Sys Rev Pharm 2020;11(9):248-259
A multifaceted review journal in the field of pharmacy

Evaluation and Comparison of the Optimization Parameters Based on Univariate and Multivariate Techniques for Estimation Atorvastatin Calcium with Novel Reverse Indirect Spectrophotometric Method

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ABSTRACT
A Novel, accurate, precise and sensitive reversal indirect spectrophotometric method for estimation of Atorvastatin Calcium (ATOR) has been proposed. The method is based on an oxidation of (ATOR) with of excess of KIO_4 as oxidant agent. Where, the remaining of oxidant KIO_4 reacts with the Para – Anisidine (P-ANS) in acidic medium, the resulting yellowish dye complex exhibit a maximum absorption at 538.0 nm, this spectrum generated revers pack at 428 nm was certified for reverse estimation. Beer's law is obeyed over the concentration range from (5-170 µg/ml) by applying univarate techniques, tow model of multivariate techniques has been applied; Central Composite Design (CCD) and Box-Benhken Design (BBD). By using multivariate techniques, ATOR was obeyed Beer's law at concentration range from (5-185µg/ ml) and (3-188µg/ ml) for (CCD) and (BBD) techniques, respectively. This method had a good accuracy (relative error RE % in the range of (±2.386%, ±2.202% and ± 0.688) good precision (relative standard deviation RSD% is better than ±2.5833%, ±2.0522% and ±1.4177), and the limit of detection (LOD) is (0.14384, 0.12617 and 0.10588 µg/ml) and the limit of quantitation (LOQ) is (0.43590, 0.38235 and 0.32085µg/ml) for univarate, (CCD) and (BBD) techniques, respectively. The method has been applied successfully for assay of Atorvastatin Calcium in pure forms and pharmaceutical preparation forms.

Keywords: Atorvastatin Calcium, spectrophotometric, univarate, multivariate, Central Composite Design (CCD), Box-Benhken Design (BBD).

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INTRODUCTION
Atorvastatin calcium ATOR is one of the most important statin drugs in the last decade [(1)]. ATOR [R-(R*,R*)]-2-(4fluorophenyl) -b, d-.dihydroxy -5-.(1-methylethyl)-3-phenyl.-4-[(phenylamino (carbonyl] -1 Hpyrrole -1-heptanoic acid, calcium salt (2:1).trihydrate. **fig.1**[(2)] is considered the most familiar drugs in reducing plasma levels of triglycerides and "bad" cholesterol (low-density lipoproteins), as well as raising the plasma levels of "good" cholesterol (high-density lipoproteins) by inhibition of the cytoplasmic enzyme HMG-CoA (3-hydroxy-3-methyl-glutaryl-coenzyme A) reductase. This enzyme is dependable for the synthesis of plasma cholesterol through the mevalonate pathway in hepatocytes [(3-5)]. A review of the literature exposed the following analytical techniques related with the determination ATOR. Reported spectrophotometric methods for assess ATOR by ion-pair complexes between ATOR with acid dyes [(6)], and with cationic pararosaniline dye [(7)] p-dimethylaminobenzaldehyde (PDMAB) in acidic medium [(8)], oxidation of ATOR by iodine and formation brown tri-iodide (I3) complex [(9)]. Kinetic spectrophotometric method [(10)], additionally to other techniques, High-Performance Thin Layer Chromatography (HPTLC) [(11, 12)], High-performance liquid chromatography (HPLC) Method [(13-15)], and Capillary Electrophoresis Method [(16)]
With the rapid growth in the number of heart medicines in local markets, producers prefer to follow appropriate rules for analysis. These rules should follow the strict local regulatory requirements. Unfortunately, and for that purpose not all reviewed approaches are rapid and accurate, the inaccuracy of that apparent when it is transferred to the quality control laboratories for the purpose of evaluation in the Standardization and quality control laboratories. The aim of this study was represented to develop a novel, accurate, precise and sensitive reversal indirect spectrophotometric method for estimation of Atorvastatin Calcium (ATOR) moreover, this method could create a chemical dual kit to assess mentioned drug.

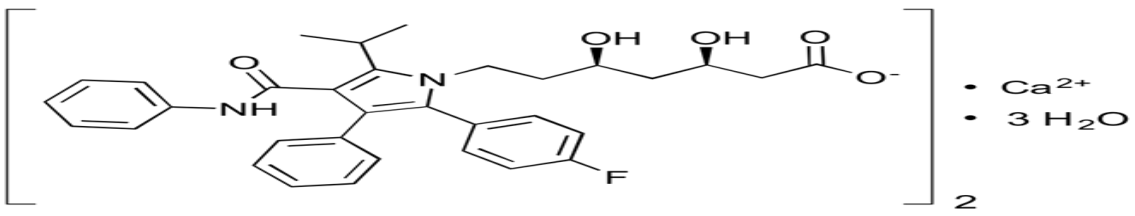

Fig 1. structure formulation of atorvastatin calcium

EXPERIMENTAL

Instrument and equipment's

SHIMADZU UV-1800 PC dual beam UV–visible spectrophotometer (Kyoto/Japan), with quartz cell match pair of 1cm path length was used and connected to Lenovo laptop. Electronic sensitive Balance Sartorius (TE214s). Steam and water bath with thermal sensor and shaker were used.

Software

- UV-Probe spectroscopy software edition (2.32).
- Design-Expert (12.0.3) 2019 software for using the processing of CCD and BBD techniques.
- Excel 2013 was useful for calculations and the signal transforms.

Reagent and Materials

An Atorvastatin calcium ATOR were helpfully provided by Pioneer Company for the pharmaceutical industry, Iraq. Its purity was labeled to be 99.90%. Aqueous solution of Para – Anisidine (P-ANS) was prepared daily. Solutions of KIO4, distilled water. HCl solution was prepared by diluting concentrated acid (Sp.G=1.18 g /L and 37% by weight) in distilled water. Methanol, Ethanol, Sodium perchlorate, Potassium iodate, Potassium chlorate, Sodium hydroxide, Potassium hydroxide, Lithium hydroxide, Ammonium hydroxide, Sulfuric acid, Nitric acid, Acetic acid, Formic acid, Ortho phosphoric acid, and extracts. All above materials were provided from sigma-Aldrich, Riedel-de Haen, Scharlau, ROMIL, PHILIP HARRIS, J.T. Baker, CHEM-SUPPLY, CDH, and MERCK. ATOR drug samples which include tablet Liponeer (20 mg Iraq-pioneer co) and Lipitor (10 mg America-Pfizer co.) were applied in this study.

