Istanbul J Pharm 50 (2): 95-102
DOI: 10.26650/IstanbulJPharm.2020.0003
Original Article

Spectrophotometric determinations of most commonly used statins in pharmaceutical preparations with 2,3-dichloro-5,6-dicyanobenzoquinone

Gamze Ergin Kızılçay[1], Sıdıka Ertürk Toker[1]

[1]Istanbul University, Faculty of Pharmacy, Department of Analytical Chemistry, Istanbul, Turkey

ORCID IDs of the authors: G.E.K. 0000-0002-1089-7195; S.E.T. 0000-0002-6827-8362

Cite this article as: Ergin Kizilcay, G., & Erturk Toker, S. (2020). Spectrophotometric determinations of most commonly used statins in pharmaceutical preparations with 2,3-dichloro-5,6-dicyanobenzoquinone. *İstanbul Journal of Pharmacy, 50* (2), 95-102.

ABSTRACT

Background and Aims: In this study, simple, accurate and precise spectrophotometric methods were developed for the determination of five 3-hydroxy-3-methylglutaryl-coenzyme-A (HMG-CoA) reductase inhibitors in pure and pharmaceutical dosage forms.

Methods: In the developed methods, atorvastatin fluvastatin, pitavastatin, rosuvastatin and simvastatin were used in this class of drugs called statins. The methods were based on the charge-transfer reaction of n-electron donor drugs with ϖ-acceptor 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ). All variables such as temperature, time, reaction medium, amount of reagent were examined for optimal formation of complexes to be given by the drugs with DDQ and optimal conditions were determined.

Results: The linearity ranges for atorvastatin, fluvastatin, pitavastatin, rosuvastatin and simvastatin were found to be 0.5-50, 1-6, 2.5-50, 5-25 and 5-50 µg/mL, respectively.

Conclusion: The proposed methods were successfully applied to both the pure and the pharmaceutical dosage forms.

Keywords: HMG-CoA reductase inhibitors, spectrophotometric determination, charge-transfer reaction, DDQ, pharmaceutical preparations

INTRODUCTION

3-Hydroxy-3-methylglutaryl-coenzyme-A (HMG-CoA) reductase inhibitors, called statins, are the most effective drugs used to prevent hypercholesterolemia and related diseases. Statins show therapeutic effects by competitively inhibiting HMG-CoA reductase, an enzyme that catalyzes the conversion of HMG-CoA to mevalonate, an early rate limiting step in cholesterol biosynthesis in the body. These agents are highly effective in reducing total cholesterol and low-density lipoprotein levels in several forms of hypercholesterolemia (Tobert, 2003; Jones et al., 2003; Caslake et al., 2003; Antal et al., 2017).

Regarding the statins that form part of this study, simvastatin is a semi-synthetic product, while the others are fully synthetic compounds (Sweetman, 2005; Antal et al., 2017).

Atorvastatin calcium (AT) (3R,5R)-7-[2-(4-fluorophenyl)-3-phenyl-4-(phenylcarbamoyl)-5-propan-2-ylpyrrol-1-yl]-3,5-dihydroxyheptanoic acid, calcium salt, Fluvastatin sodium (FL) (E,3R,5S)-7-[3-(4-fluorophenyl)-1-propan-2-ylindol-2-yl]-3,5-dihydroxyhept-6-enoic acid monosodium salt, Pitavastatin calcium (PT) (E,3R,5S)-7-[2-cyclopropyl-4-(4-fluorophenyl)quinolin-3-yl]-3,5-dihydroxy-

Address for Correspondence:
Sıdıka ERTÜRK TOKER, e-mail: serturk@yahoo.com, serturk@istanbul.edu.tr

Submitted: 13.02.2020
Revision Requested: 10.03.2020
Last Revision Received: 13.04.2020
Accepted: 03.06.2020

hept-6-enoic acid monocalcium salt, Rosuvastatin calcium (RS) (E,3R,5S)-7-[4-(4-fluorophenyl)-2-[methyl(methylsulfonyl) amino]-6-propan-2-ylpyrimidin-5-yl]-3,5-dihydroxyhept-6-enoic acid calcium salt and Simvastatin (SM) [(1S,3R,7S,8S,8aR)-8-[2-[(2R,4R)-4-hydroxy-6-oxooxan-2-yl]ethyl]-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl]-2,2-dimethylbutanoate (Figure 1) are the most commonly used statins in the treatment of hyperlipidemia (Lennernas & Fager, 1997; Terata et al., 2003; Sweetman, 2005; Antal et al., 2017). Several analytical methods such as spectrophotometric (Wang & Asgharnejad, 2000; Erk, 2002; Krishna & Sankar, 2007; Stanisz & Rafa, 2008; Saminathan, Sankar, Anandakumar, & Vetrichelvan, 2009; Gupta, Mishra, & Shah, 2009; Ashour, Bahbouh, & Khateeb, 2010; Moussa, Mohammed, & Youssef, 2010; Kokilambigai, Seetharaman, & Lakshmi, 2017), high performance liquid chromatographic (HPLC) (Carlucci, Mazzeo, Biordi, & Bologna, 1992; Ochiai, Uchiyama, Imagaki, Hata, & Kamei, 1997; Vuletic, Cindric, & Kouznjak, 2005; Sankar, Babu, Kumar, & Krishna, 2007; Gomes et al., 2009; Kaila, Ambasana, Thakkar, Saravaia, & Shah, 2010; Abdallah, 2011; Kumar, Nisha, Nirmal, Sonali, & Bagyalakshmi, 2011; Kokilambigai et al., 2017), HPTLC (Yadav et al., 2005; Sane, Kamat, Menon, Inamdar, & Mote, 2007) and electroanalytical techniques (Antal et al., 2017) have been used for the determination of these drugs, either simultaneously or alone, both as bulk drugs and as formulations (Figure 1).

