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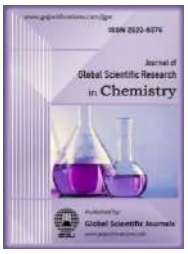

Spectrophotometric Determination of Atorvastatin Clopidogrel and Rosuvastatin Using N-Brosuccimide and Congo Red Dye

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ABSTRACT

This study aimed at development a simple, accurate, sensitive and rapid spectrophotometric method for determination of Atorvastatin (Atro), Clopidogrel (Clo) and Rosuvastatin (Ros) drug in bulk sample and in dosage, by using N-Bromosuccimide (NBS) as oxidant. The method is based on oxidation of studied drug by addition of known excess (NBS) in acidic medium (5M) Hydrochloric acid. The reaction of (Atro) and (Ros) at 45ºC while the reaction of (Clo) at room temperature 25C°. followed by determination of residual (NBS) by reacting with fixed amount of Congo red dye, measuring the absorbance at 576nm, the amount of (NBS) reacted correspond to the amount of drugs and the measure absorbance was found increase linearly with the concentration of drug which is corresponds to amount of (Ator), (Clo) and (Ros) which is corroborated by correlation coefficient of 0.9986, 0.9982 and 0.999 for drugs respectively. The system obey Beer's law for 4-35, 4-32 and 4-35 µg/ml respectively. The molar absorptivity was 3.4□104, 1.5□104 and 3.5□104 L. mol-1. cm-1, The limit of detection (LOD) and quantification (LOQ) were 0.131, 0.435, 0.143, 0.591 and 0.177, 0.433 µg.ml-1 for (Atro), (Clo) and (Ros) respectively. Comparable accuracy (3%) also the method was equally precise as shown by relative value (<2%). The method was successfully applied to the assay of (Ator), (Clo) and (Ros) in pharmaceutical preparation.

1. Introduction

Atorvastatin (ASN Fig 1) is one of the important cholesterol lowering drugs which act by the Competitive inhibition of 3-hydroxy-3-methylglutaryl coenzyme A(HMG-CoA) reeducate enzyme which catalyzed the rate-limiting step in cholesterol biosynthe sis [(1)]. Atrovastatin chemically (3R, 5R) -7- [2-(4-Flourophenyl]-3-phenyl-4- (phenyl carbamoyl) -5-propan-2-ylpyrrol-1-yl]-3, 5-dihydroxy heptanoic acid[(2)]. The drug is odorless white crystalline powder in soluble in water soluble in methanol [(3)]. Several technique have been reported for determination ASN in pharmaceutical formulation several analytical methods including spectrophotometric method (3-6). HPLC 7-9,

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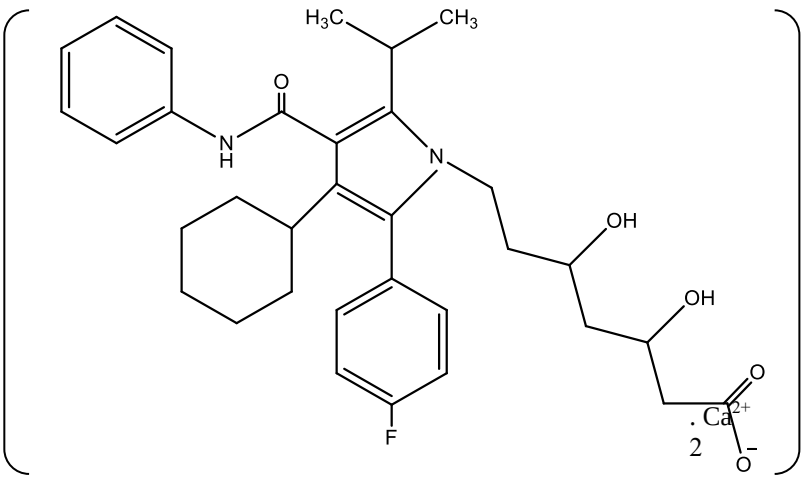

Fig. 1 structure of atorvastatin

A clopidogrel Fig 2 was introduced in 1982 and was approved for medical use in 1998. It is on the world Health Organizations List of Essential Medical use in 1998, the most effective and safe medicine needed in health system it is also used along for the prevention of thrombosis after placement of a coronary stent or as an alternative antiplatelet drug for people intolerant to asprin [10]. Survey of biochemical literature revealed that spectrophotometric method[10,11]. HPLC method[12].

