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Spectrophotometric Determination of Atorvastatin Based on Charge Transfer Reaction with Quinalizarin

Fatih Yiğit [1,a], Nurettin Tarkan [2,b], Mehmet Akdeniz [2,c], Figen Erek [3,d], Işıl Aydın [4,e]

[1] *Bursa Group Chairmanship, The Council of Forensic Medicine, Bursa, 16230, Türkiye.*
[2] *Diyarbakir Group Chairmanship, The Council of Forensic Medicine, Diyarbakir, 21070, Türkiye.*
[3] *Department of Chemistry, Faculty of Science, Dicle University, Diyarbakir, 21280, Türkiye.*
[4] *Department of Analytical Chemistry, Faculty of Pharmacy, Dicle University, Diyarbakir, 21280, Türkiye.*
**Corresponding author*

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ABSTRACT

An easy and economical spectrophotometric method has been developed for the determination of atorvastatin from tablet formulation. This method is based on the formation of a blue-colored charge transfer complex of Atorvastatin with quinalizarin in methanol medium. The blue colored complex gives maximum absorbance at 573 nm. In order to develop the quantitative analysis method of atorvastatin, several parameters such as the type of solvent, the effect of reagent concentration, and the reaction time and temperature on absorbance of complex were investigated. It was determined that the optimum Quinz solution ($0.5x10^{-3}$ M) was 3mL and the optimum temperature of the reaction was room temperature. Beer's law range of proposed method is found 10-100 $\mu g.mL^{-1}$. LOD and LOQ of the proposed method were found as 1.49 $\mu g.mL^{-1}$ and 4.98 $\mu g.mL^{-1}$, respectively. As a result, this proposed method can be used in the quantitative analysis of atorvastatin in tablet formulations.

Keywords: Quinalizarin, Atorvastatin, Spectrophotometric determination.

[a] *yfatih4478@gmail.com* *https://orcid.org/0009-0000-5822-1699*
[b] *nurettintarkan@gmail.com* *https://orcid.org/0009-0006-6494-3085*
[c] *mehmetakdeniz21@hotmail.com* *https://orcid.org/0000-0002-4435-4826*
[d] *figen.erek@dicle.edu.tr* *https://orcid.org/0000-0002-2861-5504*
[e] *iaydin@dicle.edu.tr* *https://orcid.org/0000-0001-6571-6032*

Introduction

Atorvastatin (ATV) is a member of the statins class [1,2]. In addition, it is prevent heart disease, including heart attacks and strokes [3]. There are three different salt forms of Atorvastatin: Atorvastatin Calcium, Atorvastatin Lactone and Atorvastatin Sodium **[**4**].** The molecular formula of Atorvastatin Calcium is $[C_{33}H_{35}FN_2O_5]_2.Ca.3H_2O$ with molecular weight 1209.4 grams/mol. It dissolves in methanol and is slightly soluble in ethanol [5].

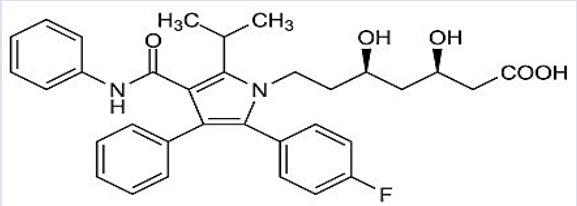


Figure 1. Chemical structure of ATV

Atorvastatin is a synthetic HMG-CoA reductase inhibitor that lowers plasma cholesterol levels and triglyceride levels [6]. Many different techniques have been used for the determination of atorvastatin. These techniques are; HPLC with electrospray tandem mass spectrometry [7-9], extractive spectrophotometry [10], LC-MS [11-16], HPTLC [17], and GC [18] for the determination of the amount of ATV in several samples.

The charge transfer complex (CTC) is formed as a result of the interaction between an electron acceptor and an electron donor. The formation of CTC is often characterized by broad-band absorbance peaks in visible spectrophotometry, however, this spectrum differs from that of both the acceptor and donor moleculars [19-22].