Standard stock solutions

0.1g of ATOR were precisely balanced and transmitted into 100-mL volumetric flasks, 50 mL of water was added to flask and heated until 40 C° 15 min with shaken to dissolve. Then the volume was completed to the mark with methanol. To obtain a concentration (1000 µg/ ml) of ATOR.

Preparation of Para – Anisidine (P-ANS)

0.1 g of P-ANS was dissolved in 5 mL of hot water and diluting to 100 mL with distilled water, to obtain a [0.1% (w/v)] of P-ANS.

Preparation of potassium periodate (KIO_4)

4.0 g of KIO_4 was dissolved in 5 mL of hot water and diluting to 100 mL with distilled water, to obtain a [4% (w/v)] of KIO_4. At the same procedure prepared others oxidant agents and used in primary test investigation.

Preparation solutions of acids

(~ 1.0 M) of acid was prepared by taken: 8.5 mL of concentrated hydrochloric acid (HCl) (11.8 M) and diluted to 100 mL in a volumetric flask using distilled water. At the same procedure prepared other acids and used in primary test investigation.

Univarate technique

The univarate techniques optimization was carefully studied in order to achieve a final complex formation reaction, thus, the highest sensitivity and maximum absorption. Eleven experiment variables were studied carefully and individually to obtain the optimum conditions of experiment and define the most significant of these. Where it included; effects of Acid Volume, Oxidant Volume, Oxidant Concentration, (p-Anisidine) Volume, (p-Anisidine) Concentration, Different Types of Acid, Different Types of Oxidant, Mixing Order, Reaction Time, oxidation Drugs Time, and Stability.

multivariate techniques

Response surface methodology (RSM)

RSM is a set of mathematical and statistical methodologies [17], is a general technique in experimental design which is widely assumed to optimize various approaches [18]. RSM can decrease the number of experiments required for evaluating various variables and their interactions [19], and thus, it is previously extensively used for optimization of formulation design. Generally, central composite design (CCD) and Box-Benhken Design (BBD) are a popular form of RSM. CCD can prepare a quadratic response surface with a minimum number of experiments and help to optimize the reactive variables and evaluate the interaction between the various variables. BBD is a more focused than CCD in effective variables and his fewer experiments than CCD [20].

Central Composite Design (CCD) [21]

In the current work, CCD was used to investigate the relations interactions between of the significant variables, which obtained from univarate technique, three levels of three-factorial CCD were presented by Design Expert 12.0.3 (DX) software. The levels were represented as minimize, middle and maximize values: As follows these factors were coded at three levels between (−1) and (+1), where (−1) matches to the minimum value, (0) to the medium, and (+1) to the maximum value of each factor. Three independent variables were examined involve: Oxidant Volume (X1), P-Anisidine Concentration (X2) and Acid Volume (X3). The factors and levels were followed according to **Table (1)**. Twenty experiments included six as a central experiment coded were performed by DX software; the factors chosen and the settings of factor levels as well as the responses are presented in **Table (2).** Illustrate the configurations of quadratic of CCD designs.

Table 1: coded levels and Un-coded of the independent variables.

Independent variable	Coded unit		
	-1	0	1
X1	$X1_{lower}$	$X1_{central}$	$X1_{upper}$
X2	$X2_{lower}$	$X2_{central}$	$X2_{upper}$
X3	$X3_{lower}$	$X3_{central}$	$X3_{upper}$

Table 2: configurations of quadratic designs used when examining three factors.

NO. of Experiment	Variables		
	X_1	X_2	X_3
1	-1	-1	-1

2	+1	-1	-1
3	-1	+1	-1
4	+1	+1	-1
5	-1	-1	+1
6	+1	-1	+1
7	-1	+1	+1
8	+1	+1	+1
9	-1	0	0
10	+1	0	0
11	0	-1	0
12	0	+1	0
13	0	0	-1
14	0	0	+1
C	0	0	0
C	0	0	0
C	0	0	0
C	0	0	0
C	0	0	0
C	0	0	0

Where C is a central experiment coded.

These coded values in the **(table 2)** where; found new factor (***xi***) are used to build a regression model to fit the experimental data and were found from equation
Where ***xi*** is the coded unit of the studied independent variable, **Xi** is its upper limit and **Xc** is its central value [22]

$$x_i = \frac{X_i - X_c}{\Delta X_i}$$

The achieving of the method was approximate by investigating the responses **(Y)** as absorptions value, which depend on input factors **X1, X2, X3**…….**Xz.** The response surface behavior was examined for the response function **(Y)** using the second-order polynomial equation. The generalized surface response model is

$$Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_4X_1X_2 + \beta_5X_1X_3 + \beta_6X_2X_3 + \beta_7X_1^2 + \beta_8X_2^2 + \beta_9X_3^2$$

Where **Y** is the response, **X'n** are the significant effects factors, **β_0** is the model intercept coefficient and **β'n** represent the level of the effect of the various factors in the model. This model can be used to predict the response for any condition within the experimental space [23-25].
The point of maximum response is calculated by taking the first derivatives of the equation of the response surface with respect to **X1**, **X2**, and **X3**, and equating them to zero. Then the equations with **X1**, **X2**, and **X3** are solved simultaneously to produce the estimated co-ordinates of the point of maximum response [26, 27]. The equations are

$$\frac{\partial y}{\partial X_1} = \beta 1 + \beta 4X2 + \beta 5X3 + 2\beta 7X1 = 0 \text{ …… (1)}$$

$$\frac{\partial y}{\partial X_2} = \beta 2 + \beta 4X1 + \beta 6X3 + 2\beta 8X2 = 0 \text{ …… (2)}$$

$$\frac{\partial y}{\partial X_3} = \beta 3 + \beta 5X1 + \beta 6X2 + 2\beta 9X3 = 0 \text{ …… (3)}$$