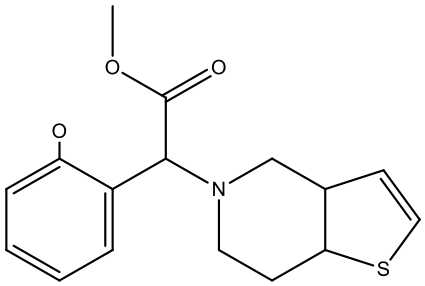

Fig 2 structure of Clopidogrel

Rosuvastatin (RSV) Fig 3 belong to a class called statin, it is a selective and competitive inhibitor of HMG-CoA reeducates and used for treatment of dyslipidemia. It is chemically the calcium salt of {E}-7-[4- (4-fluorophenyl)-6- isopropyl-2-[methyl (methyl sulfonyl) amino] pyrimidin-5y] (3R, 55) 3,5-dihydroxy-6-enoic acid, Rsv is a selective and competitive inhibitor of 3-hydroxy-3- methyl glutaryl-coenzyme A(HMG-CoA) reeducates the rate-limiting enzyme that convert 3-hydroxy methyl glut aryl coenzyme A to mevalonate, aprecursos of cholestrol [13].

The survey of analytical literature for RSV revealed several methods based on varied techniques, spectrophtometnc methods[14-19], HPLC[20-22].

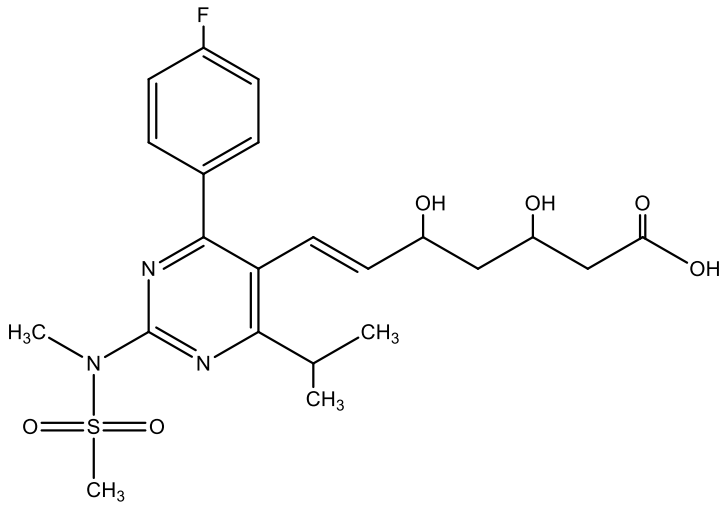

Fig 3 structure of Rosuvastatin

II - Experimental

2-1. Instrument.

All absorbance measurement. Were on

- Ta_2 – UV. Spectrophotometer produced by PG – Instruments – England using matched pair of quartz cell of 1 cm path length.
- Thermostatic water bath (15-90°) model Bs-11 product by lab companion Korea.
- pH meter 3510 - Jenway

2-2. Materials and Chemicals

All the chemical and reagent used of analytical grade and the aqueous solutions were prepared by using distilled water

PlavigrelAwa 75 mg

Atorvastatin Awa10 mg

RosatinAwa10 mg

- Hydrochloric acid (5M) has been prepared by add 45 ml of concentrated hydrochloric acid (36%) to 100ml with distilled water.
- Congo Red 500 μg/ml dye solution has been prepared by dissolving 0.25 mg of dye in 500 ml distilled water.
- N-Bromosuccimide 700 μg/ml oxidant solution has been prepared by dissolving 0.35 gram of NBS in 500 ml distilled water.

Assay method

Different aliquots of standard solution 4-35 µg ml- (Ator), 2-32 µg/ml (ClO) and 4-35 µg/ml (Ros) of pure drug were transferred into different series of 10ml volumetric flask and to each flask was then added 1.5 ml 5M Hydrochloric acid followed by 2ml of N-Bromosuccinnmide. The flasks were stoppered. The contents mixed well and allow to stand for 10 min in water bath at 45°C for (Ator and Ros) and at 25C° for clop with shaking. Finally 2 ml of Congo red solution 500 µg/ml was added to each flask, diluted to mark with distilled water mixed well and the absorbance of each solution was measured at 576nm against distilled blank after 5 minutes. Calibration graph were prepared by plotting the absorbance versus the concentration of pure drug (Fig4 – 7) The concentration of the unknown was read from the calibration graph and calculated from the regression equation derived from beer law (Table 1)