As a result of our article, scanning studies, the spectrophotometric determination of these five statins based on the charge transfer reaction with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) are not available.

When we consider other analytical methods used in drug active substance analysis, spectrophotometric methods are advantageous because of the need for a simple and cheap device and a fast measurement capacity. The accuracy and precision of the methods are also well suited for the identification of drugs in both pure and dosage forms. With this information, we aimed to develop spectrophotometric determination of five statins using DDQ reagent. DDQ has been intensively used as the reagent for visible-spectrophotometric methods of a number of n-electron donor drugs (Foster, 1969; Melby & Patai, 1970; Rao, Bhat, & Dwivedi, 1972; Kovar, Mayer, & Auterhoff, 1981; Kovar & Abdel-Hamid, 1984; Hussein, Mohamed, & Abdel-Alim, 1989; Saleh, 1998; Abdellatef, 1998; Abdel-Gawad, Issa, Fahmy, & Hussein, 1998; Saleh, Askal, Darwish, & El-Shorbagi, 2003). In these methods, the blue-purple colored DDQ^{-} radical anion formed by the interaction of the drug substances in the appropriate solvent with the reagent was measured in the visible region.

MATERIALS AND METHODS

Apparatus

In this study, measurements were made using a Shimadzu UV-160 A spectrophotometer. A 1 cm glass cell was used for the measurements.

Reagents and solutions

AT and its pharmaceutical dosage form, Ator film tablet® (20 mg of per tablets) were provided by Sanovel pharmaceuticals

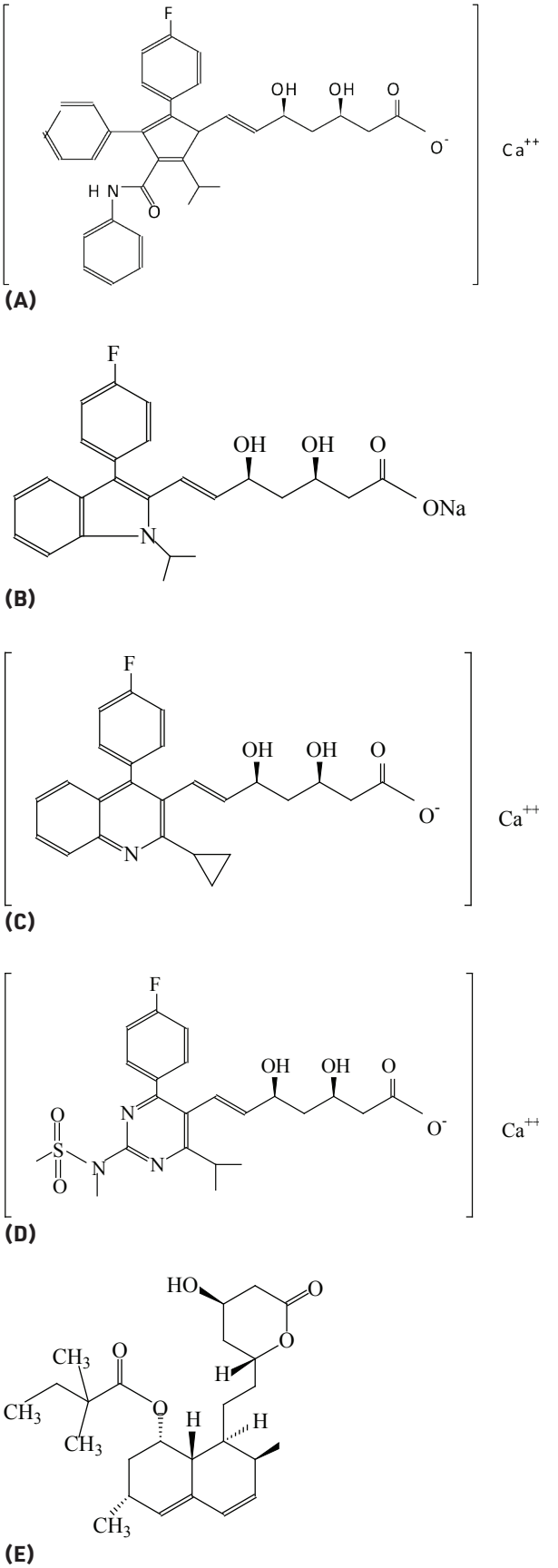

Figure 1. Chemical structure of statins, (A): AT, (B): FL, (C): PT, (D): RS, (E): SM.

