ,  $0.3097$ ,  $2.2341$ ,  $0.0428$ , and  $0.0852 \mu\text{g mL}^{-1}$ ; LOQ values were  $4.6210$ ,  $1.0322$ ,  $7.4471$ ,  $0.1427$ , and  $0.2840 \mu\text{g mL}^{-1}$  for ATV, FLV, RSV, PTV, and SMV, respectively.



**Figure 4.** Effect of reaction time on the development of the reaction product of drugs (at room temperature).



**Figure 5.** Continuous variation plots for drugs.

**Table 1.** Regression equations of developed methods.

	Regression equation*	Slope $\pm$ SD	Intercept $\pm$ SD	Correlation coefficient, r
ATV	$A = 0.0495C - 0.0182$	$0.0495 \pm 0.0012$	$-0.0182 \pm 0.0059$	0.9994
RSV	$A = 0.0677C - 0.2468$	$0.068 \pm 0.002$	$-0.2467 \pm 0.005$	0.9997
FLV	$A = 0.0752C - 0.2262$	$0.0736 \pm 0.0026$	$-0.2233 \pm 0.0078$	0.9986
SMV	$A = 0.0405C + 0.0137$	$0.0405 \pm 0.0002$	$0.0139 \pm 0.0012$	0.9994
PTV	$A = 0.3838C - 0.036$	$0.3807 \pm 0.0043$	$-0.0361 \pm 0.0055$	0.9998

\* $A = a + bC$  (where  $C$  is the concentration of drug in  $\mu\text{g mL}^{-1}$ ,  $A$  is the absorbance at  $\lambda_{\text{max}}$ ).

In the precision study, the RSD values were 0.88%–3.02% for intra-day precision and 1.22%–3.42% for inter-day precision. The obtained results indicate good precision and are summarized in Table 2.

The standard addition method was applied for recovery studies and the results obtained are shown in Table 3. The average percent recoveries obtained were 100.12%–100.81%, indicating good accuracy of the methods.

The proposed methods were stable to small variations in the procedure variables such as time of the reaction ( $5 \pm 0.5$  min) and added reagent volume ( $1.0 \pm 0.05$  mL).

**Table 2.** The results of validation parameters for proposed methods.

Parameter	Statins				
	ATV	FLV	RSV	PTV	SMV
Linearity range <sup>a</sup> ( $\mu\text{g mL}^{-1}$ )	4.0–20.0	4.0–12.0	4.0–14.0	0.8–2.4	2.5–20.0
Intra-day <sup>b</sup> , RSD %	1.77	1.09	1.58	1.38	0.88
Inter-day <sup>c</sup> , RSD %	1.90	1.54	1.77	1.73	1.22
LOD ( $\mu\text{g mL}^{-1}$ )	0.3553	0.3097	0.2225	0.0428	0.0852
LOQ ( $\mu\text{g mL}^{-1}$ )	1.1842	1.0322	0.7416	0.1427	0.2840

<sup>a</sup>Average of 6 determinations, <sup>b</sup> $n = 5$  corresponds to replicate analysis for each level

<sup>c</sup>Results of 5 different days

The applicability of the proposed method was tested by the determination of drugs in their pharmaceutical preparations. The results obtained were satisfactorily accurate and precise as indicated by the excellent % recovery and  $\text{RSD} < 2$  (Table 4).

The results obtained were compared statistically by Student's  $t$ -test (for accuracy) and the variance ratio  $F$ -test (for precision) with those obtained by the official methods for FLV<sup>47</sup>, SMV<sup>47</sup>, and ATV<sup>48</sup>, and reference methods for RSV<sup>21</sup> and PTV<sup>30</sup>. The values of  $t$ - and  $F$ -tests obtained at 95% confidence level did not exceed the theoretical tabulated values, indicating no significant difference between the methods compared (Table 5).



