MOLECULAR COMPLEXES OF ROSUVASTATIN CALCIUM WITH -ACCEPTORS

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Abstract *The molecular reaction of Rosu-Ca, with various □-acceptors like tetracyanoethylene, p-chloranilic acid, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, 2,3,5,6-tetrabromo-1,4-benzoquinone, 1,3,5- trinitrobenzene, 2,3,5,6-tetrachloro-1,4-benzoquinone, 7,7,8,8-tetracyano-quinodimethane, and 2,4,7-trinitro-9-fluorenone gives CT complexes w. The donar and acceptor correlation was determined by uv-visible spectrophotometric techniques .The obtained colored complexes was use for the development of accurate spectrophotometric methods for the determination of Rosu-Ca. The absorbances, concentrations of Rosu-Ca is in the range of 2–200 □g mL^{-1} under the optimum reaction conditions, linear relationships with good correlation coefficients (0.9984–0.9995) . The detection limit range is from 0.41 to 12.24 □g mL^{-1}. Interference could not be observed from the additives which are present in the drugs that were co-related with RoSu-Ca. These methods are successfully applied for analysis of drugs with good accuracy . The range of recovery percentages were from 99.54–100.46 ± 1.58–1.82%. The proposed methods are practical and valuable for drug analysis.*

Keywords *Rosuvastatin Calcium, Molecular complexes, Spectrophotometry ,HPLC, CT complexes.*

1. Introduction

Rosuvastatincalcium(Rosu-Ca),bis[(*E*)-7-[4-(4-fluorophenyl)-6-isopropyl-2-[methyl(methyl- sulfonyl)-amino] pyrimidin-5-yl](3*R*,5*S*)-3,5-dihydroxyhept-6-enoic acid calcium salt, is a synthetic HMG-CoA inhibitor exerts its action by specifically inhibiting the HMG-CoA reductase, the enzyme that catalyzes the conversion of HMG-CoA to mevolanate, which limits the biosynthesis of cholesterol in the body. Inhibition of the enzyme decreases de novo cholesterol synthesis, increasing expression of LDL receptors on hepatocytes. This increase the uptake of low density lipoprotine by the hepato cytes, decrease the amount of LDL-cholesterol in the blood. Rosu-Ca reduces blood levels of triglycerides and slightly increases levels of HDL-cholesterol [1–3].

{3-hydroxy-3-methylglutaryl-coenzymeA(HMG-CoA), low-density lipoprotein (LDL) Thin-layer chromatography(TLC) [4], High performance liquid chromatography (HPLC) }

A literature survey revealed that several analytical methods were reported for Rosu-Ca determination. These methods include high-performance TLC [4], HPLC [5,6], and capillaryzone Electrophoresis [7].

High performance liquid chromatography is an effective analytical

technique, which is used in pharmaceutical purpose. However it is most expensive ,time-consuming procedures for establishing the most accurate chromatographic techniques. Spectrophotometry is the most used technique in pharmaceutical industries due to its simplicity and availability in QC labs [8–13].Spectrophotometric method is used for determination of Rosu-Ca is appropriate for HPLC. The drawbacks that decrease in selectivity due to measures native light absorption of Rosu-Ca at blue-shifted uv region, which may be subjected to interferences, employment of multi-steps of non-selective oxidation reactions and tedious liq-liq extraction procedures using largevolumes of org.solvent methods based on formation of ion-pair associates. So, the development of new spectrophotometric techniques is used for the determination of Rosu-Ca in its bulk and pharmaceutical dosage forms (drugs) is essential.

The interactions between the electron-donars and electron-acceptors associated with the formation of colored CT complexes, which u absorbs radiations in the uvisible region.For the analysis of drugs ,the complex formation will provide information for the development of uv visible spectrophotometric methods [18–25] This information leads interest in employment of the CT-reaction as the basis of the development of new spectrophotometric methods for determination of Rosu-Ca.

