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Kinetic Estimation of Antihypertensive Drugs in Pharmaceutical Dosage Forms

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ABSTRACT

Sensitive kinetic spectrophotometric methods are described for the determination of Lovastatin, Atorvastatin, and Acebutalol Hcl. The method depends on oxidation of each of studied drugs with Acidic potassium permanganate. The reaction is followed spectrophotometrically by measuring the rate of change of absorbance at 526 nm. The Rate constant and fixed time (at 100 Secs) methods are utilized for construction of calibration graphs to determine the concentration of the studied drugs. The calibration graphs are linear in the concentration ranges 40.4-404 μgmL^{-1} 55.8- 558 μgmL^{-1} and 33.1-331 μgmL^{-1} using the rate constant and fixed time methods, respectively.

Keywords: Lovastatin, Atorvastatin, Acebutalol Hcl, Kinetic spectrophotometry and KMno4.

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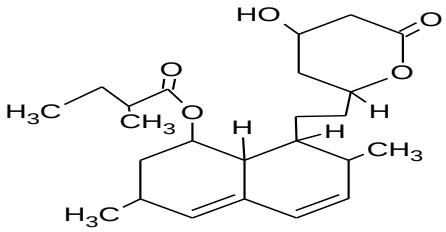
INTRODUCTION

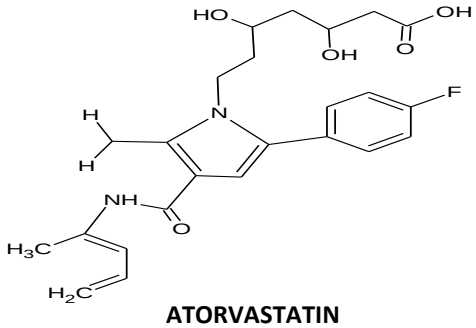
Lovastatin is a potent inhibitor of 3- hydroxyl – methyl-3-glutaryl-coenzyme A (HMG-coA) reductase, the rate controlling enzyme in cholesterol bio synthesis [1, 2]. Hence, it has been proved effective in lowering the plasma cholesterol level in both animals and humans. There are numerous reports in the literature describing the susceptibility of this and other "statin" drugs to oxidative degradation [3], in the European and USP 26 Pharmacopoeias [4, 5] different HPLC procedures are recommended for the determination of the drug in crude and in the single component formulations. Using UV-spectrophotometry, a considerable number of methods have been reported for the determination of lovastatin in pharmaceutical dosage forms [6-11].

Atorvastatin Calcium (ATV) is chemically [R (R*, R*)] -2- (4-Fluorophenyl) - β, -dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamin)carbonyl}-1H-pyrrole-1-heptanoicacid calcium salt trihydtrate. Atorvastatin calcium is an inhibitor of -3-methylglutaryl coenzyme A (HMG—Co A) reductase. A literature survey regarding quantitative analysis of these drugs revealed that attempts were made to develop analytical methods for ATV using extractive Spectrophotometry[12], HPLC,[13-17], GC-MS [18] ,LC-MS [19] ,LC-electrospray tandem mass spectrometry [20-22], and HPTLC [23], methods, while for estimation of ATV and aspirin combination HPLC [24], method had been reported. The low cost and the case of operation make the spectrophotometric techniques highly desirable for the determination of Atorvastatin in pharmaceuticals.

Acebutalol hydrochloride, (RS)-3-acetyl-4-[2-hydroxy-3-(1-methylethylamino) propoxy] butyranilide hydrochloride, is a cardioselective beta blocker used in the Management of hypertension, angina pectoris and cardiac aeehythmias [25]. There are several methods reported in the literature which includes spectrophotometry [26-30], HPLC [31], GC [32], capillary electrophoresis [33], and potentiometry using ISE [34], for Acebutalol. Literature survey revealed that no Kinetic spectrophotometric methods are known for the determination of Lovastatin, Atorvastatin and Acebutalol Hcl. The proposed methods are simple, accurate and easy to apply to routine used. The structures of studied Antihypertensive drugs are shown in Fig.1.