Box-Behnken design (BBD)
With the same methodology for the (CCD) technique (BBD) was applied with more focused for levels of three factors mentioned above. This should allow evaluating the influences of the factors, obtaining the optimum condition for desirable response [28]. The BBD requires only three levels for each factor as major advantages and does not represent configurations in which all factors are simultaneously at the highest or lowest levels. The BBD has been applied in many optimization studies of spectroanalytical methods [29-31]. To calculate number of experiments (N) required by Box-Behnken Design (BBD) is defined as the following equation [32]:

$$N = 2P (P - 1) + Cp$$

Where **P** is number of factors and **Cp** is the number of central points. The central points are used to find the experimental error. Three factors were depended in BBD technique: Oxidant Volume (X1), P-Anisidine Concentration (X2) and Acid Volume (X3), based on the above equation, it was found that the number of experiments required by BBD technique is fifteen experiments, including three experiments as a central experiment. The factors chosen and the sites of factor levels as well as the responses are presented in **Table (3).** Illustrate coded factor levels of a three-variable system by a BBD technique.

Table (3) Coded factor levels of a three-variable system by a BBD-

NO. of Experiment	Variables		
	X1	X2	X3
1	-1	-1	0
2	+1	-1	0
3	-1	+1	0
4	+1	+1	0
5	-1	0	-1
6	+1	0	-1
7	-1	0	+1
8	+1	0	+1

9	0	-1	-1
10	0	+1	-1
11	0	-1	+1
12	0	+1	+1
C	0	0	0
C	0	0	0
C	0	0	0

Where C is a central experiment coded.

GENERAL RECOMMENDED PROCEDURE

When P-ANS is oxidized by any oxidizing agent, it produces a yellowish orange solution that is more stable in acidic media.

The reveres indirect spectrophotometric method needs the subsequent step: the first step take series of 10 mL volume flasks; differing amounts of stock ATOR solution were added to involve the concentration range from (10-100 µg/ml), second step add (1ml) of oxidant agent at all flasks and wait 5 min, the third step add the P-ANS reagent to the mixtures.Then add the acid, previously completion the volumes to the mark with distilled water. The absorbance of the resulting (yellowish) colored solution was measured at (λmax) 538nm against the blank, this spectrum generated revers pack at 428 nm was certified for reverse estimation, which was planned in the similar style.

RESULTS AND DISCUSSION

Univarate technique

Eleven experiment variables were studied carefully and individually to obtain the optimum conditions of experiment (P-ANS oxidation) and define the most significant of these. The experiment variables include:

Effect of Acid Volume

Various volume of acid is added to fixed volume and concentration of P-ANS Reagent 1.5 mL, 0.08 % w/v respectively. **Fig 1**. It is observable from the figure that the optimum volume that gives a maximum and reproducible color formation was obtained by using 1.5 mL of (3 % w/v) KIO_4 solution in water. Investigated when addition 0.6 mL of acids solutions was obtained to the maximum clear peak.

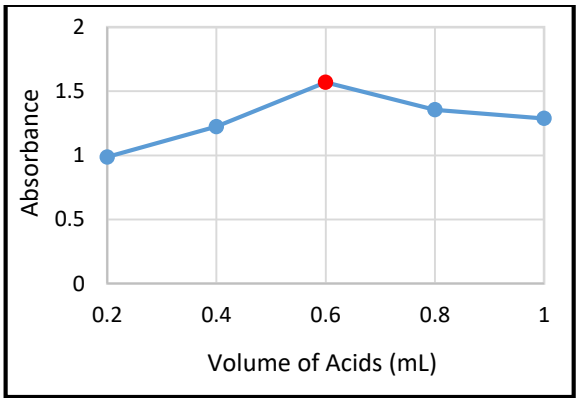

Fig 1: Effect of Acid Volume on the absorbance

Effect of Oxidant Volume

Several volumes of the oxidant agents have been observed for the reason of generating intense colored dye with a strong color contrast. The results in **(Fig 2)** show that, Thus, 1.5 mL of oxidant was private a highest intensity in the test.

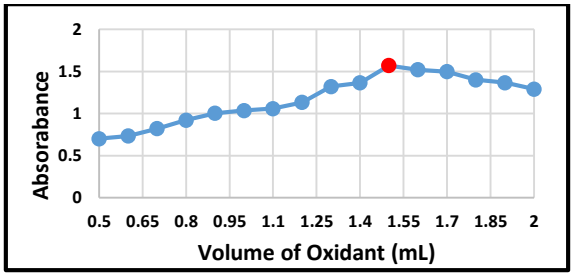

Fig 2: Effect of Oxidant Volume on the absorbance

Effect of Oxidant Concentration

Different concentrations of oxidant reagents are added to specified drug concentrations **(Fig 3)**. Investigated when addition 1.5 mL of (3 % w/v) of oxidant reagent was obtained to the maximum clear peak.

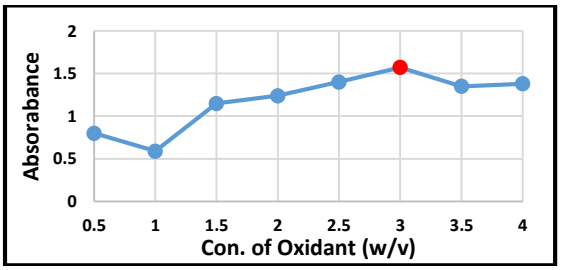

Fig 3: Effect of Oxidant Concentration on the absorbance

Effect (P-ANS) Volume

Several volumes of the (P-ANS) Volume have been experiential for the reason of generating intense colored dye with a strong color contrast. The results in **(Fig 4)** show that 1.6 mL of (0.08 % w/v) P-ANS was optimum volume, in the 0.6 mL of acid (1N), the absorbance would be faintly decreased. Thus, 1.6 mL was resulted as the optimum volume and it was private a highest intensity in the test.