2. Results and Discussion

Spectral Characteristics of the Reaction

Interaction of Rosu-Ca (Figure 1) with polycyanoquinone, polyhaloquinone and □-acceptors (Figure 2) in non-polar solvents such as dichloro ethane was found to form colored CT complexes with low molar absorptivity readings.

Figure 1. Chemical structures of Rosuvastatin calcium (Rosu-Ca) with co-formulated drugs.

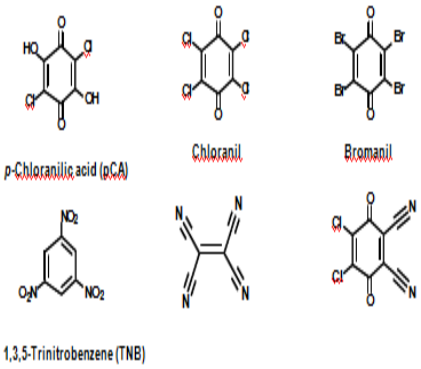
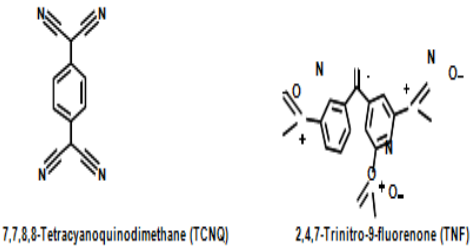
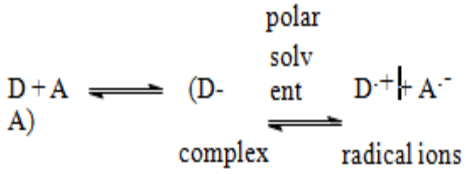


Figure 2. Chemical structures of the polyhaloquinone , polycyanoquinone □-acceptors



In presence of Polar solvents like methanol or aceto nitrile, Rosu-Ca (D) electron donor reacts with acceptor moiety (A) forms intensely colored radical ions with high molar absorptivity values, acc. to the below scheme:



Due to the high ionizing power of polar solvents, the complex(D-A)produced, forms the peaks in the absorption spectra of Rosu-Ca-acceptor reaction mixtures which are similar to radical anions of the acceptors formed from the iodide reduction method [26].

The interaction of Rosu-Ca , □-acceptors at room temp. formed colored chromogens

Which has different absorption max. at 838nm, 431nm, 458nm, 515nm, 409nm, 492nm, 457nm, and 406 nm for TCNQ, TNB, DDQ, pCA, TCNE, Bromonil, Chloronil, and TNF. (Fig. 3–5).

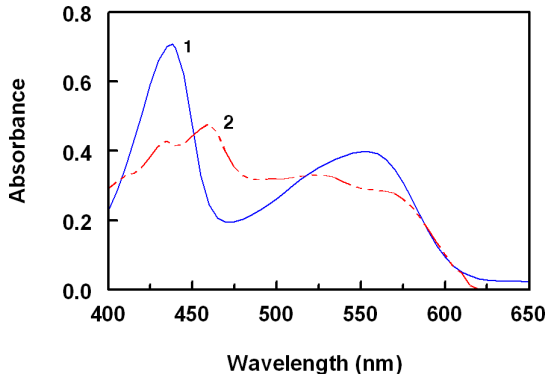
In aceto nitrile the chromogen reacts with TCNQ and forms greenish-blue colored radical anion,

which shows absorption max. at 832nm, 823nm, 760nm, and 740 nm (Figure 4). The obtained bands

formation of the radical anion TCNQ ,may be formed by the clevage of an original donor-acceptor (D-A) complex with Rosu-Ca. This was promoted by the high ionizing power of acetonitrile.

The Rosu-Ca complex with TNB shows 2 absorption max. at 435nm , 550 nm (Fig. 3). The intensity of the first max. is at 1.5 folds the second one. So the readings were carried out at 435 nm, where higher sensitivity was marked.