LOVASTATIN



ATORVASTATIN

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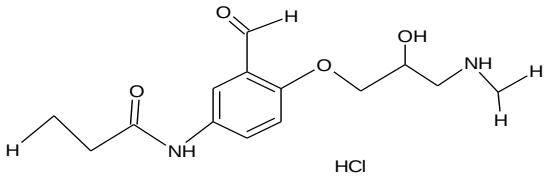

ACEBUTALOL HCL

MATERIALS AND METHODS

EXPERIMENTAL

Apparatus

A Peltier Accessory (Temperature controlled) Varian Cary 50 model UV-Vis spectrophotometer equipped with 1 cm quartz cell was used for all spectral measurements. Systronics pH meter were used for the accurate pH determinations.

Materials and reagents

All the materials were of analytical reagent grade, and the solutions were prepared with double distilled water, samples of Lovastatin, Atorvastatin, and Acebutalol Hcl were generously supplied by Cipla Pharmaceuticals pvt. Ltd, Goa. Potassium permanganate (Merck, Germany) 0.001 M solution was prepared by dissolving 0.0395g $KMnO_4$ in 100 ml of double distilled water, followed by boiling and filtration through sintered glass. Potassium permanganate solution should be freshly prepared and its molarity was checked titrimetrically. Sodium hydroxide (Merck, Germany), 2M NaOH was prepared by dissolving 8g of NaOH in 100mL of double distilled water. 2 M perchloric acid was prepared by dissolving 17.5 mL of $HClO_4$ in 100 ml of double distilled water. 2M $NaClO_4$: was prepared by dissolving equal proportions of 2M NaOH and 2M $HClO_4$. 10 % Acetic acid is prepared by dissolving 10 ml Acetic acid in 100 ml double distilled water.

Preparations of standard solution

A Working standard solution of 0.01 M Lovastatin, Atorvastatin, and Acebutalol Hcl were prepared by dissolving 0. 404 g, 0. 558 g, and 0. 331g in 100 mL of 10% Acetic acid.

Kinetic procedure for Antihypertensive drugs.

All kinetic measurements were performed under pseudo first order conditions where Lovastatin, Atorvastatin, and Acebutalol Hcl used were at least 10 fold excess over permanganate at a constant ionic strength of 0.4 mol dm^{-3}.The reaction was initiated by mixing previously thermostatted solutions of $KMnO_4$ and Lovastatin, Atorvastatin, and Acebutalol Hcl., which also contained the required quantities of $HClO_4$ and $NaClO_4$ to maintain the required acidity and ionic strength respectively. The temperature

maintained at 25 ±0.1^{0}CThe course of the reaction was followed by monitoring the decrease in the absorbance of $KMnO_4$ at 526nm for Lovastatin, Atorvastatin, and Acebutalol Hcl in acidic medium.

Preparation of dosage forms sample solutions

Twenty tablets were weighed and finely powdered. Quantities of the mixed powder equivalent to 100 mg of pure drugs were transferred into a 100 ml calibrated flask. Dissolved in about 30 ml of acetone was added and the mixture was shaken for 5 min. The mixture was filtered using Whatman No. 42 filter paper and the filtrate was evaporated to dryness on a water bath. The residue was washed thoroughly several times with water before dissolving it in 10 % Acetic acid. The solution was then transferred into a 50 ml volumetric flask, made up to the mark with 10 % Acetic acid and suitable aliquot was then subjected to analysis using the procedure described under method 2.5 after diluting to 0.01 M solution. The cream is dissolved in 10 % Acetic acid and then the procedure was continued as described under tablets after diluting to 0.01 M Solution.

RESULT AND DISCUSSIONS

Potassium per magnate as strong oxidizing agent has been used in oxidimetric analytical method for determination of many compounds. During the course of the reaction, the valence of manganese changes. The heptavalent manganese ion changes to the green color (Mn VI), while in neutral and acid medium, the permanganate is reduced to color less (Mn II). The behaviour of permanganate was the basis for its uses in its development of spectrophotometric method. The absorption spectrum of aqueous potassium permanganate solution in acidic medium exhibited an absorption band at 526 nm. The different variables that affect the formation of manganate ion were studied and optimized. Calibration graph of various kinetic procedures are given below.

Recommended Kinetic Procedure.

The rate constant, Fixed time methods and Fixed concentration method were used for determining Antihypertensive drugs namely, Lovastatin, Atorvastatin, and Acebutalol HCl., and the best method was choosen based on applicability, the slope of the calibration graph, the intercept and the Correlation coefficient (r).