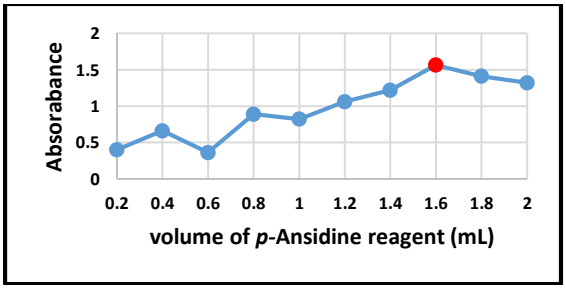

Fig 4: Effect of P-ANS Volume on the absorbance

Effect of (P-ANS) Concentration

Different (P-ANS) reagent concentrations are added to mixtures of drug and oxidant **(fig 5)**. From the figure it is apparent that the optimal concentration that gives maximum and provable color production was achieved by using 1.5 mL of (3 % w/v) of oxidant reagent with fixed concentration of drugs was adding in the acidic medium

represented for 0.6 mL of (1N) HCl acid. Investigated show that 1.6 mL of (0.08 % w/v) P-ANS was optimum concentration.

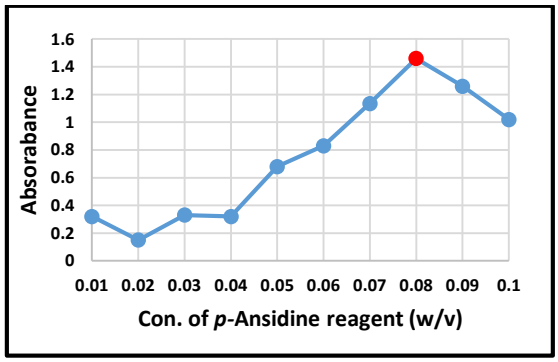

Fig 5: Effect of P-ANS Concentration on the absorbance

Effect of Different Types of Acid

At fixed concentration (1N) of deferent types of acids (H_2SO_4, HCl and HNO_3) were used to develop the sensitivity of method. It was found that hydrochloric acid gave the maximum color intensity among other acids **(Fig 6)** consequently, it was selected for subsequent experiments.

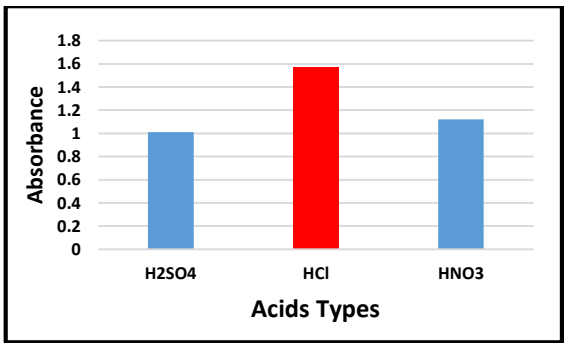

Fig 6: Effect of Different Types of Acid on the absorbance

Effect of Different Types of Oxidant

At fixed concentration (3 % w/v) of deferent types of oxidants ($NaClO_4$, KIO_4, $KClO_3$ and KIO_3) were used to develop the sensitivity of method. It was found that (KIO_4) oxidant provided the maximum color intensity among other oxidants, **(Fig 7)** subsequently, it was chosen for subsequent experiments.

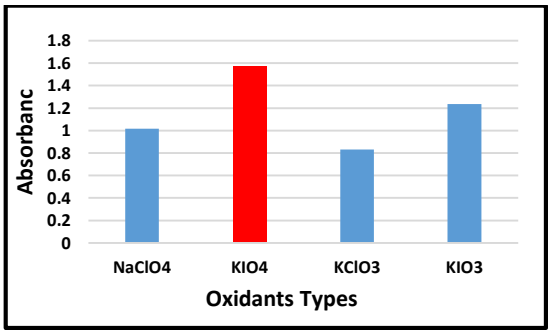

Fig 7: Effect of Different Types of Oxidant on the absorbance

Effect of Reagent Mixing Order

Various sequence of addition of reagents were investigated **(Fig 8)**. It was found that when the reaction components were mixed in the following order (**Drugs + Oxidant + P-ANS + acid**) under general procedure the best result was obtained.

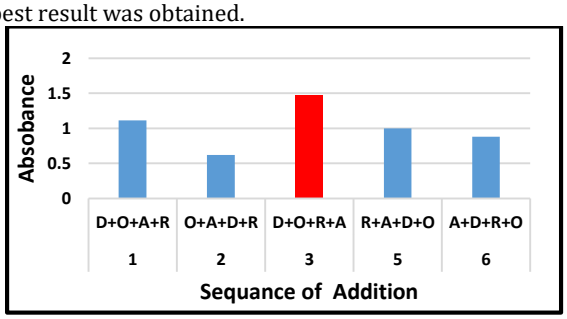

Fig 8: Effect of reagent mixing order (**O:** *oxidant* ***D****: Drug,* ***R****: Reagent,* ***A****: Acid*)

Effect of Reaction Time

The complex was measured at different times and it was observed after 10 minutes is the most suitable time for the measurement the complex. **(Fig 9)**.

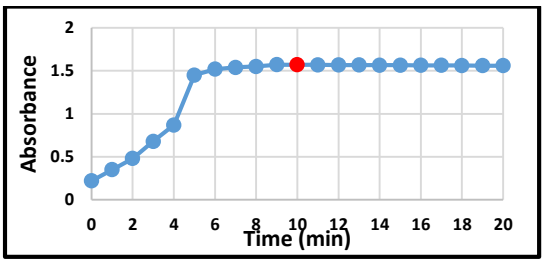

Fig 9: The effect of reaction time for absorbance

Effect of oxidation Drugs Time

There are no significant differences due to the oxidation time, so a 10 min was adopted as the standard time for the successive analyses. **(Fig 10)** shown effect of the time for oxidizing drugs by KIO_4 as oxidant agent.