**Table 3.** The results of recovery studies obtained with standard addition method.

Statins			Total amount found <sup>a</sup> ( $\mu\text{g mL}^{-1}$ ) (Mean $\pm$ S.D. <sup>b</sup> )	Recovery (%)	RSD (%)
ATV <sup>1</sup>	8.0	2.0	10.031 $\pm$ 0.051	100.31	0.51
		4.0	12.031 $\pm$ 0.04	100.26	0.35
		6.0	14.057 $\pm$ 0.03	100.41	0.22
FLV <sup>2</sup>	4.0	2.0	6.044 $\pm$ 0.033	100.74	0.55
		4.0	8.047 $\pm$ 0.026	100.59	0.33
		6.0	10.024 $\pm$ 0.020	100.25	0.20
RSV <sup>3</sup>	6.0	2.0	8.032 $\pm$ 0.053	100.41	0.66
		4.0	10.061 $\pm$ 0.074	100.61	0.73
		6.0	12.060 $\pm$ 0.030	100.50	0.24
PTV <sup>4</sup>	1.0	0.2	1.209 $\pm$ 0.006	100.81	0.54
		0.4	1.401 $\pm$ 0.007	100.12	0.55
		0.8	1.807 $\pm$ 0.003	100.40	0.22
SMV <sup>5</sup>	5.0	3.0	8.048 $\pm$ 0.037	100.61	0.46
		5.0	10.056 $\pm$ 0.049	100.56	0.49
		7.0	12.048 $\pm$ 0.062	100.40	0.51

<sup>1</sup>Ator tablet®, containing 20 mg of ATV per, tablet

<sup>2</sup>Lescol tablet®, containing 40 mg of FLV per tablet

<sup>3</sup>Crestor tablet®, containing 20 mg of RSV per tablet

<sup>4</sup>Livalo tablet®, containing 2 mg of PTV per tablet

<sup>5</sup>Zocor tablet®, containing 20 mg of SMV per tablet

<sup>a</sup>Five independent analyses, <sup>b</sup>Standard deviation

**Table 4.** The results of analysis of drugs in tablets.

Statins	Mean <sup>a</sup> $\pm$ S.D. <sup>b</sup>	Recovery (%)	RSD (%)
ATV <sup>1</sup>	19.96 $\pm$ 0.185	99.65	0.927
FLV <sup>2</sup>	40.574 $\pm$ 0.359	101.436	0.884
RSV <sup>3</sup>	19.69 $\pm$ 0.277	98.437	1.406
PTV <sup>4</sup>	1.988 $\pm$ 0.033	99.390	1.659
SMV <sup>5</sup>	20.023 $\pm$ 0.273	100.115	1.363

<sup>1</sup>Ator tablet®, containing 20 mg of ATV per tablet

<sup>2</sup>Lescol tablet®, containing 40 mg of FLV per tablet

<sup>3</sup>Crestor tablet®, containing 20 mg of RSV per tablet

<sup>4</sup>Livalo tablet®, containing 2 mg of PTV per tablet

<sup>5</sup>Zocor tablet®, containing 20 mg of SMV per tablet

<sup>a</sup>Five independent analyses, <sup>b</sup>Standard deviation

**Table 5.** Statistical evaluations of the results obtained by proposed and reference methods for the assay of drugs in pharmaceutical preparations ( $n = 5$ ).

Statistical value	ATV		FLV		RSV		PTV		SMV	
	Proposed method <sup>a</sup>	Ref. Method <sup>48</sup>	Proposed Method <sup>b</sup>	Ref. Method <sup>47</sup>	Proposed Method <sup>c</sup>	Ref. Method <sup>21</sup>	Proposed Method <sup>d</sup>	Ref. Method <sup>47</sup>	Proposed method <sup>e</sup>	Ref. Method <sup>30</sup>
Mean $\pm$ SD	19.98 $\pm$ 0.167	20.01 $\pm$ 0.345	40.25 $\pm$ 0.289	39.93 $\pm$ 0.367	19.94 $\pm$ 0.298	20.31 $\pm$ 0.375	2.004 $\pm$ 0.023	2.028 $\pm$ 0.04	20.07 $\pm$ 0.198	19.88 $\pm$ 0.256
Recovery (%)	99.9	100.05	100.62	99.82	99.70	101.55	100.2	101.4	100.35	99.4
RSD (%)	0.835	1.72	0.718	0.919	1.494	1.846	1.147	1.923	0.986	1.287
t-test of significance <sup>**</sup>	1.13		1.12		0.29		1.28		1.13	
F-test of significance <sup>**</sup>	1.28		0.703		2.37		1.39		1.28	