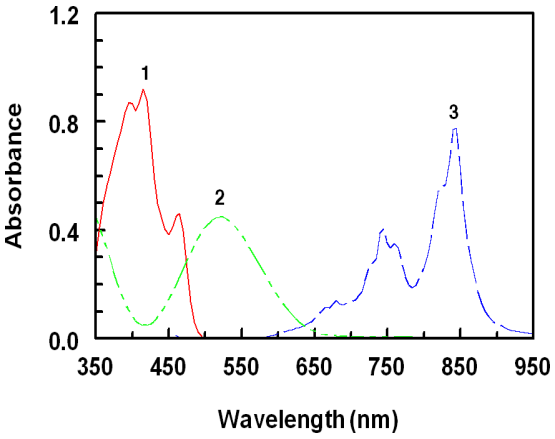
Fig.3. The absorption spectra of CTcomplexes of Rosu-Ca, TNB (1) and DDQ (2) Concentrations of Rosu-Ca were 50□g mL^{-1} and 15 □g mL^{-1}. In case of TNB and DDQ, respectively. Solutions were prepared in acetonitrile for reaction with TNB and in methanol for reaction with DDQ.



The 3 ionic forms of Chloranilic acid (pCA) exists as orange- yellow at a low p^H, purple colour stable at p^H – 3, violet colour stable at high p^H;

Since these interactions of Rosu-Ca , pCA in aceto nitrile forms a purple complex(Fig. 4)

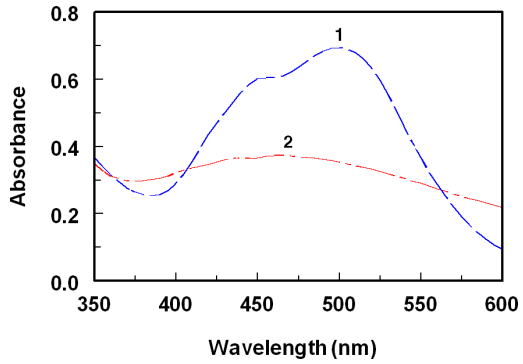
Fig. 4. The absorption spectra of CT complexe of Rosu-Ca, TCNE (1), pCA (2), and TCNQ (3).The concentration of Rosu-Ca are 53□g mL^{-1}, 75□g mL^{-1}, and 24 □g mL^{-1} in case of TCNE, pCA, and TCNQ, respectively. Solutions were prepared with all acceptors in aceto nitrile.



the characteristic absorption band of TCNE radical anion reported max. in aceto nitrile at 435 nm was not seen (Figure 4).But, a duplet at 396 nm and 416 nm was appeared which is similar to the 1,2,3,3-pentacyanopropeneide (PCNP) anion, and

is preferable than TCNE anion,not only in quantitative analysis,but also shows more molar absorptivity [26]. It results max. of Rosu-Ca , DDQ (Fig 3), Bromonil, chloronil (Fig. 5), and TNF are same as that of radical anions and the acceptors are formed by the method of reduction, coincide into the values obtained [27,28].

Fig. 5. The absorption spectra of CT complexe of Rosu-Ca , Bromonil (1) and Chloronil (2).The concentrations of Rosu-Ca are 82□g mL^{-1}, and 155 □g mL^{-1} Solutions were prepared with all acceptors in aceto nitrile.



The 8 acceptors used in the present analytical work may be illustrated to their difference in electron affinities and the conditions used in the reaction. Bromanil, chloranil, and TNF gave relatively weak molar absorptivity values (Tab.1). It was explained on the basis of weak □-acceptors which shows less electron affinities than TCNQ ,DDQ [29].

Optimization of Reaction Conditions

The high concentration of reagents are useful for getting equilibrium and minimum time required for attaining max. absorbance at particular wave lengths. Different solvents are used to carry out the reactions.Methanol gives max. sensitivity in case of DDQ and aceto nitrile is considered as suitable solvent for remaining acceptors. This is because of its max. sensitivity, and was attributed for high dielectric constant of aceto nitrile which gives max yield of radical anions [30].