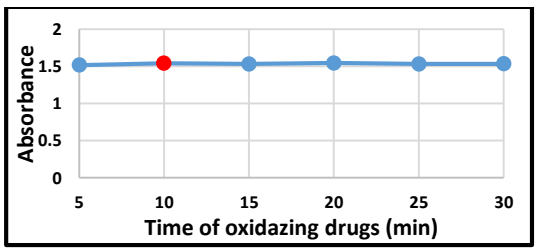

Fig 10: Effect of oxidation drugs time for absorbance

Effect of Stability

The effect of time on the colored complex generated was investigated by allowing the reaction to proceed for various times. The results shown in (**Fig 11)** prove that stable the complex remains at least 60 minutes.

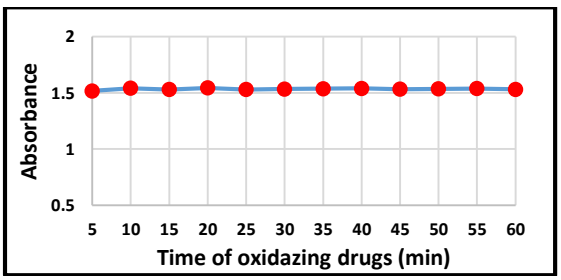

Fig 11: Effect of Stability for absorbance

Multivariate techniques

The multivariate method was used to validate optimal conditions obtained through the univariate technique. The most three significant parameters (Oxidant Volume (X1), P-ANS Concentration (X2) and acid volume (X3)) have been optimized using the multivariate techniques done by Central Composite Design (CCD) and Box-Benhken Design (BBD) manners, Whereas the other experimental parameters that have slight effects on the evaluated signal have been kept at the optimum values achieved by the univariate technique.

Central Composite Design (CCD)

For evaluating the absorbance response of each chosen parameter was required selecting the value carefully. The values of these parameters were selected within the limits specified for each of the parameters affecting the measured absorption of colored products. The number of experiments was reduced to 20 experiments **table (5)**, the encoded of independent parameters ware illustrated in **table (4)**. The surface response model was applied to investigation the effect of the three parameters were chosen to develop an optimal response (**Fig 12 to 14**), shown the relationship between the three parameters.

Table 4: Uncoded and coded levels of the three independent parameters

Code	Independent Parameters	Coded unit		
		-1	0	1
X1	Oxidant Volume (mL)	0.5	1.25	2
X2	P-ANS Con.(w/v)	0.02	0.06	0.1
X3	Acid Volume (mL)	0.2	0.6	1

Table 5: The Central Composite Design (CCD) with three independent parameters (uncoded parameter) and their experimental absorption values.

NO. of Experiment	Parameters			Absorbance
	X1	X2	X3	
1	2	0.02	0.2	0.8333
***2**	1.25	0.06	0.6	1.4967
3	1.25	0.1	0.6	1.3452
4	2	0.1	0.2	1.1133
5	2	0.1	1	1.2080
6	0.5	0.1	1	1.0301
7	0.5	0.02	0.2	0.6667
8	2	0.06	0.6	1.4480
***9**	1.25	0.06	0.6	**1.5233▲**
***10**	1.25	0.06	0.6	1.5033
***11**	1.25	0.06	0.6	1.4867
12	0.5	0.1	0.2	0.9153
13	0.5	0.06	0.6	1.2367
***14**	1.25	0.06	0.6	1.5114
15	2	0.02	1	0.9333
***16**	1.25	0.06	0.6	1.5211
17	1.25	0.06	1	1.4333
18	0.5	0.02	1	0.7567
***19**	1.25	0.06	0.6	1.4929
20	1.25	0.06	0.2	1.3367

* Central point coded value equal 0

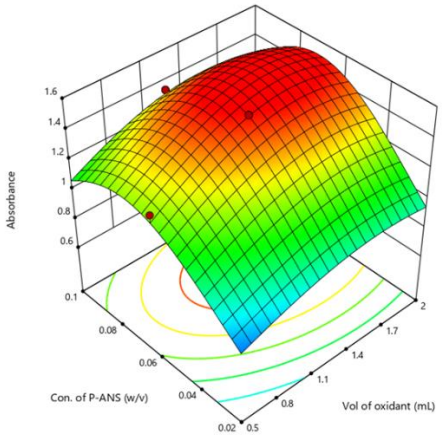

Fig 12: 3D response surface for the absorbance of oxidation P-ANS by KIO4 as an oxidant agent (at constant optimum value of acid: 0.6 mL (1N))

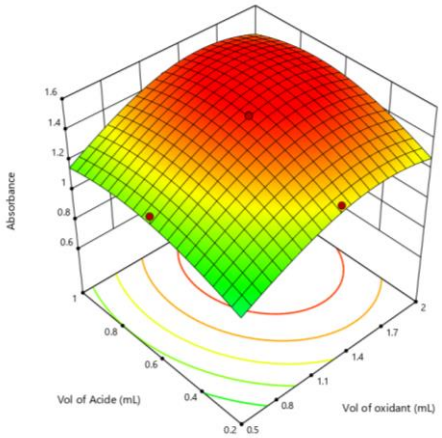

Fig 13: 3D response surface for the absorbance of oxidation P-ANS by KIO_4 as an oxidant agent (at constant optimum value of

KIO_4: 1.25 mL, 3 w/v)

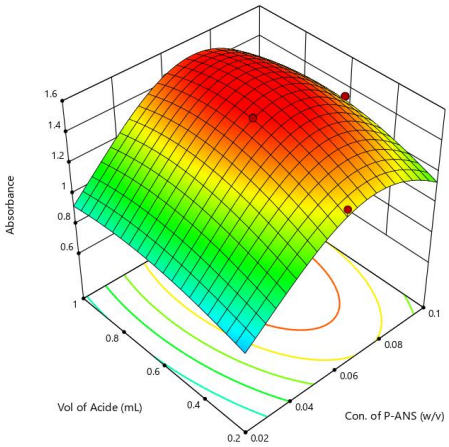

Fig 14: 3D response surface for the absorbance of oxidation P-ANS by KIO4 as an oxidant agent (at constant optimum value of P-ANS: 1.6 mL, 0.06 w/v)

Box-Benhken Design (BBD

Box-Benhken Design (BBD) have fewer design points (experiments) and more advantage than central composite designs (CCD), thus, they are less number to run (cost-effective, rapid and sensitive) with the same number of parameters. BBD is distinguished from CCD because it focuses carefully on the values of independent affecting parameters in order to obtain the ideal response. **Table (6)** reported the box-benhken design coded levels of the three independent parameters.