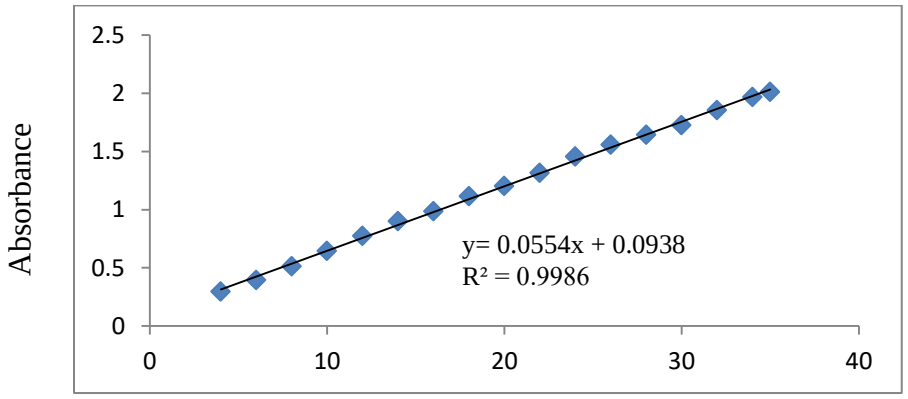

Fig.4: Calibration curve for determination Atorvastatin

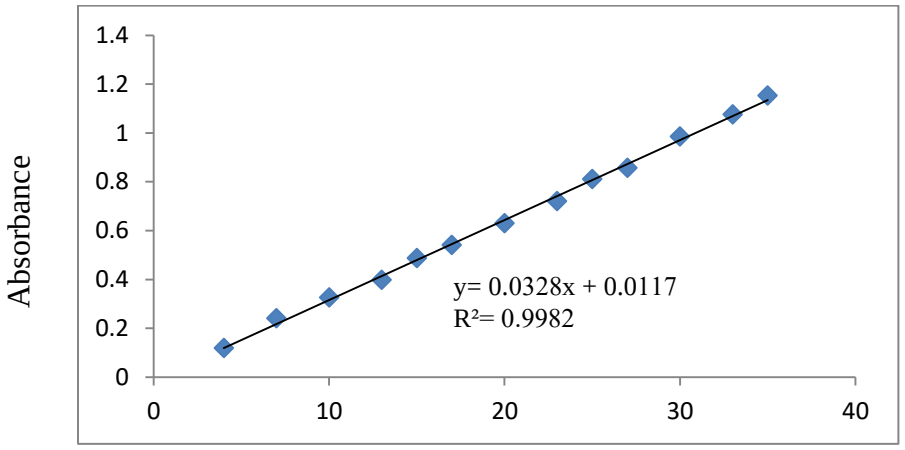

Fig.5: Calibration curve for clopidogrel

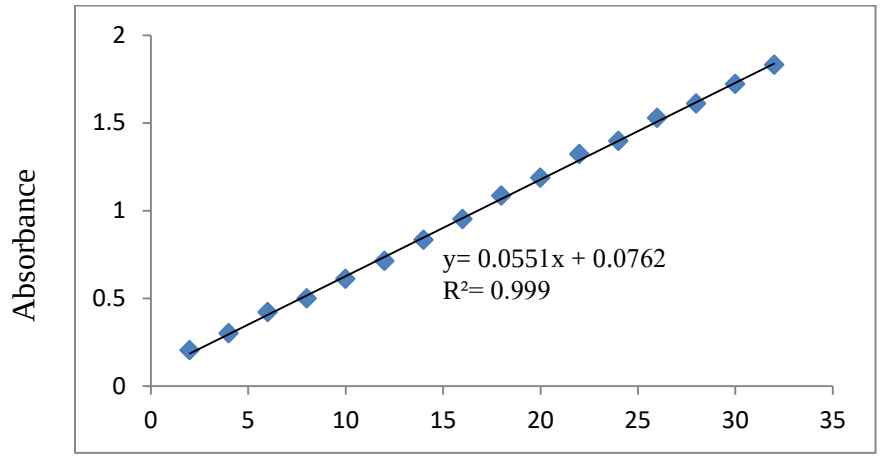

Fig.6: calibration curve for Rosuvastatin

Result and Discusion

Each method developed for quantification of drugs has been validated in terms of precision accuracy, limit of detection, limit of quantification, linearity, selectivity and ruggedness. The Beer's law limit, slope, intercept, correlation coefficient, sandell's sensitivity and Regression equations.

For each drug are tablet in (table 1) to assess the precision each experiment was repeatd at least 5 time and accuracy is estimated in terms of percent recovery and percent RSD. Excellent percent recovery and RSD being less than 2.5 for each drug demonstrates accuracy and precision of the methods.

Table 1: Analytical and regression parameters of spectrophotometric method.