The color developed was obtained y with DDQ, PCA after 10–50 min with remaining acceptors (Tab. 1). These colors are stable at room temp. for mimimum of 30 min.

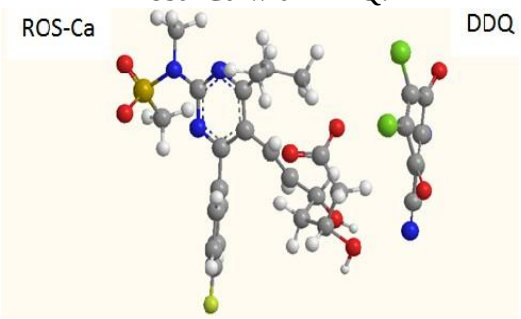
Molar Ratio of the Reaction, Molecular Modeling, and Proposing the Site of Interaction

Job's method was used for calculating the molar ratio of Rosu-Ca to DDQ. From the conclusions Rosu-Ca:DDQ ratio is 1:2. It indicates 2 moles of DDQ with 1 mole of Rosu-Ca.The reaction was postulated as 1:1 ratio when we Consider the divalent Ca ion for DDQ with Rosu anion with only 1 site of addition instead of having more than one possible electron-donating site. The high electron density in Rosu molecule ispresent on the 2 O_2 atoms of the sulfonamide group. The total charges on each of the 2 O_2 atoms of the sulfonamide anion are −0.95643 and −0.96832. DDQ reacts with sulfonamide group of Rosu to produce CT complex (Fig. 6). In sulfonamide group, sulfur donate a lone pair of electrons to the 2 O_2 atomswhich leads the development of negative charges on the O_2 atoms, and form chargetransfer complex[29].

Tab. 1. Optimum conditions for CT reactions of Rosu-Ca with π-acceptors and values of molar absorptivities.

Acceptor[a]	Condition				Molar absorptivity
	Reagent conc. (mg mL^{-1})	Solvent	Time (min)	λ_{max} (nm)	($\varepsilon \times 10^{-4}$)
pCA	4	Acetonitrile	At once[b]	518	1.4
DDQ (1.9)	2	Methanol	At once[b]	460	3.0
TCNE (2.2)	2	Acetonitrile	15	412	1.8
TNB (0.7)	4	Acetonitrile	30	435	0.64
TCNQ (1.7)	1	Acetonitrile	15	840	4.0
Bromanil (1.37)	5	Acetonitrile	5	498	0.88
Chloranil (1.37)	5	Acetonitrile	5	460	2.2
TNF (1.1)	5	Acetonitrile	60	412	0.25

Fig. 6. Energy-minimized CT complex of Rosu-Ca with DDQ.



Validation and Development of Analytical Methods

Linearity, Sensitivity and Calibration Curves

Under optimum conditions, the calibration curvesof Rosu-Ca with different reagents used in the present work. The results are obtained by using the Least-Square method.By using Beer's law plots (n = 5) the intercepts are correlated with coefficients in concentration range of 2□g mL^{-1} –200 □g mL^{-1} (Tab. 2). The LOD and LOQ are calculated by [31] using the formula: LOD or LOQ = □SDa/b, where □ = 3 for limits of detection(LOD) and 10 for limits of quantitation (LOQ), SDa is the standard deviation of the intercept, and b is the slope. Based on this the three replicate measurements and the limits of detection are found 0.44□g mL^{-1}–12.20 □g mL^{-1}.[limits of detection (LOD) and limits of quantitation (LOQ)]

PRECISION

For analyzing 6 replicates of each sample as a batch in a single assay run, & the between-assays precision were calculated by analyzing the similar sample, as triplicate, in 2 separate assayruns. The assays gave good results and the relative standard deviations were almost less than 2% (Tab. 3).