Charge transfer interactions, which were first discovered by Benessi Hildebrend(1949), were developed and their importance increased over time with the explanations of valence bonds and molecular orbitals. These non-covalent interactions are hydrophobic, electrostatic, hydrogen bond interactions. These interactions are the basic steps in molecular recognition in the field of biology and chemistry [23]. Charge transfer interactions are also important from a pharmacological perspective. The activity of pharmacological compounds can be determined [24]. These interactions can also be used in protein-ligand recognition and drug design [25].

Quinalizarin (1,2,5,8-tetrahydroxyanthraquinone) is a chromogenic reagent and one of the tetrahydroxyanthraquinone isomers derived by replacing four hydrogen atoms with four hydroxyl (OH) groups. It is used in the pigment and dye industry and is used as an indicator, being orange in neutral/acidic medium, blue in slightly basic medium and purple in strongly basic medium [26-30].

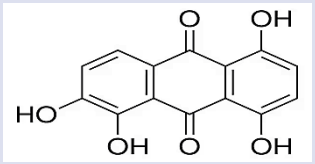


Figure 2. Chemical structure of Quinalizarin

The aim of this study was developed a simple, rapid, economic, validated spectrophotometric method for the determination of ATV in pharmaceutical formulation. The method depends on the reaction between the ATV (π-acceptor group) and quinalizarin (donor group) in the methanol to yield charge transfer complex that is measured at 573nm. In order to optimize the method, parameters such as solvent type, quinalizarin concentration, reaction time and temperature were examined. Once the optimum conditions were determined, the method was validated. Then, the resulting charge transfer complex was characterized.

Material and Methods

Instrumentations

All the absorption spectral measurements were made by using UV-1800 UV/Visible Scanning Spectrophotometer (Shimadzu Ltd) equipped with 1 cm matched quartz cells.

Materials and Reagents

All solvents (methanol, ethanol, acetonitrile, chloroform, acetone) used in this work were of HPLC grade.

The powder used as the atorvastatin reference standard was supplied by Abdi İbrahim Drug Comp.-Turkey. According to the manufacturer's method, its purity was 92.39 %. The Cholvast tablets used (Biofarma product, Turkey), pharmaceutical dosage form of Atorvastatin 10 mg per tablet, were purchased from commercial source with label content. Sigma Aldrich brand Quinalizarin, (1,2,5,8-tetrahydroxy-anthraquinone, Quin) was used without purification.

Stock Solutions

Atorvastatin stock solution was prepared as 500 µg/mL by dissolving in Methanol. Standard ATV solutions to be used in the analysis were prepared by diluting this stock solution.

5. 10^{-4} $molL^{-1}$ stock solution of quinalizarin, daily, was prepared in 100 mL of methanol.

Determining the Calibration Range of Proposed Method

Standard working solutions in the range of 10-100 µg/mL were prepared in 10 mL volumetric flasks, then 3 mL (0.5 x 10^{-3} M) Quin solution was added to the flasks. The mixture was then shaken well and made up to a final volume of 10 mL with methanol. The flasks were shaken at room temperature for 30 minutes, after which the absorbance of all solutions was measured at 573 nm against a blank of reagent solutions containing Quin. A linear regression plot was plotted using absorbance data versus atorvastatin concentration.

Test Procedure for Tablets

The colvast tablet formulation containing 10 mg of ATV was purchased from a local pharmacy. 10 tablets were independently weighed (average 152 mg per tablet), then 10 tablets were ground into a glass mortar. A portion of the powder sample equivalent to 10 mg of atorvastatin was transferred to a 50 mL beaker. 20 mL of methanol was added to the beaker and the mixture was shaken. The solution was then kept in an ultrasonic bath for 10 minutes. This solution was filtered, then the solution was transferred into a flask and the volume was made up to 50 mL with methanol. Solutions containing ATV at concentrations within the working range were prepared in 10 mL flask and the recommended method was applied to these solutions. The contents of the tablets were determined from the absorbance of the solutions with the help of the calibration equation. The developed process for the analysis of atorvastatin from the pharmaceutical formulation (Cholvast tablets) was applied.