Parameter	Ator	Clop	Res
λ (max)	576	576	576
Beer's law (µg/ ml)	4-35	4 -32	4-35
Molar absorptivity 1 mol^{-1} cm^{-1}	3.4 x 10^4	1.5 x 10^4	3.5 x 10^4
Sandel index µg /cm^2	0.016	0.0214	0.0137
Limit of detection µg /ml	0.131	0.143	0.177
Limit of quantification µg /ml	0.435	0.591	0.433
Regression equation	0.0554 x + 0.0938	0.0328 x + 0.0117	0.0551 x + 0.0762
Intercept (a)	0.0938	0.0117	0.0762
Slope (b)	0.0554	0.0328	0.0551
Correlation coefficient	0.9986	0.9982	0.999

Tab. 2: Determination of accuracy and precision of the methods on pure drugs sample

Drug	Taken mg/ µl	Found mg /µl	Recovery (%)	RSD%
Atorvastatin	5	4.980	99.60	1.22
	10	9.949	99.49	0.68
	15	15.024	100.16	0.75
	20	20.039	10.19	0.67
Rosuvastatin	5	4.89	97.80	1.18
	10	9.76	97.60	0.98
	15	15.16	101.06	0.85
	20	20.14	100.70	0.63
Clopidogrel	5	4.85	97.00	1.16
	10	9.97	99.70	1.06
	15	14.96	99.73	0.97
	20	19.72	98.60	0.87

*average of six determination

Procedure for the tablets

Atorvastin and Rosvastatin

To determine the content of atorvastatin in pharmaceutical preparation 10 tablet of (Atorvastatin Awa)(RosatinAwa) 10 mg were weighted and finely powdered a portion powder equivalent to one table t which content 10 mg of drug was dissolved in 10 mg of ethanol and transfer to 100 ml. volumetric flask and complete the volume by distilled water, the solution filtrate by what man No.41 filter paper this solution was further diluted as necessary to complet the analysis following the recomended procedure.

Clopidogrel

To determine the content clopidogrel in pharmaceutical prpartion 10 tablet of (PlavigrelAwa 75mg) 75mg were weighted to weight of one tablet. Which content 75mg of drug was dissolved in 10 ml of ethanol and trans fer to 100 ml volumetric flask, complte the volume by distilled water, then the solution filtrate by whatsman No.41 filler paper this solution was further diluted as necessary to complet the analysis following the recommended procedure. The result tablet in table 3.

Tab. 3: Results of assay of tablets by the proposed methods statistical evalution and recovery experiments by standard addition method

Pharmaceutical tablet	Drug in tablet (mg)	Drug added µg/ ml	Total found µg /ml	Recovery %
Atorvastatin	10	5	5.12	102.40
		10	10.15	101.50
		15	14.89	99.26
		20	19.95	99.75
Clop	75	5	4.86	97.20
		10	9.97	99.70
		15	15.22	101.46
		20	19.93	99.65
Ros	10	5	5.10	102.00
		10	9.98	99.80
		15	15.17	101.13
		20	19.85	99.25

Tab. 4: t-test and f-test value for pharmaceutical analysis

Drug	Recovery %		texp	F test
	Present method	Standard method		
Atorvastatin	101.80	101.52	0.98	1.84
	102.10	99.72		
	98.86	99.33		
	98.30	98.62		
Rosuvstatin	102.00	101.14	0.95	2.98
	99.80	997.90		
	101.13	101.26		
	99.25	97.20		
clopidogrel	101.90	95.14	1.637	5.13
	101.53	98.98		
	99.35	101.24		
	101.00	99.28		

Factor that influence the sensitivity and the color produce by the reaction of N-bromosuccinamide, drug, and congo red in the prescences of hydrochlonic acid have been thoroughly investigated. Atypical spectrum for colored dye formed in acidic medium versus reagent blank is shown in figure 8.