(Istanbul, Turkey). FL and its pharmaceutical dosage form, Lescol capsul® (40 mg of per capsul) were obtained from Novartis pharmaceuticals (Istanbul, Turkey). PT and its pharmaceutical dosage form, Livalo film tablet® (2 mg of per tablets) were provided by Kowa pharmaceuticals (Japan). RS and its pharmaceutical dosage form, Crestor film tablet®, (20 mg of per tablets) were obtained from Astra Zeneca pharmaceuticals (Istanbul, Turkey). SM and its pharmaceutical dosage form, Zocor film tablet® (20 mg of per tablets) were obtained from Nobel pharmaceuticals (Istanbul, Turkey). DDQ was purchased from Merck (Darmstadt, Germany). In this study, analytical-reagent grade chemicals and solvents were used.

Stock solutions were prepared separately as follows: 2.0 mg/mL RS and 1.0 mg/mL SM solutions (equivalent to the same milligram of drug base) were prepared by dissolving in acetonitrile. 1.0 mg/mL FL solution (equivalent to the same milligram of drug base) was prepared by dissolving in a methanol-acetonitrile mixture (1: 9). 2.0 mg/mL. AT and PT solutions (equivalent to the same milligram of drug base) were prepared using dimethylsulfoxide as solvent.

1, 2 and 3 mg/mL. DDQ solution was prepared in acetonitrile (for RS, FL and SM) and in dimethylsulfoxide (DMSO) (for AT and PT). These solutions were freshly prepared every day.

Choice of solvent

During the selection of the most suitable solvent in the reactions and measurements, different solvents such as acetone, acetonitrile, chloroform, 1,4-dioxane, DMSO, ethanol, methanol and methylene chloride were tested. As a result of solvent selection studies, the most suitable solvents were determined to be acetonitrile for fluvastatin, rosuvastatin and simvastatin and DMSO for atorvastatin and pitavastatin.

Reagent concentration

The effect of DDQ concentration (%, w / v) on the efficiency of the reaction was investigated. Therefore, a constant concentration of statin was selected and various concentrations (%, w / v) of DDQ solution were added thereto. Measurements were made at the end of the same waiting period.

Reaction time and temperature

In order to find the optimum reaction temperature, the reactions were carried out at room temperature, 50, 60, 70 and 80 °C and the absorbance values were read.

Similarly, in order to find the optimal reaction time, the reactions were determined by monitoring the absorbance values for 10, 20 and 30 minutes at the temperature at which the highest absorbance was obtained for each substance.

Stoichiometry of the reaction

The molar ratios of drug-DDQ for each statin were examined according to Job's continuous variation method (Job, 1928).

General procedure

Appropriate volumes of stock solutions or drug solutions were taken up in a 5 mL volumetric flask, 1 mL of DDQ solution was added and the volume was adjusted to the volume with acetonitrile or DMSO. The reaction mixtures were allowed to stand for 20 min at 70°C for SM, 30 min at 80°C for FL, 20 min at 80°C for RS, 20 min at 90 for AT and PT. Then the absorbance of the formed complexes was measured at 459 nm (for FL, RS and SM) and 469 nm (for AT and PT) against reagent blank obtained in a similar way .

Assay

Ten capsules or tablets were weighed separately and the average capsule/tablet weight was calculated. The tablets or capsule contents were powdered using a mortar. The amount of powder, equivalent to one tablet weight per drug, was accurately weighed and transferred to a 100 mL volumetric flask. Then the appropriate amount of acetonitrile for RS and SM, acetonitrile-methanol mixture (9: 1) for FL or DMSO for AT and PT was transferred to the flask. The mixtures were shaken mechanically for five minutes and sonicated in an ultrasonic bath for 30 min. Then they were filled to the volume with the above solvents and filtered through a filter. A suitable portion of the filtrate was determined as defined in the General Procedure.

Method validation

Validation studies were planned according to the International Conference on Harmonization guidelines (ICH, 2005).

The selectivity of the method was performed with a mixture of common tablet excipients such as cellulose, glucose, lactose, starch, talc, magnesium stearate, titanium dioxide etc.

The calibration graph was created in accordance with Beer's laws with absorbance values (5 determinations per level) measured at 5 concentration levels of each statin.