Table 6: Uncoded and coded levels of the three independent parameters

Code	Independent Parameters	Coded unit		
		-1	0	1
X1	Oxidant Volume (mL)	1.25	1.5	1.75
X2	P-ANS Con.(w/v)	0.04	0.07	0.1
X3	Acid Volume (mL)	0.4	0.6	0.8

The number of experiments required to investigate the three parameters previously mentioned at three levels will be 27 (3^3). However, by using the Box-Benhken Design (BBD), this was reduced to 17 experiments **table (7)**, (including five center point replicates each had the coded value 0)

Table 7: The Box-Benhken Design (BBD) with three independent parameters (uncoded parameter) and their experimental absorption values.

NO. of Experiment	Parameters			Absorbance
	X1	X2	X3	
1	1.25	0.07	0.8	1.2117
2	1.75	0.04	0.6	1.0333
3	1.5	0.1	0.8	1.1900
***4**	1.5	0.07	0.6	1.5100
5	1.5	0.04	0.4	1.0700
6	1.5	0.04	0.8	1.0267
7	1.75	0.07	0.8	1.3433
***8**	1.5	0.07	0.6	1.5367
9	1.25	0.07	0.4	1.3633
10	1.75	0.07	0.4	1.3180
11	1.25	0.1	0.6	1.3180
12	1.75	0.1	0.6	1.2370
***13**	1.5	0.07	0.6	1.5773▲
***14**	1.5	0.07	0.6	1.5567
15	1.5	0.1	0.4	1.2137
16	1.25	0.04	0.6	0.9933
***17**	1.5	0.07	0.6	1.5527

* Central point coded value equal 0

The surface response model was applied on BBD method to investigation the effect of the three independent parameters were chosen to develop an optimal response **(Fig 15 to 17)** shown the relationship between three parameters.

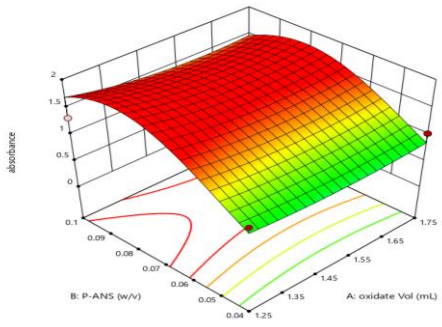

Fig 15: 3D response surface for the absorbance of oxidation P-ANS by KIO4 as an oxidant agent (at constant optimum value of acid: 0.6 mL (1N))

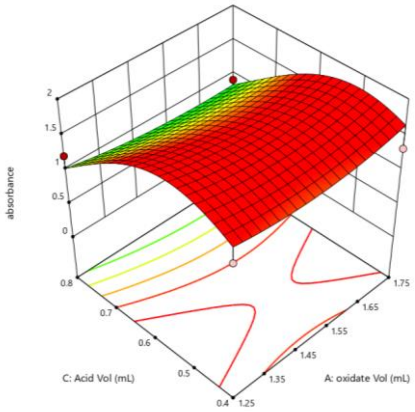

Fig 16: 3D response surface for the absorbance of oxidation P-ANS by KIO_4 as an oxidant agent (at constant optimum value of KIO_4: 1.25 mL, 3 w/v)

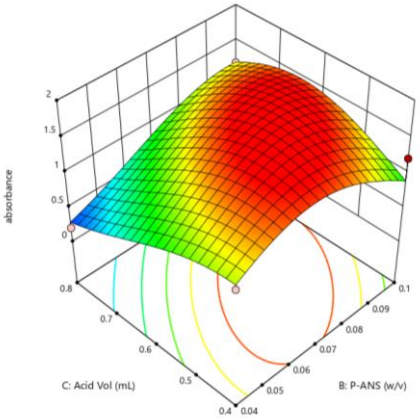

Fig 17: 3D response surface for the absorbance of oxidation P-ANS by KIO4 as an oxidant agent (at constant optimum value of P-ANS: 1.6 mL, 0.07w/v)

Spectrum and compositions values

After the identification of the most appropriate conditions that were obtained from univariate technique. As well as the results obtained from multivariate methods, Central Composite Design (CCD) and Box-Behnken Design (BBD). The indirect reverse spectrophotometric method is so easy to use in the determination of ATOR in their pure form and pharmaceutical formulations via reaction of P-ANS with potassium periodate (KIO_4) in the acidic medium. **(Fig 18)** shown the spectrum absorption of reaction by using univariate method, CCD and BBD. The spectrum was scanning from 350 to 800 nm. **Table (8)** reported the optimum conditions of indirect reverse spectrophotometric methods for determination ATRO, drug by the mentioned techniques.

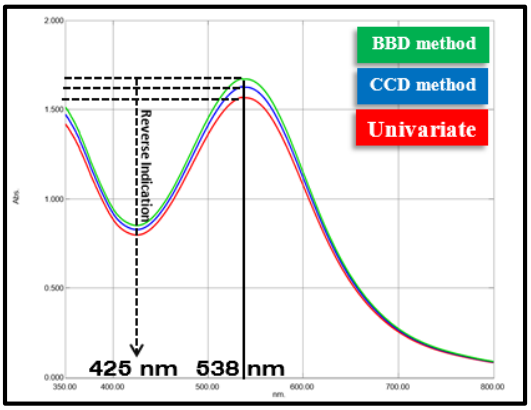

Fig 18: Absorption spectra of KIO_4 with P-ANS Reagent in the acidic medium by using univariate method, CCD and BBD.

Table 8: The optimum conditions of indirect reverse spectrophotometric method for determination ATRO.