Results and Discussions

The Effect of the Solvent Nature

The study of the effect of the solvent is the first parameter to be examined. It plays a role in increasing the interaction of the solvent reagent with the drug. It is also important in the stability of the CTC formed [31]. As can be seen in Table 1, the most sensitive result was obtained with methanol. It was decided to use methanol as a solvent in our other studies, probably because of its high capacity to form hydrogen bonds with the quinalizarin [32-34].

Table 1. Effect of the solvent(N=3)

Solvents	Absorbance±SD
Methanol	0.512±0.007
Acetonitrile	0.265±0.021
Ethanol	0.192±0.073
Acetone	0.251±0.025
Chloroform	0.064±0.008

Effect of Quin Concentration

Since the maximum formation of the complex from the analyte depends on the amount of reagent in the solution and the associated equilibrium, it is an important parameter to examine the concentration of the reagent in the solution(32-34). Studies were conducted by taking the concentration of ATV as 75 µg/mL. 0.5-5mL of Quin solution (0.5x10^{-3} M) was added to these ATV solutions and completed with methanol to 10 mL. The absorbance of ATV-Quin CTC was measured. Looking at the results in Figure 1, it was seen that the addition of 3 mL of the reagent solution (0.5x10^{-3} M) was sufficient to bring the color exactly to the desired intensity.

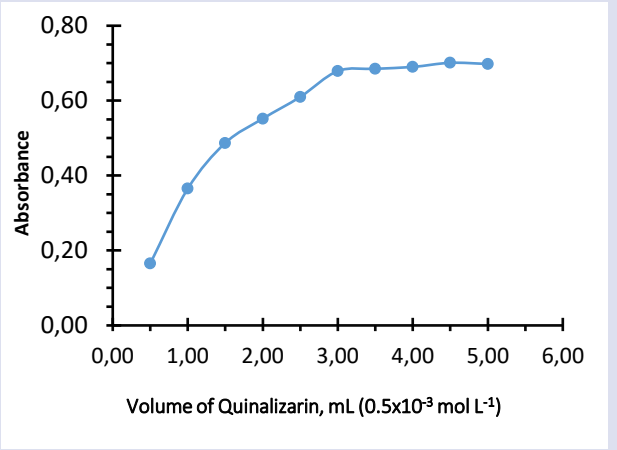


Figure 3. The effect of reagent volume

In Figure 3., a remarkable increase in absorbance continues up to 3 mL, while the amount of reagent added after this point increases the absorbance slightly and after remains constant.

Effect of the Reaction Time and Temperature

The optimum reaction time was determined by constantly monitoring the absorbance of the solution consisting of 75 µg/mL ATV and $0.5x10^{-3}$ M Quin reagent at different temperatures and at the optimum wavelength. As seen in Figure 4., it was observed that the absorbance of CTC decreased in the range of 30-60 °C over 30 min. The absorbance was measured every 30 minutes from the starting point of the experiment, the decrease in absorbance values continued, and the experiment was terminated here because the decrease in absorbance continued after 150 minutes. It was observed that the absorbance of colored CTC was both most stable and maximum at 25±2 °C(laboratory temperature). Subsequent experiments were carried out at 25±2 °C(laboratory temperature)