The limits of quantitation (LOQ) and limits of detection (LOD) were calculated using the following formulas:

$LOQ = 10SDa / b$

$LOD = 3SDa / b$

(SDa is the standard deviation of the intercept and b is the slope)

The interday and intraday precision were studied by analysis of standards for same day and five different days (each n=5).

The accuracy of the proposed method was determined by standard addition method. Three different concentrations of standard statin solutions were added over different amounts of sample solutions and these mixtures were analysed. The recovery percentage of the standard added to the test samples was calculated using the following formula:

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

Where C_t is the total concentration of the analyte; C_u is the concentration of the analyte present in the formulation; and C_a is the concentration of the analyte added to the formulation.

The robustness of the recommended method was evaluated by examining the effect of small changes in reaction conditions such as reaction time (5±0.5 min) and additional reagent volume (1.0±0.05 mL).

The proposed methods were examined by determining statins in drug dosage forms for applicability of the methods.

RESULTS AND DISCUSSION

AT FL, PT, RS and SM are the drugs most commonly used to prevent hypercholesterolemia and related diseases. , A current rise in cardiovascular disease has increased the need for these drugs and has led many pharmaceutical companies to add statins to their products. This has also increased the need for simple, accurate and precise methods for the determination of these substances.

Since statins are very popular drugs, there have been many studies to date on their analysis in pharmaceutical preparations. However, visible spectrophotometric determination of these drugs in pharmaceutical preparations based on charge transfer reaction with DDQ has not yet been published. Therefore, visible spectrophotometric analyses using DDQ reagent were developed for the determination of these drugs in pharmaceutical preparations. The developed methods are based on the formation of ion pair complexes of these p-acceptor drugs with DDQ, the n-electron donor. For many years, π –acceptors have been known to produce charge transfer complexes and radical anions with various electron donors (Foster, 1969; Melby & Patai, 1970; Rao et al., 1972; Kovar et al., 1981; Kovar & Abdel-Hamid, 1984; Hussein et al., 1989; Saleh, 1998; Abdellatef, 1998; Abdel-Gawad et al., 1998; Saleh et al., 2003; Antal et al., 2017).

Blue chromogens were obtained from the reaction of statins with DDQ in acetonitrile or DMSO. Those obtained in acetonitrile or DMSO from these chromagens showed maximum absorption at 459 and 469 nm, respectively (Figure 2).

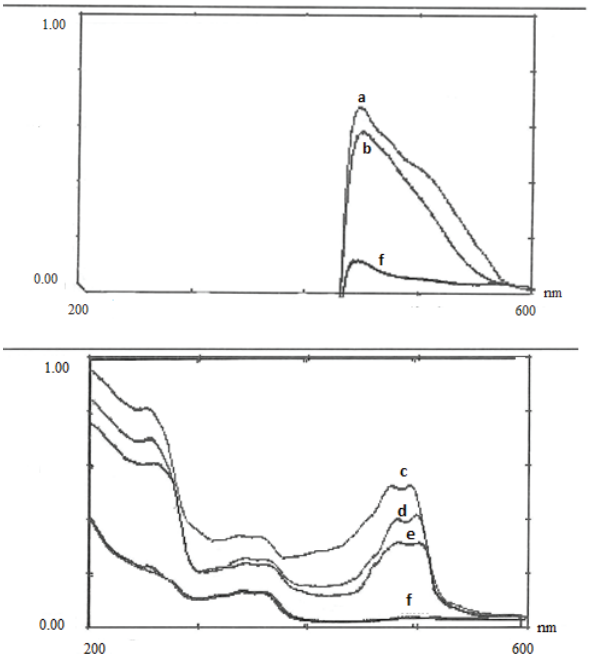

Figure 2. Absorption spectrum of charger transfer complex between drugs and DDQ reagent a: PT; 20 µg mL^{-1}, b: AT; 40 µg mL^{-1}, c: FL; 2.5 µg mL^{-1}, d: RS; 10 µg mL^{-1}, e: SM; 10 µg mL^{-1}, f: reagent blank.

In order to determine the optimum conditions, the effect of different parameters on chromogens was examined.

Choice of solvent

In solvent selection studies, a number of solvents were tested as mentioned above in the experimental section. The most suitable solvent was found to be acetonitrile for FL, RS and SM, and DMSO for AT and PT.

Reagent concentration

In the reagent quantification experiments, various concentrations of the DDQ solution (by volume) were studied by adding to a constant statin concentration. 1.0 mg/mL (w/v) DDQ solution was found to be sufficient for the quantitative determination of PT, 2.0 mg/mL (w/v) DDQ solution was found to be sufficient for the quantitative determination of SM and RS; 3.0 mg/mL (w/v) DDQ solution was found to be sufficient for the quantitative determination of FL.

Reaction time and temperature

The optimum reaction temperature and time was determined for each drug by following the absorbance values at 50, 60, 70, 80 and 90 °C, at 10, 20 and 30 minutes. The reaction temperature and time appropriate for each substance are shown in Table 1 (below).