<sup>a</sup>Ator tablet® (20 mg ATV) <sup>b</sup>Lescol tablet® (40 mg FLV) <sup>c</sup>Crestor tablet® (20 mg RSV) <sup>d</sup>Livalo tablet® (2 mg PTV) <sup>e</sup>Zocor tablet® (20 mg SMV)

\* Five independent analyses \*\* $p = 0.05$ ,  $t = 2.23$ ,  $F = 5.05$

#### 4. Conclusion

The aim of this study was to develop simple, fast, validated, and very economical spectrophotometric methods for analyzing ATV, FLV, RSV, PTV, and SMV in their pharmaceutical preparations. The developed methods were based on a charge transfer reaction with TCNQ reagent. The proposed methods are nearly the same<sup>11,16,18</sup> or more sensitive<sup>10,12–15,19</sup> and time saving<sup>9,13,14</sup> than other published spectrophotometric methods. Moreover, the proposed methods are less expensive than the application of HPLC techniques.<sup>20–30</sup> The described methods are suitable for the determination of statins in pharmaceutical formulations without interference from excipients and could be easily applied in quality control laboratories for the routine analysis of these drugs in raw material and pharmaceutical formulations.

#### References

1. Tobert, J. A. *Nat. Rev. Drug Discov.* **2003**, *2*, 517–526.
2. Jones, P. H.; Davidson, M. H.; Stein, E. A.; Bays, H. E.; McKenney, J. M.; Miller, E. *Am. J. Cardiol.* **2003**, *92*, 152–160.
3. Caslake, M. J.; Stewart, G.; Day, S. P.; Daly, E. *Atherosclerosis* **2003**, *171*, 245–253.
4. Endo, A. *J. Lipid Res* **1992**, *33* 1569–1582.
5. Erturk, S.; Onal, A.; Çetin, S. M. *J. Chromatogr. B* **2003**, *793*, 193–205.
6. Sweetman, S. C. *Martindale: The Complete Drug Reference*, 34th Ed., Pharmaceutical Press, London, 2004.
7. Terata, Y.; Saito, T.; Fujiwara, Y.; Hasegawa, H.; Miura, H.; Watanabe, H.; Chiba, Y.; Kibira, S.; Miura, M. *Pharmacology* **2003**, *68*, 169–176.
8. Lennernas, H.; Fager, G. *Clin Pharmacokinet.* **1997**, *32* 403–425.
9. Nagaraju, P.; Pasumarthy; Gopal, N. V.; Srinivas, V. D. N.; Padma, S. V. N. *Asian J. Res. Chem.* **2008**, *1*, 64–66.
10. Erk, N. *Anal. Lett.* **2003**, *36*, 2699–2711.
11. Nagaraj; Vipul K.; Rajshree, M. *Anal. Sci.* **2007**, *23*, 445–451.
12. Stanis, B.; Rafa, W. *Chem. Anal-Warsaw* **2008**, *53*, 417–428.
13. Ashour, S.; Bahbouh, M.; Khateeb, M. *Int. J. Biomed. Sci.* **2010**, *6*, 19–26.
14. Saminathan, J.; Ksankar, A. S.; Anandakumar, K.; Vetrichelvan, T. *E-J. Chem.* **2009**, *6*, 1233–1239.
15. Erk, N. *Pharmazie* **2002**, *57*, 817–819.
16. Moussa, B. A.; Mohamed, M. F.; Youssef, N. F. *Eur. J. Chem.* **2010**, *1*, 348–351.
17. Wang, L.; Asgharnejad, M. *J. Pharmaceut. Biomed.* **2000**, *21*, 1243–1248.
18. Gupta, A.; Mishra, P.; Shah, K. *E-J. Chem.* **2009**, *6*, 89–92.
19. Krishna, M. V.; Sankar, D. G. *E-J. Chem.* **2007**, *4*, 272–278.
20. Sankar, G. D.; Babu, J. P.; Kumar, A. B.; Krishna, V. M. *Acta Cienc. Indica, Ser. Chem.* **2007**, *33*, 1–4.
21. Gomes, F.; Garcia, P.; Alves, J.; Singh, A.; Kedor-Hackmann, E.; Santoro, M. *Anal. Lett.* **2009**, *42*, 1784–1804.
22. Altuntas, T. G.; Erk, N. *J. Liq. Chromatogr. R. T.* **2004**, *27*, 83–93.
23. Pasha, M. K.; Muzeeb, S.; Basha, S. J. S.; Shashikumar, D.; Mullangi, R.; Srinivas, N. R. *Biomed. Chromatogr.* **2006** *20*, 282–293.
24. Seshachalam, U.; Kothapally, C. B. *J. Liq. Chromatogr. R. T.* **2008**, *31*, 714–721.
25. Vuletic, M.; Cindric, M.; Kouznejak, J. D. *J. Pharmaceut. Biomed.* **2005**, *37*, 715–721.
26. Carlucci, G.; Mazzeo, P. *J. Pharmaceut. Biomed.* **1992**, *10* 693–697.
27. Ochiai, H.; Chiyama, N. K.; Imagaki, S. *J. Chromatogr. B* **1997**, *694*, 211–217.