SPECIFICITY AND INTERFERENCE

The advantages of the present assay are the measurements that are performed in the visible region which are away from the ULTRA VOILET-absorbing interfering substances that may be co-extracted from dosage forms which contain Rosu-Ca. Potential interferences of fenofibrate and ezetimibe drugs are studied in a ratio which are present in the combined dosage forms. In the proposed assay, no interferences of these drugs are identified with Rosu-Ca. The CT reaction for Rosu-Ca was attributed to its electron-donating basic character which permit the CT, rather than another drug, which do not have the basic property which is required to aquire CT reaction. Interference was not observed from earlier.

Tab. 2. Determination of Rosu-Ca based on its CT reaction with various π-acceptors by using Quantitative parameters.

Acceptor	Range ($\mu g\ mL^{-1}$)	Intercept	Slope	Correlation coefficient	LOD ($\mu g\ mL^{-1}$)	LOQ ($\mu g\ mL^{-1}$)
TCNQ	5–50	0.0032	0.0388	0.9995	0.41	1.37
TNB	4–30	0.0171	0.0137	0.9992	1.52	5.07
DDQ	2–40	0.0146	0.0104	0.9990	1.14	3.80
pCA	10–150	0.0211	0.0061	0.9987	1.82	6.07
TCNE	5–60	0.0062	0.0183	0.9989	4.32	14.39
Bromanil	25–100	0.0074	0.0087	0.9993	5.11	17.02
Chloranil	40–200	0.0171	0.0021	0.9984	12.24	40.76

Tab 3. Determination of Rosu-Ca based on its CT reaction with different acceptors by precision of proposed methods.

Acceptor-based method	ROS-Ca ($\mu g\ mL^{-1}$)	Within-assay, n = 6 Mean ($\mu g\ mL^{-1} \pm SD$)	RSD	Between-assays, n = 6 ($\mu g\ mL^{-1} \pm SD$)	RSD
pCA	30	48.78 ± 0.41	0.84	50.05 ± 0.84	1.68
DDQ	20	20.51 ± 0.25	1.22	19.56 ± 0.36	1.84
TCNE	40	40.57 ± 0.75	1.85	38.96 ± 0.75	1.93
TNB	20	20.73 ± 0.23	1.11	19.55 ± 0.35	1.79
TCNQ	40	38.78 ± 0.16	0.41	38.04 ± 0.29	0.76
Bromanil	80	81.57 ± 0.84	1.03	78.24 ± 0.81	1.04
Chloranil	200	198.78 ± 2.69	1.35	201.05 ± 2.08	1.03

ROBUSTNESS AND RUGGEDNESS

Robustness is calculated by the influence of slight difference in experimental variables: acceptor reagent concentrations,reaction time on the analytical performance of the method. During the experiments, one experimental parameter was changed and another parameters remains same, each time recovery percentage was calculated. The slight changes did not affect the results and the recovery percentages are found 96.25–102.41% ± 0.64–1.81%..The ruggedness was calculated by implementing the procedures by using2 different instruments in different laboratories at different time.The obtained results from lab-to-lab and day-to-day are found to be reproducible as RSD do not exceed 2%.

APPLICATION OF THE METHOD TO THE ANALYSIS OF TABLETS

The proposed and obtained reported method are [14] appliedfor the determination of Rosu-Ca and for its tablets. The results obtained are compared with those obtained by the reported method. The obtained readings of the labeled amount are 98.44–100.36 ± 1.48–1.81% (Tab. 4). There is no significant differences in the *T*- and F-tests which are identified between the calculated and theoretical readings of both methods at 95% confidence level. This indicates similar precision and accuracy in the analysis of Rosu-Ca and its capsules.