Table 1. The results of optimum temperature and time

Statins	Optimum Temperature	Optimum Time
SM	70 °C	20 min
FL	80 °C	30 min
RS	80 °C	20 min
AT	90 °C	20 min
PT	90 °C	20 min

Stoichiometry of the reaction

Using the equimolar drug and DDQ solution, the reaction stoichiometry was examined separately for each drug. The mole ratios (drug/reagent) were found 1:1 for SM and for PT (one molecule of reacts with one molecule of DDQ), 5:1 for RS and AT (five molecules of reacts with one molecule of DDQ) and 1:2 for FL (one molecule of FL with two molecules of DDQ).

Method validation

In method validation studies, there was no interaction from the additions and excipients, e.g. lactose, glucose, fructose, magnesium stearate and starch.

Linear relationships between the absorbance and the concentration were found to be in the ranges of 0.5-50, 1-6, 2.5-50, 5-25, 5-50 µg/mL for AT, FL, PT, RS and SM, respectively. The regression equation parameters of the developed methods are shown in Table 2.

LOD values of the methods were calculated to be 1.13, 0.33, 1.84, 1.61 and 0.27 µg/mL; and additionally, LOQ values of the methods were calculated to be 3.77, 1.10, 6.14, 5.37 and 0.90 µg/mL for AT, FL, RS, PT and SM, respectively.

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

Parameter	Statins				
	AT	FL	RS	PT	SM
Linearity range[a] (mg mL^{-1})	0.5-50.0	1.0-6.0	5.0-25.0	2.5-50.0	5.0-50.0
Regression equation*	A=0.0106C-0.192	A=0.1264C-0.0341	A=0.0423C-0.0586	A=0.0132C-0.1941	A=0.0191C-0.0141
Slope ± SD	0.0106± 0.0012	0.1264± 0.0026	0.0423±0.002	0.0132±0.0043	0.0191±0.0002
Intercept ± SD	-0.192± 0.0059	-0.0341± 0.0078	-0.0586±0.005	-0.1941± 0.0055	-0.0141±0.0012
Correlation coefficient, r	0.9993	0.9998	0.9992	0.9985	0.9998
LOD (mg mL^{-1})	1.13	0.33	1.84	1.61	0.27
LOQ (mg mL^{-1})	3.77	1.10	6.14	5.37	0.90

[a]Average of six determinations
[b]n=6 correspond to replicate analysis for each level
*Results of six different days
**$A = a + bC$ (where C is the concentration of drug in mg mL^{-1}, A is the absorbance at λ_{max}).

In the precision study, the RSD values were found te be 0.14-1.69% for intra-day precision and 0.25-1.88% for inter-day precision. The obtained results indicate good repeatability and reproducibility as outlined in Table 3.

Accuracy studies were conducted using the standard addition method and the obtained results are shown in Table 4. The average recovery percentage obtained was between 99.7-101.7%, which showed good accuracy of the methods.

In studies on the robustness of the proposed methods, small changes in the procedure variables such as reaction time (5±0.5 minutes), and added reagent volume (1.0±0.05 mL) were examined and it was found that the methods were not affected by these changes.

The proposed methods were applied to the quantification of the pharmaceutical preparations of these drugs and the results obtained are shown in Table 5. The results obtained by the methods were satisfactorily accurate and precise with excellent % recovery and RSD values.

Table 3. The results of precision studies by obtained with proposed method.

Statins	Amount taken (mg mL^{-1})	Intraday RSD[a] (%)	Interday RSD[a] (%)
AT	2.5	1.60	1.88
	10.0	1.69	1.83
	30.0	1.26	1.40
FL	1.0	1.11	1.42
	3.0	1.05	1.28
	6.0	0.43	0.64
RS	5.0	0.85	1.54
	10.0	0.25	0.74
	25.0	0.33	0.58
PT	2.5	1.13	1.33
	25.0	0.14	0.33
	50.0	0.20	0.25
SM	5.0	0.89	1.59
	25.0	0.36	0.40
	50.0	0.34	0.47

[a]Relative standard deviation

Table 4. The results of recovery studies obtained with standard addition method.