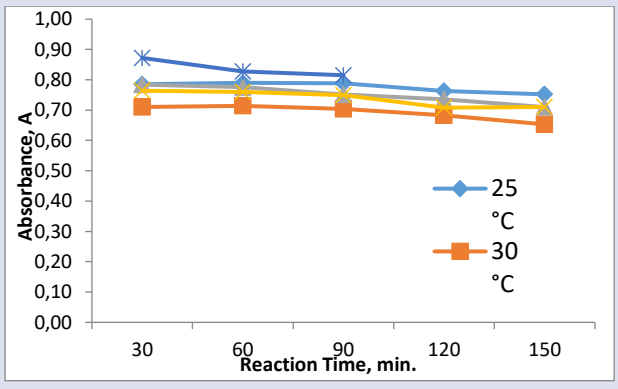


Figure 4. The effect of reaction time and temperature

Stoichiometry of the Charge Transfer Complex

Job's method was used to determine the stoichiometry of ATV-Quinalizarin CTC. Solutions of the studied atorvastatin drug and quinalizarin reagent were prepared in equal concentrations[35]. While changing the concentration ratio of ATV and Quin, the absorbances of the mixtures, at the optimum wavelength, were measured for each point of the experiment against the prepared blank solution (Figure 5). The molar ratio (ATV:Quin) that provides the best absorbance in the reaction; was 0,7.

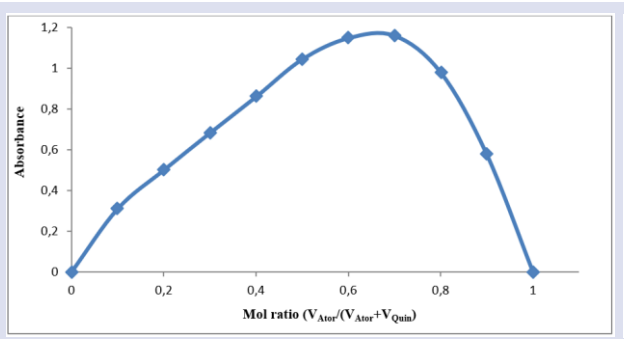


Figure 5. The Stochiometry of CTC

Quin in methanol showed a maximum absorption band at 490 nm, while a solution of atorvastatin in methanol showed no absorbance in the 400-700 nm range. The CTC obtained by mixing the two solutions formed a new characteristic band at 573 nm.

Validation of Proposed Method

The linear regression equation was obtained by using the International Harmonization Conference resolutions method [36]. In the concentration range indicated for atorvastatin, the method showed that the correlation coefficient was linear. As a result of the linear correlation analysis of the data, the following equation was found.

According to the International Harmonization Conference resolutions method, the process of determining the lowest measurable concentration was calculated as "Detection limit and Detection limit" [36]. As seen in Table 2, LOD and LOQ of the proposed method were found as 1.49 µg/mL and 4.98 µg/mL, respectively.

Table 2. Optimum conditions and analytical parameters

Parameters	Values
λ_{maks}, (nm)	573
Working range (µg/mL)	10-100
Correlation coefficient (r^2)	0.9964
Regression equation	y=0.0097x+0.0646
Slope	0.0097
Interception	0.0646
LOD (µg/mL)	1.49
LOQ (µg/mL)	4.98
Molar Absorptivity $L.mol^{-1}.cm^{-1}$	$6.05x10^3$

The Accuracy of the proposed method

The accuracy of the proposed method was tested by recovery % and t test. As seen in Table 3, the percentage of the relative standard deviation (%RSD) value was less than 1% for the method, indicating that the precision values for the developed method were good [36]. The accuracy of the proposed method was calculated with a percent relative error (RE%) of less than 1% and the value indicates the accuracy of the proposed method [36].

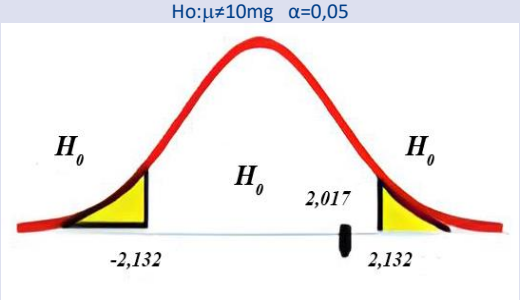
Table 3. The accuracy of the proposed method for ATV

Taken Conc. (µg/mL)	Found Conc[a]. (µg/mL)	±SD	RSD %	RE[b] %	Recovery %
20	20,13	0,092	0,46	0.63	100.63
60	60,39	0,121	0,20	0.65	100.65
100	99,27	0,111	0,11	0.73	99.27

[a] Meaning of 10 different values detected. [b] RE – Relative error; SD – Standard deviation; RSD – Relative standard deviation

The proposed method was used for the detection of atorvastatin in pharmaceutical dosage forms and the results are shown in Table 4. Statistical value at 95% confidence level was obtained from the t-test. The fact that the recovery value is high and the t^b value is greater than the t_c value shows that the proposed method is reliable and accurate [36].