Rate constant method

Pseudo-first order rate constants were calculated for Lovastatin, Atorvastatin, and Acebutalol Hcl Concentrations in the range from 40.4-242.4 μgmL^{-1} 55.8- 390.6 μgmL^{-1} and 66.2-297.6 μgmL^{-1} and are presented in (Table.1). A plot of K_{obs} versus [Lovastatin, Atorvastatin, and Acebutalol Hcl.] is drawn, which was used as a calibration graph (Fig 2).

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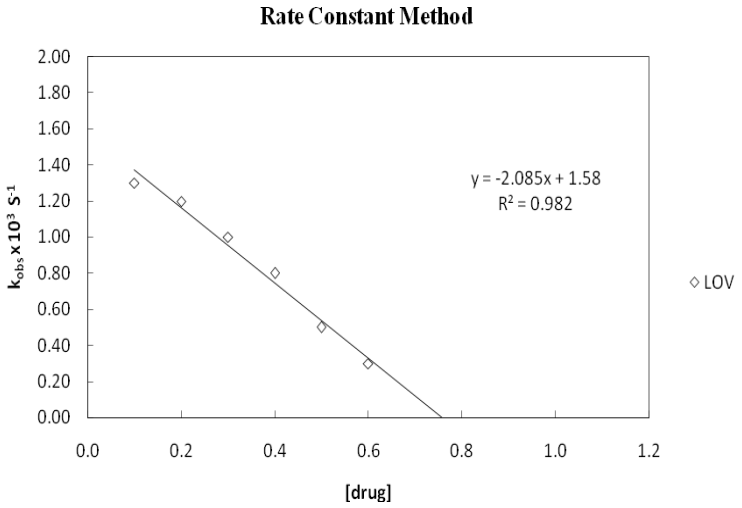
Table 1. Various Kinetic methods for the determination of Antihypertensive drugs. (LOV- Lovastatin, ATR-Atorvastatin, ACB – Acebutalol)

[Drugs]x10^3/ mol dm^{-3}	Rate constant method K_{obs} x 10^3 / S^{-1}			Fixed time method (t = 100 s) Abs.		
	LOV	ATR	ACB Hcl	LOV	ATR	ACB Hcl
0.1	1.3	1.2	1.9	5.8	4.4	3.9
0.2	1.2	1.1	1.8	5.3	4.0	3.6
0.3	1.0	1.0	1.6	4.9	3.7	3.3
0.4	0.8	0.8	1.5	4.4	3.4	2.9
0.5	0.5	0.7	1.3	4.2	3.2	2.5
0.6	0.3	0.6	1.1	3.9	3.0	2.2
0.7	-	0.5	0.9	3.5	2.6	1.9
0.8	-	-	0.7	3.1	2.2	1.6
0.9	-	-	-	2.8	1.9	1.4
1.0	-	-	-	2.4	1.7	1.0

Experimental and calculated

Fig. 2. Calibration graph of Antihypertensive drugs by Rate constant method



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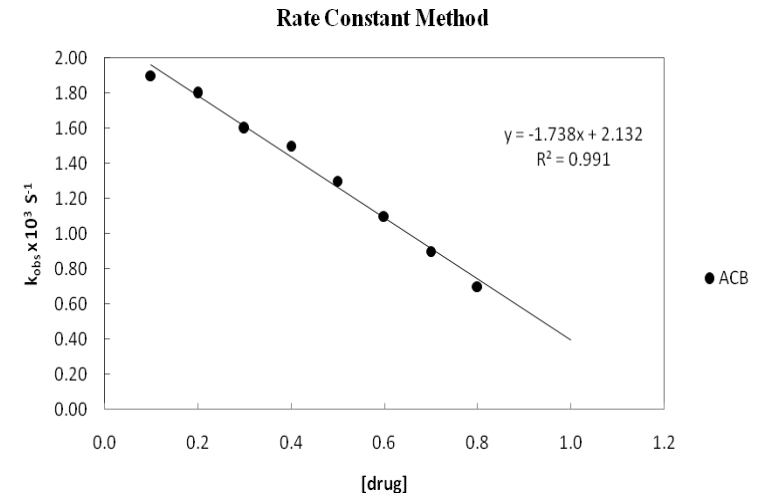