Statins	Amount taken (mg mL^{-1})	Amount added (mg mL^{-1})	Total amount found[a] (mg mL^{-1}) (Mean± S.D[b])	Recovery (%)	RSD (%)
AT[1]	10.0	10	19.984±0.402	99.7	1.84
		20	30.09±0.558	100.5	1.63
		30	39.73±0.698	101.2	1.80
FL[2]	1.0	1.0	2.022±0.013	101.1	0.65
		2.0	3.036±0.027	100.9	0.91
		4.0	5.094±0.017	101.7	0.30
RS[3]	5.0	2.5	7.54±0.041	100.6	0.43
		5.0	10.11±0.039	100.8	0.54
		15	20.44±0.227	101.2	0.42
PT[4]	10.0	2.5	12.53±0.032	100.3	0.26
		15	25.13±0.083	100.4	0.24
		35	45.16±0.075	100.3	0.14
SM[5]	10.0	5.0	15.12±0.054	100.6	0.42
		15.0	25.24±0.108	100.8	0.53
		35.0	45.19±0.111	100.5	0.19

[1]Ator tablet®, containing 20 mg of AT per tablets
[2] Lescol tablet®, containing 40 mg of FL per tablets
[3] Crestor tablet®, containing 20 mg of RS per tablets
[4] Livalo tablet®, containing 2 mg of PT per tablets
[5] Zocor tablet®, containing 20 mg of SM per tablets
[a]Six independent analyses.
[b]Standard deviation

Table 5. The results analysis of drugs in tablets.

Statins	Mean[a] ± S.D[b]	Recovery (%)	RSD (%)
AT[1]	19.69 ±0.49	99.45	2.52
FL[2]	39.97±0.4	100.279	1.000
RS[3]	19.902±0.0482	99.40	0.2420
PT[4]	1.97±0.05	99.25	2.56
SM[5]	20.002±0.62	99.91	3.1

[1]Ator tablet®, containing 20 mg of AT per tablets
[2]Lescol tablet®, containing 40 mg of FL per tablets
[3]Crestor tablet®, containing 20 mg of RS per tablets
[4]Livalo tablet®, containing 2 mg of PT per tablets
[5]Zocor tablet®, containing 20 mg of SM per tablets
[a]Six independent analyses.
[b]Standard deviation

The obtained results were statistically compared by the student's *t*-test (for accuracy) and the variance ratio *F*-test (for precision) with the results obtained by the official methods for AT (USP, 2009), FL (USP, 2007) and SM (USP, 2007), reference methods for RS (Gomes et al., 2009) and PT (Kumar et al., 2011). It was observed that the values of t- and F-tests obtained at 95% confidence level did not exceed the theoretical table value and there was no significant difference between the methods compared (Table 6).

CONCLUSION

This study aimed to develop validated spectrophotometric methods, which are also fast, simple and economical, to analyse AT, FL, RS, PT and SM in pharmaceutical preparations. The methods developed are based on the charge transfer reaction of these drugs with DDQ reagent.

The sensitivity of the proposed methods is almost the same (Gupta et al., 2009; Moussa et al., 2010; Kokilambigai et al., 2017), or more sensitive, when compared with some previously published methods (Erk, 2002; Stanisz & Rafa, 2008; Saminathan et al., 2009; Ashour et al., 2010; Kokilambigai et al., 2017). Additionally, the proposed methods are faster than some previously published methods (Saminathan et al., 2009; Ashour et al., 2010; Kokilambigai et al., 2017).

Furthermore, the proposed methods are cheaper than the HPLC techniques (Carlucci et al., 1992; Ochiai et al., 1997; Vuletic et al., 2005; Sankar et al., 2007; Gomes et al.,

Table 6. Statistical evaluations of the results obtained by proposed and reference methods for the assay of drugs in pharmaceutical preparations (*n*=5)

Statistical value	AT		FL		RS		PT		SM	
	Proposed method[a]	Ref. Method[48]	Proposed Method[b]	Ref. Method[47]	Proposed Method[c]	Ref. Method[21]	Proposed Method[d]	Ref. Method[47]	Proposed method[e]	Ref. Method[30]
Mean*± SD	19.69±0.49	20.01±0.345	39.97 ±0.4	39.93 ±0.367	19.902±0,0482	20.31±0.375	1.97±0.05	2.028±0.04	20.002±0.62	19.88±0.256
Recovery (%)	99.45	100.05	100.279	99.82	99.40	101.55	99.25	101.4	99.91	99.4
RSD (%)	2.52	1.72	1.0	0.919	0.2420	1.846	2.56	1.923	3.1	1.287
t-test of significance**	1.8945		0.8685		1.387		1.747		0.3982	
F-test of significance**	0.559		0.45767		0.659		0.79957		1.10596	

[a]Ator tablet® (20 mg AT), [b]Lescol tablet® (40 mg FL), [c]Crestor tablet® (20 mg RS), [d]Livalo tablet® (2 mg PT), [e]Zocor tablet® (20 mg SM),
* Five independent analyses. **p = 0.05, t = 2.228, F = 5.05

2009; Kaila et al., 2010; Abdallah, 2011; Kumar et al., 2011; Kokilambigai et al., 2017).

The methods described can safely be used to determine statins in pharmaceutical formulations without intervention from excipients and can easily be applied in quality control laboratories for routine analysis of these drugs in raw materials and pharmaceutical formulations.

Peer-review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- S.E.T.; Data Acquisition- G.E.K.; Data Analysis/Interpretation- G.E.K.; Drafting Manuscript- S.E.T.; Critical Revision of Manuscript- S.E.T.; Final Approval and Accountability- G.E.K., S.E.T.; Supervision- S.E.T.

Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: It was supported by the Istanbul University Research Fund.

Acknowledgement: This article was supported by the Research Fund of Istanbul University (Project No : 23661).

REFERENCES

- Abdallah, O. M. (2011). RP-HPLC determination of three anti-hyperlipidemic drugs in spiked human plasma and dosage forms. *E-Journal of Chemistry,* 8, 753–761.
- Abdel-Gawad, F. M., Issa, Y. M., Fahmy, H. M., & Hussein, H. M. (1998). Spectrophotometric determination of ciprofloxacin in pure form and in tablets through charge-transfer complexation reactions. *Microchimica Acta,* 130, 35–40.
- Abdellatef, H. E. (1998). Utility of certain π-acceptors for the spectrophotometric determination of perindopril. *Journal of Pharmaceutical and Biomedical Analysis*, 17, 1267–1271.
- Antal, I., Koneracka, M., Zavisova, V., Kubovcikova, M., Kormosh, Zh., & Kopcansky, P. (2017). Statins determination: A review of electrochemical techniques. *Critical Reviews in Analytical Chemistry,* 47, 474–489.
- Ashour, S., Bahbouh, M., & Khateeb, M. (2010). Kinetic spectrophotometric determination of fluvastatin in pharmaceutical preparations. *International Journal of Biomedical Science,* 6, 19–26.
- Carlucci, G., Mazzeo, P., Biordi, L., & Bologna, M. (1992). Simultaneous determination of simvastatin and its hydroxy acid form in human plasma by high performance liquid chromatography with UV detection. *Journal of Pharmaceutical and Biomedical Analysis*, 10, 693–697.
- Caslake, M. J., Stewart, G., Day, S. P., Daly, E., McTaggart, F., Chapman, M. J. … Packard, C. J. (2003). Phenotype-dependent and -independent actions of rosuvastatin on atherogenic lipoprotein subfractions in hyperlipidaemia. *Atherosclerosis,* 171, 245–253.
- Erk, N. (2002). Rapid spectrophotometric method for quantitative determination of simvastatin and fluvastatin in human serum and pharmaceutical formulations. *Phamazie,* 57, 817–819.
- Foster, R. (1969). *Organic charge-transfer complexes*. London, UK: Academic Press.
- Gomes, F., Garcia, P., Alves, J., Singh, A., Kedor-Hackmann, E., & Santoro, M. (2009). Development and validation of stability – indicating HPLC methods for quantitative determination of pravastatin, fluvastatin, atorvastatin and rosuvastatin in pharmaceuticals. *Analytical Letters,* 42, 1784–1804.
- Gupta, A., Mishra, P., & Shah, K. (2009). Simple UV spectrophotometric determination of rosuvastatin calcium in pure form and in pharmaceutical formulations. *E-Journal of Chemistry,* 6, 89–92.