Table 4. t-test for pharmaceutical preparation of ATV

Commercial Brand		Found Calculated ±SD
Cholvast 10 mg (ATV)	Ho:µ=10mg, v=n-1=4 Ho:µ≠10mg α=0,05 	10,01 ± 0,108 t^b = 2,132 t_c= 2,017 Recovery % = % 101 b: Theoretical t value to 95% confidence level c: Calculated average value of five determinations.

Precision

After the optimum experimental conditions were determined, the test was carried out for the stability of the CTC formed against time. For this, a complex was formed at 3 different concentrations, and the absorbance values in the morning, noon and evening for 3 days are shown in Table 5. As a result of the experiment, it was observed that the decreasing trend in intraday and interday absorbance values continued at all different concentrations. So, it is thought that the structure of the CTC is caused by the deterioration over time.

Table 5. The intra-day and inter-day of the proposed method for ATV (N=3)

Taken Conc. (µg/mL)	Found Conc.(µg/mL) Intra-day ±SD			Found Conc. (µg/mL) Inter-day±SD
	Day-1	Day-2	Day-3	
20	20.21±0.84	19.22±1.02	18.36±0.67	19.26±0.93
60	60.90±0.79	60.49±1.23	56.57±0.91	59.32±2.39
100	99.53±1.2	96.09±1.42	95.40±1.51	97.01±2.21

Robustness

The robustness of the proposed method was evaluated the effect of small changes in the parameters reaction time (30 ± 5 min), volume of added reagent (3.0 ± 0.1 mL). As seen in Table 6, none of the small changes in these parameters significantly affected the determination of atorvastatin.

Table 6. The effect of small change in Quin volume and reaction temperature on absorbance of CTC.

Changed parameters		Recovery %	±SD	RSD%
Quin Volume (mL)	3,0 + 0.1	99.8	0.02	0.28
	3,0 – 0.1	99,9	0.11	0.19
Reaction Time (°C)	30 + 5	99.6	0.22	0.482
	30 – 5	100.4	0.18	0.524

Conclusions

The original goal of this research was to demonstrate the formation of charge transfer (π-π) complex between the important Quin, a π-electron rich aromatic molecule, and an electron deficient aromatic acceptor molecule (ATV). So, the proposed method is based on the easily formed CTC between the atorvastatin and quinalizarin in methanol. The formation of complex was substantiated by UV-visible spectroscopy. In addition, this research has shown that the concept of π-π complex formation is a viable method for the development of ATV and other drugs. The linear working range, detection and quantification limits of the method were found to be 10-100 µg/mL, 1.49 µg/mL and 4.98 µg/mL, respectively. This easy, economical method based on CTC reaction can be easily applied for analysis of atorvastatin drug from pharmaceutical formulations.

Conflict of interest

All authors declared no conflict of interest regarding this paper.

Reference

[1] Budavari S., *The Merk Index*. 13th ed. Whitehouse Station, N.J. Merk & Co., Inc. (1997), 148.

[2] Sweetman S.C., Martindale, *The Complete Drug Reference*. 34th ed. London, The Pharmaceutical Press, (2005), 868.

[3] About Atorvastatine, Available at: https://www.nhs.uk/medicines/atorvastatin/about-atorvastatin/ Retrieved November 4, 2023

[4] "Lipitor becomes world's top-selling drug"**,** Crain's New York Business, (2011), 12-28

[5] Wei J., Chen S., Fu H., Wang X., Li H., Lin J., Xu F., Changliang H., Xiaoxia L., Huaqiao T., Gang S., Wei Z., Measurement and correlation of solubility data for atorvastatin calcium in pure and binary solvent systems from 293.15 K to 328.15 K, *Journal of Molecular Liquids*, 324, (2021) 115124.