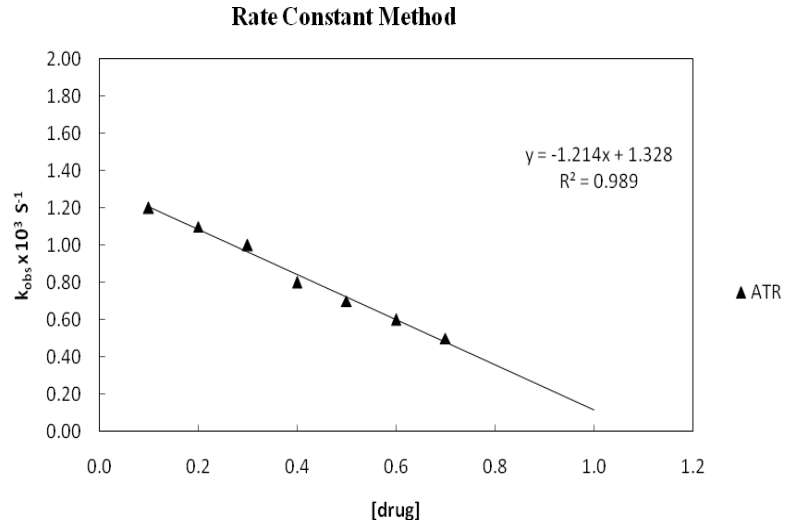

Fixed time method

A pre-selected time (100secs) was fixed and the absorbance was measured for different concentrations of drugs (Table 1). A plot of the absorbance versus the initial concentration of Antihypertensive was drawn, which was linear and could be used as a calibration graph (Fig 3).The range of the drug concentrations giving the most acceptable calibration graph with the above was 40.4-404 µgmL^{-1} 55.8- 558 µgmL^{-1} and 33.1-331 µgmL^{-1}(Table 2).

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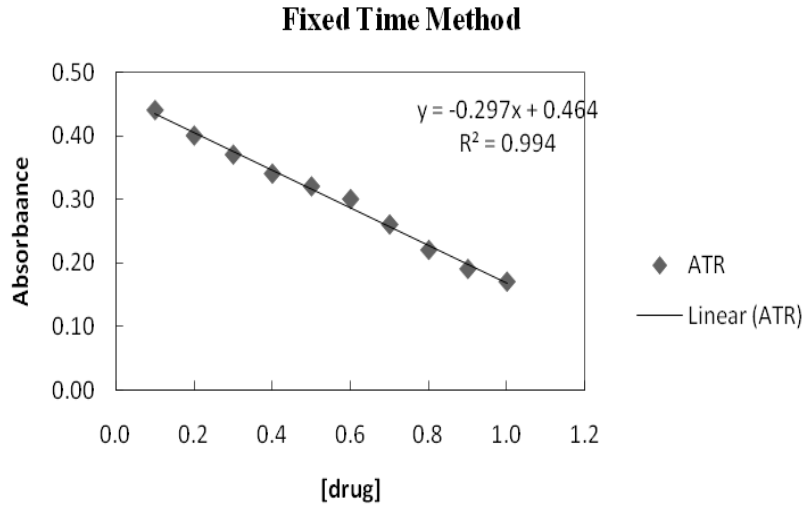
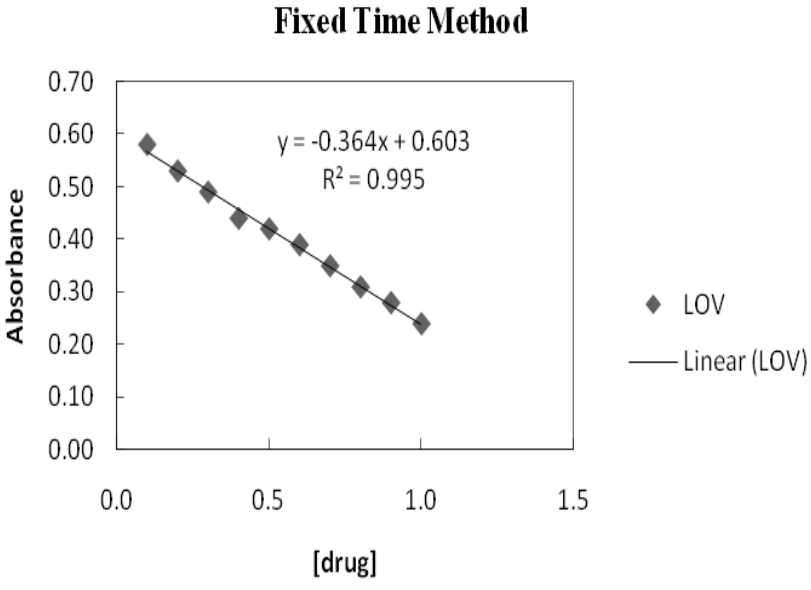
Table2: Analytical parameters of Antihypertensive drugs with Acidic KMno4.