- Hussein, S. A., Mohamed, A. M. I., & Abdel-Alim, A. A. M. (1989). Utility of certain π-acceptors for the spectrophotometric determination of gliclazide and tolazamide. *Analyst,* 114, 1129–1131.
- International Conference on Harmonization. (2005). *ICH Q2 (R1) harmonised tripartite guideline, validation of analytical procedures: Text and methodology.* Geneva.
- Job, P. (1928). Formation and stability of inorganic complexes in solution. *Annales de Chimie*, 9, 113–203.
- Jones, P. H., Davidson, M. H., Stein, E. A., Bays, H. E., McKenney, J. M., Miller, E. … STELLAR Study Group. (2003). Comparison of the efficacy and safety of rosuvastatin versus atorvastatin, simvastatin, and pravastatin across doses (STELLAR* Trial). *The American Journal of Cardiology,* 92, 152–160.
- Kaila, H. O., Ambasana, M. A., Thakkar, R. S., Saravaia, H. T., & Shah, A. K. (2010). A new improved RP-HPLC method for assay of rosuvastatin calcium in tablets. *Indian Journal of Pharmaceutical Sciences,* 72, 592–598.
- Kokilambigai, K. S., Seetharaman, R., & Lakshmi, K.S. (2017). Critical review on the analytical techniques for the determination of the oldest statin-atorvastatin-in bulk, pharmaceutical formulations and biological fluids. *Critical Reviews in Analytical Chemistry,* 47, 538–555.
- Kovar, K. A., & Abdel-Hamid, M. E. (1984). Charge-transfer complexes 2. molecular-complexes and radicals of drugs containing the imidazoline ring. *Archiv der Pharmazie,* 317, 246–250.
- Kovar, K. A., Mayer, W., & Auterhoff, H. (1981). Molecular complexes and radicals of procaine. *Archiv der Pharmazie,* 314, 447–458.
- Krishna, M.V., & Sankar, D. G. (2007). Adaptation of color reactions for spectrophotometric determination of pitavastatin calcium in bulk drugs and in pharmaceutical formulations. *E-Journal of Chemistry,* 4, 272–278.
- Kumar, N. S., Nisha, N., Nirmal, J., Sonali, N., & Bagyalakshmi, J. (2011). HPLC determination of pitavastatin calcium in pharmaceutical dosage forms. *Pharmaceutica Analytica Acta,* 2, 1–4.
- Lennernas, H., & Fager, G. (1997). Pharmacodynamics and pharmacokinetics of the HMG-CoA reductase inhibitors. *Clinical Pharmacokinetics,* 32, 403–425.
- Melby, L. R., & Patai, S. (1970). *The Chemistry of the Cyano Group*. New York: Interscience.
- Moussa, B. A., Mohamed, M. F., & Youssef, N. F. (2010). Derivative spectrophotometric method for simultaneous determination of ezetimibe and simvastatin in combined tablets. *European Journal of Chemistry,* 1, 348–351.
- Ochiai, H., Uchiyama, N., Imagaki, K., Hata, S., & Kamei, T. (1997). Determination of simvastatin and its active metabolite in human plasma by column-switching high performance liquid chromatography with fluorescence detection after derivatization with 1-bromoacetylpyrene. *Journal of Chromatography B,* 694, 211–217.
- Rao, C. N.R., Bhat, S. N., & Dwivedi, P. C. (1972). *Applied Spectroscopy Reviews* 5. New York, NY: Brame E.D.
- Saleh, G. A. (1998). Charge-transfer complexes of barbiturates and phenytoin. *Talanta,* 46, 111–121.
- Saleh, G. A., Askal, H. F., Darwish, L. A., & El-Shorbagi, A. N. (2003). Spectroscopic analytical study for the charge-transfer complexation of certain cephalosporins with chloranilic acid. *Analytical Sciences,* 19, 281–287.
- Saminathan, J., Sankar, A. S. K., Anandakumar, K., & Vetrichelvan, T. (2009). Simple UV spectrophotometric method for the determination of fluvastatin sodium in bulk and pharmaceutical formulations. *E-Journal of Chemistry,* 6, 1233–1239.
- Sane, R. T., Kamat, S. S., Menon, S. N., Inamdar, S. R., & Mote, M. R. (2007). Determination of rosuvastatin calcium in its bulk drug and pharmaceutical preparations by highperformance thin-layer chromatography. *JPC Journal of Planar Chromatography -Modern TLC,* 18, 194–198.
- Sankar, G. D., Babu, J. P., Kumar, A. B., & Krishna, V. M. (2007). RP-HPLC method for the estimation of Lomitapide calcium in bulk and pharmaceutical dosage form. *Acta Ciencia Indica, Series Chemistry,* 33,1–4.
- Stanisz, B., & Rafa, W. (2008). Development and validation of UV derivative spectrophotometric method for determination of atorvastatin in tablets. *Chemia Analityczna-Warsaw,* 53, 417–428.
- Sweetman, S.C. (2005). *Martindale the complete drug reference.* United Kingdom:34th Ed., Royal Pharmaceutical Society of Great Britain.
- Terata, Y., Saito, T., Fujiwara, Y., Hasegawa, H., Miura, H., Watanabe, H. … Miura, M. (2003). Pitavastatin inhibits upregulation of intermediate conductance calcium-activated potassium channels and coronary arteriolar remodeling induced by long-term blockade of nitric oxide synthesis. *Pharmacology,* 68, 169–176.
- Tobert, J. A. (2003). Lovastatin and beyond: the history of the HMG-CoA reductase inhibitors. *Nature Reviews Drug Discovery*, 2, 517–526.
- United States Pharmacopoeia Convention Inc. (2007). *United States Pharmacopoeia National Formulary, USP 30-NF 25*. Rockville, Maryland, USA.
- United States Pharmacopoeia Convention Inc. (2009). *United States Pharmacopoeia National Formulary, USP 32-NF 27*. Rockville, Maryland, USA.
- Vuletic, M., Cindric, M., & Kouznjak, J. D. (2005). Identification of unknown impurities of atorvastatin calcium substances and tablets by liquid chromatography/ tandem mass spectrometry. *Journal of Pharmaceutical and Biomedical Analysis,* 37, 715–721.
- Wang, L., & Asgharnejad, M. (2000). Second-derivative UV spectrometric determination of simvastatin in its tablet dosage form. *Journal of Pharmaceutical and Biomedical Analysis*, 21, 1243–1248.
- Yadav, S., Mhaske, D. V., Kakad, A. B., Patil, B. D., Kadam, S. S., & Dhaneshwar, S. R. (2005). A simple and sensitive HPTLC method for determination of content uniformity of atorvastatin calcium tablet. *Indian Journal of Pharmaceutical Sciences,* 67, 182–186.