[6] Andrew P. Lea and Donna McTavish, Atorvastatin:A Review of its Pharmacology and Therapeutic Potential in the Management of Hyperlipidaemias, Drugs, 53, (5) (1997) 828-47

[7] Jemal M., Ouyang Z., Chen B.C., Teitz D., Quantitation of the acid and lactone forms of atorvastatin and its biotransformation products in human serum by highperformance liquid chromatography with electrospray tandem mass spectrometry, *Rapid Commun Mass Spectrom*, 13 (1999) 1003-1015.

[8] Miao X.S., Metcalfe C.D, Determination of cholesterol lowering stain dugs in aqueous samples using liquid chromatography-electrospray ionization tandem mass spectrometry, *J Chromatography A*, 998 (2003) 133–41.

[9] Bullen W.W., Miller R.A., Hayes R.N., Development and validation of a high performance liquid chromatography-tandem mass spectrometry assay for atorvastatin, ortho-hydroxy atorvastatin and para-hydroxy Atorvastatin in human, dog and rat plasma, *J Am Soc Mass Spectrom*, 10 (1999) 55–66.

[10] Erk N., Extractive spectrophotometric determination of atorvastatin in bulk and pharmaceutical formulations, *Anal Lett*., 36 (2003) 2699–711

[11] Altuntas T.G., Erk N., Liquid chromatographic determination of atorvastatin in bulk drug, tablets and human plasma, *J Liq Chromatogr Relat Technol*., 27 (2004) 83–93.

[12] Verd J.C., Peris C., Alergret M., Diaz C., Hernandez Z.G., Sanchez R.M., Different effect of simvastatin and atorvastatin on key enzyme involved in VLDL synthesis and catabolism on high fat /cholesterol rabbit, *Br J Pharmacol,*. 127 (1999) 1479–85.

[13] Erturk S., Aktas E.S, Ersoy L., Ficicioglu S., A HPLC method for the determination of atorvastatin and its impurities in bulk drugs and tablets, *J Pharm Biomed Anal*., 33 (9) (2003) 1017–1023

[14] Shen H.R., Liz D., Zhong M.K., HPLC assay and pharmacokinetic study of atorvastatin in beagle dog after oral administration of atorvastatin self-micro emulsifying drug delivery system", Pharmazie, 61 (2006) 18–20.

[15] Bleske B.E., Willis R.A., Anthony M., Casselberry N., Datwani M., Uhley V.E., The effect of pravastatin and atorvastatin on coenzyme Q10, Am Heart J., 142 (2001) 262.

[16] Nirogi R.V., Kandikere V.N., Shukla M., Mudigonda M., Maurya S., Boosi R., Yerramilli A., Simultaneous quantification of atorvastatin and active metabolites in human plasma by LC-MS using rosuvastatin as internal standard, *Biomed Chromatogr*., 20 (2006). 924–36.

[17] Yadav S.S., Mhaske D.V., Kakad A.B., Patil B.D., Kadam S.S., Dhaneshwar S.R., Simple and sensitive HPTLC method for determination of content uniformity of atorvastatin calcium tablet, Indian J Pharm Sci, 67 (2005) 182–8.

[18] McKenney J.M., McCormick L.S., Weiss S., Koren M., Kafonek S., Blanck D.M., A randomized trial of the effects of atorvastatin and niacin in patients with combined hyperlipidaemic or isolated hypertriglyceridemia, collaborative atorvastatin study group, *Am J Med*., 104 (1998)137–43.

[19] Hassib H.B., Issa Y. M., Conductimetric studies of charge transfer complexes of some benzylidine aniline schiff's bases with substituted–benzoquinones, Egypt, *J. Chem*., 39 (1996) 329–338.

[20] Mulliken R. S., Molecular compounds and their spectra. III. The interaction of electron donors and acceptors, *Phys. Chem*., 56 (1952) 801–822.