Methods	Drugs	Linear Range(µgmL^{-1})	Intercept	Correlation coefficient(R^2)	LOD	LOQ	Sandell's sensitivity
A. Fixed time method	Lovastatin	40.4-404	0.6079	0.9978	0.0014	0.0013	0.0000070
	Atorvastatin	55.8-558	0.4701	0.9969	0.0015	0.0033	0.0000093
	Acebutalol Hcl	33.1-331	0.4237	0.9992	0.0052	0.0043	0.0000109
B. Rate constant method	Lovastatin	40.4-242.4	0.0127	0.9929	0.0827	0.2506	0.003765
	Atorvastatin	55.8-390.6	0.0104	0.9987	0.3342	1.0129	0.0036380
	Acebutalol Hcl	66.2-297.9	0.0098	0.9966	0.0641	0.1944	0.0024573





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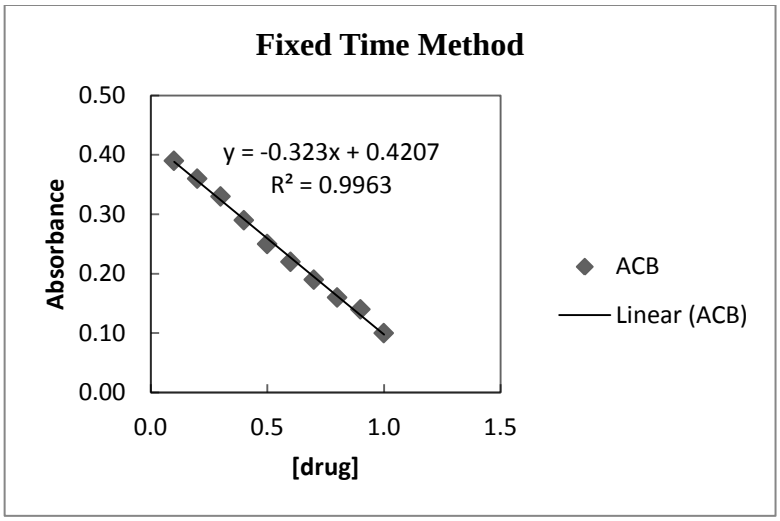

Fixed Concentration method

A preselected value of the absorbance and the time was fixed and the time was measured for different Drugs concentrations. The range of the drug concentrations giving the most acceptable calibration graph with the above was very limited which could be a disadvantage.

Initial Rate method

In this method, graphs of the rate (at the beginning of the reaction) versus the drug concentration were not easy to obtain because the reaction was fast. Thus, the tangents to the curves at zero time were not easy to draw. This method was therefore abandoned. The best correlation coefficient was obtained for the fixed time method, and the value of the slope was also high. Even though the range was limited compared to the rate-constant method, the Fixed-time method was found to be more applicable.

The performance of the proposed method was judged by calculating the student t-test and variance ratio F-test. At the 95% confidence level, the calculated t- test and F-values do not exceed the theoretical values, indicating that there is no significant difference between the proposed method and the official method. From an analytical point of view, it is concluded that the described procedure allows for the determination of Antihypertensive drugs in pure and pharmaceutical dosage forms. Unlike the spectrofluorometer, as well as gas chromatographic and HPLC procedures, the instrument is simple and inexpensive. Its importance lies in the chemical reaction upon which the procedure is based, rather than sophistication of the instrument. This aspect of the kinetic method of determination is of major interest in analytical pharmacy, since it offers a distinct possibility for the assay of a particular component in complex dosage formulations.

Validation of the proposed method

Concentration range is established by confirming that the analytical kinetic procedures provides a suitable degree of precision, accuracy and linearity when applied to the sample containing the amount of analyte within or at the extreme of the specified of the range of the analytical procedure. In this work,, concentrations ranging from 40.4-242.4 µgmL-1 55.8- 390.6 µgmL-1 and 66.2-297.6 µg/ml were studied for the investigated drugs in the Rate constant method and concentration ranging from 40.4-404 µgmL-1 55.8- 558 µgmL-1 and 33.1-331 µg/ml were studied for the investigated drugs in the constant time method (at preselected fixed time for 100 secs) . The whole sets of experiments were carried out through this range to ensure the validation of the proposed procedure. Linear calibration graphs were obtained for all the studied drugs by plotting the logarithm of rate constant method of the reaction versus Absorbance of molar concentration of analyte in the sample within the specific range.