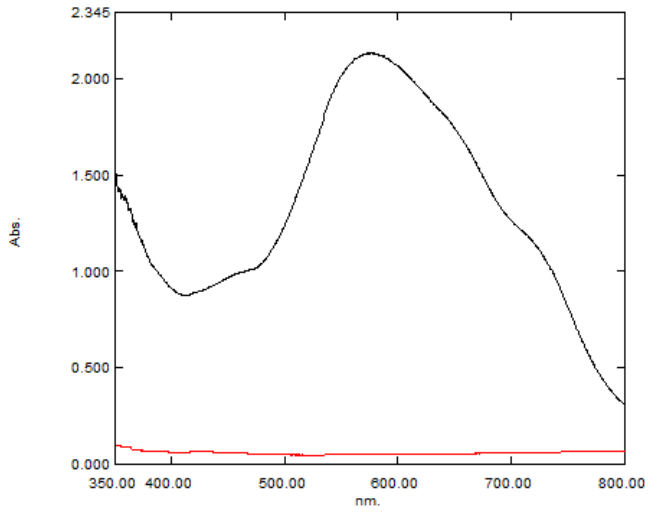

Fig 7. Absorption spectrum for Congo red in acidic medium

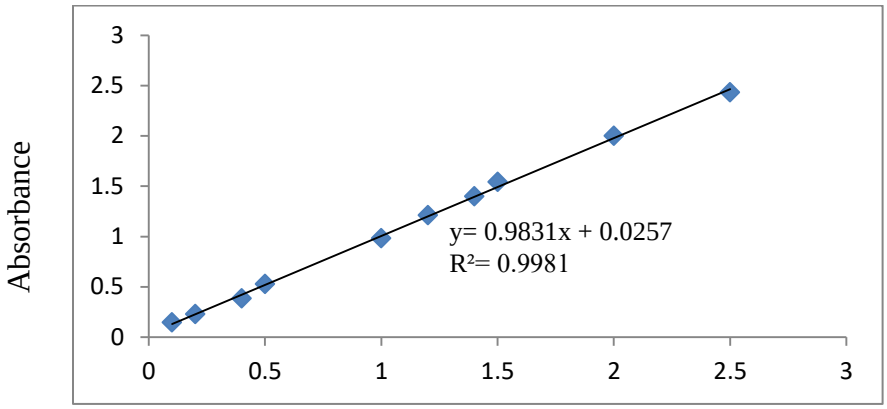

Fig.8 : Calibration curve for Congo red by in Hydrochloric medium

The optimization condition for the proposed method was important to have complete reaction formation, highest sensitivity and maximum absorbance.

Effect of dye concentration

In order to know the best amount of dye obey the Beer's law, increasing volume of 0.1-3 ml Congo red 500 µg /ml in 10 ml volumetric flask containing 1 ml hydrochloric acid. The absorbance was measured at 576 nm. and it's found that the best quantity obey Beer's law was 2 ml. Fig8.

Effect of NBS concentration

In order to know the best volume of NBS 700 µg /ml in the Congo red absorbance different volume was studied by measuring the absorbance of the color of congo red. The result showed that 2 ml bleach the color completely fig. 9.

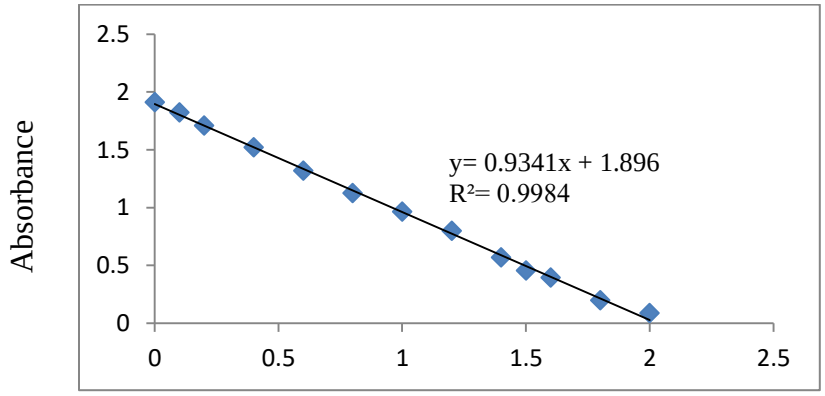

Fig.9 : Effect of NBS in Congo red dye

Effect of acid concentration

The effect of acid concentration on the measured species was investigated by following the assay procedure. The effect of 5 M it HCl of different concentration

(1 , 2 , 3 , 4 , 5 , and 6 M) was studied by keeping all other condition stable the absorbance showed that 5M is the best.

Effect of heat

In order to obtain the highest and most stable absorbance the effect of heating on the oxidation reaction of drugs was catalyzed by heating water bath 20-60° C that the best degree for oxidation of (Atro), (Ros) is 45°C while the (Clo) oxidation occur in 25°C.

Application to formulations

The proposed methods were applied to the determination of drugs in tablet. The result in (table 3) showed that the method is successful for determination of drugs and that the recipients in the dosage forms do not interfere Statistical analysis of the result using t test and F test revealed no significant difference between the proposed method and standard addition method at the 95% confidence level with respect to accuracy and precision table 4.

Conclusion

Simple, sensitive, rapid methods for the determination of drugs have been developed and vali date. The methods deperd on the use of simple and cheap chemical.

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