Tab. 4. The proposed methods for determination of Rosu-Ca in its tablets

Method	Label claim (% ± SD) [a]	t-values [b]	F-values [b]
pCA	99.58 ± 1.77	1.55	2.41
DDQ	100.21 ± 1.43	1.26	1.57
TCNE	99.54 ± 1.58	1.86	1.92
TNB	99.82 ± 0.78	0.83	0.47
TCNQ	100.09 ± 1.96	0.61	2.20
Bromanil	100.29 ± 1.32	1.72	1.34
Chloranil	100.46 ± 1.82	2.10	2.55
Reported [c]	99.95 ± 1.14		

3. Experimental Section

APPARATUS

UV-visible spectrophotometer(Double beam)with1-cm quartz cells (UV-1601 PC; Shimadzu, Kyoto, Japan) has used for the spectrophotometric measurements.

Chemicals and Reagents

Rosu-Ca ,Ezetimibe Fenofibrate ,7,7,8,8-tetracyanoquinodimethane(TCNQ), . 1,3,5-Trinitrobenzene (TNB) 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) 1,4-benzoquinone (chloranilic acid, pCA)

Tetracyanoethylene (TCNE), 2,4,7-Trinitro-9-fluorenone (TNF) 2,3,5,6-tetrachloro-1,4- benzoquinone (chloranil) , 2,3,5,6-Tetrabromo-1,4-benzoquinone (bromanil)The purities of the investigated compounds were >99%, and the solutions were stable for minimum of one week when kept refrigerated.

Preparation of Standard Solutions and Sample Tablet Solutions

Preparation of Std. Stock Rosu-Ca Solution

In 50-ml std. flask, Rosu-Ca (100 mg) was weighed and dissolved in methanol (2 mL. and the stock solution (2 mg mL^{-1}) is diluted with suitable solvents to get exact concentrations that present in the linear range of each method.

Preparation of Tablets Sample Solution

20 tablets were weighed and crushed into powdered. From this 50 mg of Rosu-Ca is taken into a 25-mL std. flask and dissolved in methanol (2 mL) for 5 min, addiding to the volume with the corresponding solvent.The solution is shaken well for 15 min, and filtered. The top level of the filtrate was rejected, and a known volume of the filtrate was diluted with a suitable solvent to form exact concentrations.

General Analytical Procedure

1ml of sample solution of Rosu-Ca (20–2,000 □g mL^{-1}) wis transferred into 10-ml std. flasks. 1ml of acceptor is added, the reaction at room temperature (25 ± 2 □C) for 5 min for pCA, DDQ, Bromonil and Chloronil, 15 min for TCNQ and TCNE, 30 min for TNB, and for 60 min forTNF.. The absorbances was measured at a wavelengths of max. absorption (840, 435, 460, 518, 412, 498, 460, and 412 nm for TCNQ, TNB, DDQ, pCA, TCNE,Bromonil, Chloronil, and TNF, respectively) against blank reagent used similarly.

Determination of Molar Ratio

In Job's method equimolar solutions of Rosu-Ca , reagents are prepared. The solutions concentrations are 4.7 □ 10^{-3} M ,in acetonitrile for TCNQ, 1.8 □ 10^{-2} M in acetonitrile for TNB, 8.6 □ 10^{-3} M in methanol for DDQ,1.7 □ 10^{-2} M in acetonitrile for pCA, 1.4 □ 10^{-2} M in acetonitrile for TCNE, 1.4 □ 10^{-2} M in acetonitrile for Bromonil, and 4 □ 10^{-2} M in acetonitrile for Chloronil.

Conclusions

The CT reaction of Rosu-Ca with □-electron acceptors has been investigated and resulted complexes were studied by UV-visible spectrophotometry. The CT complexes formed are used to get accurate spectrophotometric methods for the analysis of Rosu-Ca in pure form and in capsule form. These methods have more advantages: highly sophisticated apparatus are not needed, they are fast and rapid, and are highly sensitive. So, these methods are practical and useful for daily application in QC.

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