[21] Al-Attas A.S., Habeeb M.M., Al-Raimi D.S., Spectrophotometric determination of some amino heterocyclic donors through charge transfer complex formation with chloranilic acid in acetonitrile, *J. Mol. Liq.*, 148 (2009) 58–66.

[22] Amano M., Yamamura Y., Sumita M., Yasazuka S., Kawaji H., Atake T., Saito K., Calorimetric and dielectric study of organic ferroelectrics, phenazine-chloranilic acid, and its bromo analog, *J. Chem. Phys.,* 130 (2009) 034503.

[23] Powell E. , Lee Y.H., Partch R., Dennis D., Morey T., Varshney M., Pi-Pi complexation of bupivacaine and analogues with aromatic receptors: implications for overdose remediation, *Int J Nanomedicine*, 2 (3) (2007) 449-59.

[24] Yousef T.A., Ezzeldin E., A Abdel-Aziz H., H Al-Agamy M., Mostafa G.A.E., Charge Transfer Complex of Neostigmine with 2,3-Dichloro-5,6-Dicyano-1,4-Benzoquinone: Synthesis, Spectroscopic Characterization, Antimicrobial Activity, and Theoretical Study, *Drug Des Devel Ther*., 14 (2020) 4115–4129

[25] Meyer E.A., Castellano R.K., Diederich F. Interactions with Aromatic Rings in Chemical and Biological Recognition, *Angew.Chem.Int.Ed.,* 42 (2003) 1210 – 125.

[26] Barbosa J., Bosch E., Carrera R., A comparative study of some hydroxyanthraquinones as acid-base indicators, *Talanta*. 32 (11) (1985) 1077– 1081.

[27] Banerjee N. L. and Sinha B. C., Extraction spectrophotometric method for determination of aluminium in silicates, *Talanta*. 37 (10) (1990) 1017–1020.

[28] Gouda A. A., Cloud point extraction, preconcentration and spectrophotometric determination of trace amount of manganese(II) in water and food samples, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy,* 131 (2014) 138–144.

[29] Amin A. S., El-Sharjawy A.-A. M., Kassem M. A, Determination of thalliumat ultra-trace levels in water and biological samples using solid phase spectrophotometry, *Spectrochimica Acta A: Molecular and Biomolecular Spectroscopy,* 110 (2013) 262– 268.

[30] Gouda A.A., Malah Z.A., Development and validation of sensitive spectrophotometric method for determination of two antiepileptics in pharmaceutical formulations, *Spectrochimica Acta A: Molecular and Biomolecular Spectroscopy.* 105 (2013) 488–496.

[31] Ayad M.M., El-Hefnawy G.B., Bahlol G.W., Charge transfer complexes of methylnaphthalene derivatives with TCNE in CCl_4, *Spectrochimica Acta Part A*, 58 (2002) 161–166.

[32] Mohamed E.M., Frag Y.Z., Hathoot A.A., Shalaby E.A., Spectrophotometric determination of fenoprofen calcium drug in pure and pharmaceutical preparations. Spectroscopic characterization of the charge transfer solid complexes, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy,* 189 (2018) 357–365.

[33] Gouda A.A., Kasssem M., Novel spectrophotometric methods for determination of desloratidine in pharmaceutical formulations based on charge transfer react, *Arabian Journal of Chemistry*, 9 (2012) 1712–1720

[34] Alzoman N. Z., Sultan M. A., Maher H. M., Alshehri M. M., Wani T. A., Darwish I. A. Analytical Study for the Charge-Transfer Complexes of Rosuvastatin Calcium with π-Acceptors, *Molecules*, 18 (2013) 7711-7725.

[35] Job P., Recherches sur la formation decomplexes mineeraux en solution, et sur leur stabilitee(Formationand stability of inorganic complexes in solution), *Anal. Chem.,* 9 (1928) 133–203

[36] ICH-Q2, International Conference on Harmonisation of Technical Requirements for Registration Of Pharmaceuticals For Human Use. Validation of analytical Procedures: Text And Methodology Q2 (R1), Step 4 Version, (2005).