Parameters	Range Selected	Optimum Conditions		
		Uni	CCD	BBD
λ_{max} (nm)	350-800	538 as a base line to revers pack at (425)		
Acid Vol. (mL)	0.2 - 1.0	0.6	0.6	0.6
Oxidant Vol. (mL)	0.5 - 2	1.5	1.25	1.5
Oxidant Con.n (w/v)	0.5 - 4 %	3	3	3
P-ANS Vol. (mL)	0.2 - 2	1.6	1.6	1.6
P-ANS Con. (w/v)	0.01 - 0.1 %	.008	0.06	0.07
Types of Acid	HCl, HNO_3, and H_2SO_4	HCl		
Types of Oxidant	KIO_4, $NaClO_4$, KIO_3 & $KClO_3$	KIO_4		
Mixing Order*	O, D, R and A	D+O with very well shaking, then add +R+A		
Reaction Time (min)	0 - 30	10		
Oxidation Time (min)	5-30	10		
Stability	0 – 50	50		

Calibration Curves of ATOR

By applying the experimental optimum conditions were obtained by univariate technique and multivariate methods, **(Fig 19)** shown the spectrum of ATOR **(Figs 20, 21, and 22)** shown the linearity range of ATRO by using univariate, CCD and BBD techniques respectively. **Table (9)** reported the analytical data of ATOR determination by mentioned techniques.

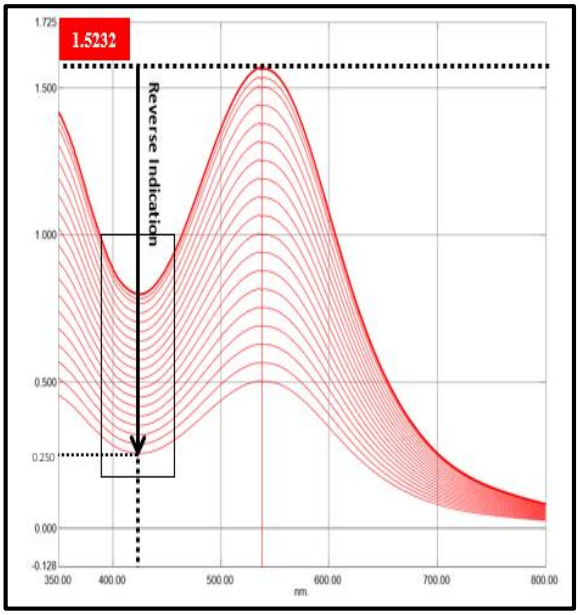

Fig 19: visible spectrum of ATOR,

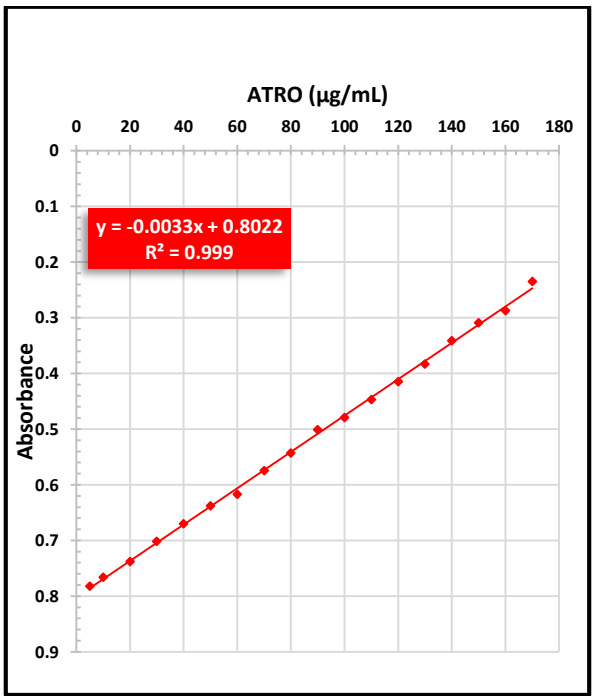

Fig 20: Linearity of ATOR was determination by using univariate optimal condition.

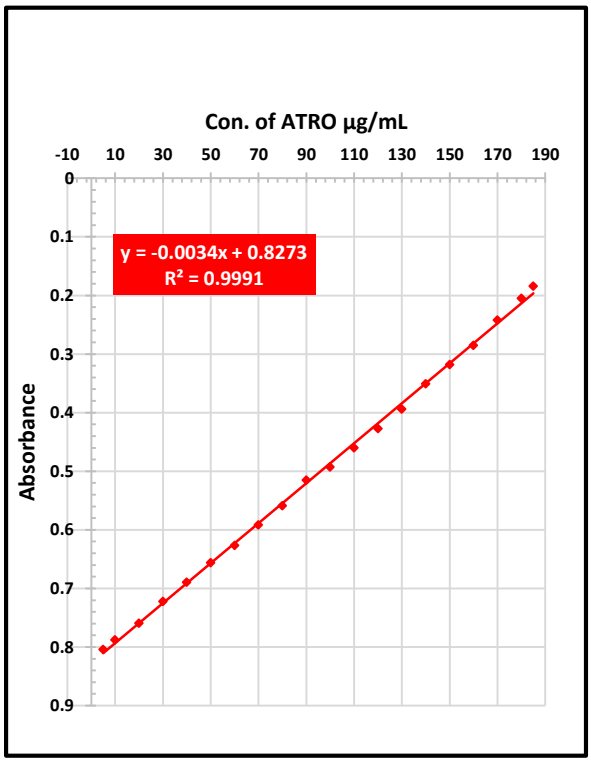

Fig 21: Linearity of ATOR was determination by using CCD optimal condition.

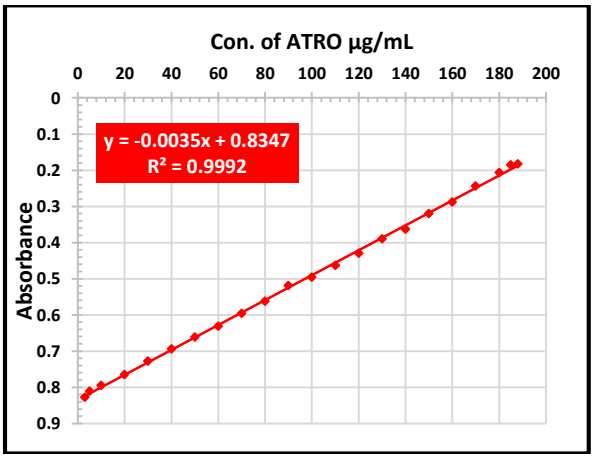

Fig (22): Linearity of ATOR was determination by using BBD optimal condition.