Precision was checked at three concentration levels. Eight replicate measurements were recorded at each concentration level. The calculated relative standard deviation were all below 2.5% indicating excellent precision of the proposed procedures at both level of repeatability and intermediate precision.

Limit of detection (LOD): was calculated based on standard deviation of response and the slope of calibration curve. The limit of detection was expressed as,

$$\mathrm{LOD} = \frac{3\sigma}{S}$$

Where σ is the standard deviation of intercept s is the slope of calibration curve. The results were summarized in (Tables 3) indicating good sensitivity of the proposed method .According to USP XXV guidelines, the calculated LOD values should be further validated by laboratory experiments. In our work, good results were obtained where the calculated LOD equations were actually detected in these experiments.

Limit of Quantification (LOQ): was calculated based on standard deviation of intercept and slope of calibration curve. In this method, the limit of quantization is expressed as

$$\mathrm{LOQ} = \frac{10\,\sigma}{S}$$

The results were summarized in (Table 3) indicating the good sensitivity of the proposed method. According to USP XXV guidelines, the calculated LOQ values should be further validated by laboratory experiments. In our work, good results were obtained where the calculated LOQ equations were actually quantities in these experiments.

The Fixed time method and rate constant methods of the proposed kinetic spectrophotometric method for the investigated drugs have been tested on commercial pharmaceutical dosage forms. The concentration of investigated drugs was computed from its responding regression equations. The results of proposed method (Fixed time and rate constant methods) were statistically compared with those of reported methods,

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in respect to accuracy and precision. The obtained mean recovery values were recorded in (Table 3), which ensures that there is no interference of other additives present in the studied formulations.

In the t- and F- tests, no significant differences were found between the calculated and theoretical values of both the proposed and the reported methods at 95% confidence level. This indicates good precision and accuracy in the analysis of investigated Antihypertensive drugs in dosage forms.

Table 3: Analysis of dosage forms by proposed and reference methods (Found values[a] ± % SD)

Drug	Labelled	Found (X ± SD)	
		Proposed method	Reference method
LOVASTATIN			
Lestric (Ranbaxy)	10 mg/Tab	9.8 ± 0.21 t = 0.21, F = 1.9	10.3 ± 0.43
Lostatin (Dr.Reddy,s)	20 mg/Tab	19.5 ± 0.95 t = 0.32, F =0.89	19.98 ± 0.13
Lovacard (Cipla)	20 mg/Tab	19.5 ± 0.084 t = 0.52, F = 0.79	19.98 ± 0.23
Lovex (Lupin)	10mg/Tab	9.9 ± 0.8 t = 0.29, F = 1.11	10.2 ± 1.27
ATORVASTATIN			
Atorcor (Dr.Reddy,s)	5 mg/Tab	4.9 ± 0.5 t = 0.89, F = 0.11	5.2 ± 0.5
Atofast (Intra lab)	10 mg/Tab	9.9 ± 0.41 t = 0.51, F = 0.96	10.02 ± 0.41
Atorin (Medley)	20 mg/Tab	19.87 ± 0.54 t = 0.32, F = 0.17	19.99 ± 0.14
Alip (Talent)	10 mg/Tab	9.7± 0.3 t = 0.69, F = 1.21	10.12± 0.71
ACEBUTALOL HCL			
Lopressor (ACBH)	400mg/Tab	401.1 ± 0.6 t=0.70, F= 1.135	400.23 ± 0.76
Tenormin (ACBH)	200 mg/Tab	200.4 ± 0.5 t=0.40, F= 1.	201.3 ± 0.95

CONCLUSION

The Fixed time and rate constant methods can be easily applied for determination of investigated Antihypertensive drugs in pure and dosage forms that do not require elaborate treatment and tedious extraction of chromophore produced. The proposed methods (Fixed time & rate constant method) are sensitive enough to enable determination of lower amounts of drug; these advantages encourage the application of proposed method in routine quality control of investigated analgesic drugs in industrial laboratories. Finally our methods provides advantage of improving selectivity, avoiding interference of colored and/ or are turbidity background of samples because it measures the increase in absorbance with time against blank treated similarly.

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