28. Abdallah, O. M. *E-J. Chem.* **2011**, *8(2)*, 753–761.
29. Kaila, H. O.; Ambasana, M. A.; Thakkar, R. S.; Saravaia, H. T.; Shah, A. K. *Indian J. Pharm. Sci.* **2010**, *72*, 592–598.
30. Kumar, N. S.; Nisha, N.; Nirmal, J.; Sonali, N.; Bagyalakshmi, J. *Pharm. Anal. Acta* **2011**, *2*, 1–4.
31. Sane, R. T.; Kamat, S. S.; Menon, S. N.; Inamdar, S. R.; Mote, M. R. *J. Planar Chromatogr. -Mod. TLC* **2005**, *18*, 194–198.
32. Yadav, S.; Mhaske, D.; Kakad, A.; Patil, B.; Kadam, S.; Dhaneshwar, S. *Indian J. Pharm. Sci.* **2005**, *67*, 182–186.
33. Erk, N. *Crit. Rev. Anal. Chem.* **2004**, *34*, 1–7.
34. Dogan-Topal, B.; Uslu, B.; Özkan, S. A. *Comb. Chem. High T. Scr.* **2007**, *10*, 571–582.
35. Saleh, G. A. *Talanta* **1998**, *46*, 111–121.
36. Kovar, K. A.; Abdel-Hamid, M. E. *Arch. Pharm.* **1984**, *317*, 246–250.
37. Foster, R. *Organic Charge-Transfer Complexes*, Academic Press, London, 1969.
38. Melby, L. R.; Patai, S. *The Chemistry of the Cyano Group*, Interscience, New York, 1970.
39. Rao, C. N. R.; Bhat, S. N.; Dwivedi, P. C. *Applied Spectroscopy Reviews*, 5, Brame, E.D., ed, Marcel Dekker, New York, 1972.
40. Kovar, K. A.; Mayer, W.; Auterhoff, H. *Arch. Pharm.* **1981**, *314*, 447–458.
41. Abdellatef, H. E. *J. Pharmaceut. Biomed.* **1998**, *17*, 1267–1271.
42. Saleh, G. A.; Askal, H. F.; Darwish, L. A.; El-Shorbagi, A. N. A. *Anal. Sci.* **2003**, *19*, 281–287.
43. Abdel-Gawad, F. M.; Issa, Y. M.; Fahmy, H. M.; Hussein, H. M. *Microchim. Acta* **1998**, *130*, 35–40.
44. Hussein, S. A.; Mohamed, A. M.; Abdel-Alim, M. *Analyst* **1989**, *114*, 1129–1131.
45. Job, P. *Anal. Chem.* **1928**, *9*, 113–203.
46. ICH, Q2 (R1), Harmonised Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology, International Conference on Harmonization, Geneva, 2005.
47. *United States Pharmacopoeia National Formulary, USP 30-NF 25*, United States Pharmacopoeia Convention Inc., Rockville, Maryland, USA; 2007.
48. *United States Pharmacopoeia National Formulary, USP 32-NF 27*, United States Pharmacopoeia Convention Inc., Rockville, Maryland, USA; 2009.