Precision and Accuracy

In the proposed methods, the accuracy and precision were confirmed by examining five replicate samples of the mentioned drugs in four various levels of concentration. **Table (10)**, summarizes the values of the recovery %, relative error % and precision was obtained by univariate and multivariate (CCD & BBD) optimal conditions methods, respectively. These values determine a good accuracy and precision of the proposed techniques.

Table 9 analytical data of ATOR determination by univariate, CCD and BBD techniques.

parameter	Values		
	Univirate	CCD	BBD
Invers λ	425 nm		
Product Color	Yellowish Orange		
Linearity Con. Range (µg/mL)	(5-170)	(5-185)	(3-188)
Regression Equation	y = -0.0033[ATRO] + 0.8022	y = -0.0034[ATRO] + 0.8273	y = -0.0035[ATRO] + 0.8347
Calibration Sensitivity (µg/	-0.0033	-0.0034	-0.0035
Correlation Coefficient (r)	-0.9994	-0.9995	-0.9996
Correlation of Linearity (r2)	0.9990	0.9991	0.9992
Detection Limit (µg/mL)	0.14384	0.12617	0.10588
Quantification Limit (µg/mL)	0.43590	0.38235	0.32085

Table 10: summarizes the values of the recovery %, relative error % and precision was obtained by univariate and multivariate (CCD & BBD) optimal conditions methods

Method	Taken	Found*	Rec.%	R.E%	R.S.D %
Uni	**30**	30.5360	101.78	1.787	2.5833
	50	51.1928	102.38	2.386	1.2091
	80	79.3166	99.146	-0.854	2.5457
	120	121.178	100.98	0.982	1.9834
CCD	**30**	30.1340	100.44	0.447	1.0564
	50	51.1008	102.20	2.202	2.0522
	80	79.8660	99.833	-0.168	1.4215
	120	120.586	100.48	0.488	0.9890
BBD	**30**	30.1520	100.50	0.507	1.4177
	50	50.3440	100.68	0.688	1.1133
	80	79.9560	99.945	-0.055	1.0772
	120	120.340	100.28	0.283	0.7411

* Average of five estimations

Study the Interferences Effects.

The amount of interference by some additives that frequently added in pharmaceutical preparations was analyzed by measuring the absorption of a mixture that contained 80 µg.mL^{-1} of ATOR drug with 100 µg.mL^{-1} from additives (Glucose, Lactose, Sucrose, Talc, and Starch). **Table (11)** showed the interference study for additives.

Table 11: Percent recovery for 80 µg. mL^{-1} of ATOR drug in the presence of 100 µg. mL^{-1} of additives for the experimental proposed methods.

Taken µg. mL^{1}	Additives	Found* µg. mL^{1}	Rec.%
80	Glucose	80.232	100.290
80	Lactose	79.452	99.315
80	Sucrose	80.223	100.279
80	Talc	80.134	100.168
80	Starch	78.218	97.773

* Average of three estimations

Application in pharmaceutical sample

By the proposed methods (univariate and multivariate techniques) were applied to the determination of three concentrations levels of Liponeer as pharmaceutical preparations. The results obtained are agreeably accurate and precise as specified by the excellent percentage recovery. **Table (12)** shown the data analyses results of Liponeer

Table 12: Determination of ATOR in pharmaceutical forms by proposed methods.

Methods	Taken µg. mL ^{1}	Found* µg. mL ^{1}	Rec. %	R. S. D.%
UNi	**30**	29.956	99.856	0.8872
	60	59.806	99.678	0.7144
	90	89.970	99.967	0.7399
CCD	**30**	30.246	100.82	0.7109
	60	60.603	101.00	0.9029
	90	90.056	100.06	0.3912
BBD	**30**	30.066	100.22	0.7011
	60	60.193	100.32	0.3145
	90	90.280	100.31	0.2449

* Average of three estimations

ANOVA test

ANOVA test was achieved on the indication sample at a 95% confidence level for matching univarite, CCD, and BBD techniques. The results are described in **Table (13)**. There is no significant variance disturbing these techniques since computed F-values are less than the critical F-values.

Table 13: **ANOVA** results in one-way implementation of the methods suggested to the pharmaceutical.

SUMMARY of ANOVA: Single Factor						
Method	**Coun.**	**Sum**	**Average**	**Variance**		
Uni.	3	282.2234	70.5558	1538.62		
CCD	3	281.6868	70.4217	1534.01		
BBD	4	280.792	70.198	1535.76		
ANOVA						
Source	**SS**	**df**	**MS**	**F**	**P-value**	**F crit**
Between Groups	0.2615	2	0.1307	8.53*10-5	0.9999	4.2565
Within Groups	13825.2188	7	1536.135			
Total	13825.4803	9				

SS, summation of squares; df, degree unit of freedom; MS, mean squares. a Degree of freedom for between groups: h-1; Within Groups: h (n-1); Total: hn-1; h, number of methods; n, number of samples of each method.

CONCLUSION

A novel, simple, rapid and sensitive method is proposed for the analysis the represented methods is so modest to achieve. The methods depend on the oxidation ATOR by excess amount of KIO_4. The P-ANS was reaction with unreacted residual of KIO4 in acid medium. The obtained solution is measuring absorbance at 425nm as reverse pack. The parameters affecting the reaction was optimized using univariate, CCD and BBD techniques with benefit purpose. The proposed method was extended to the quantification of ATOR in commercial tablets. The percentage recoveries of ATOR was found in the range of 99.67 -101%. The applicability of the developed method was evaluated through the determination of ATOR in several laboratory sample and in pharmaceutical tablets with excellent accuracy and precision. Then, the presented methodology is satisfactory for the routine quality control analysis.

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