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SIMPLE SPECTROPHOTOMETRIC METHODS FOR SIMULTANEOUS DETERMINATION OF LOSARTAN POTASSIUM AND ATORVASTATIN CALCIUM IN COMBINED DOSAGE FORMS

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ABSTRACT

New UV spectrophotometric methods were developed and validated for simultaneous determination of losartan potassium and atorvastatin calcium in bulk and marketed formulation by using simultaneous equation method (method-I) and Q-value analysis method (method-II). In method-I, 207 nm and 246 nm wavelengths (λ_{max} of losartan potassium and atorvastatin calcium respectively) were selected for measurement of absorbance for both the drugs; where as in the method-II, 237.4 nm (as an iso-absorptive point) and 246 nm wavelengths (λ_{max} of any of the two drugs) were selected for measurement of absorptivity. Both the drugs show linearity in a concentration range of 0.5-20 µg/ml at their respective λ_{max} and at the isoabsorptive point. Accuracy, precision and recovery studies were done by QC samples covering lower, medium and high concentrations of the linearity range. The relative standard deviation for accuracy, precision studies were found to be within the acceptance range (<2%). Recovery of losartan potassium and atorvastatin calcium were found to be >91.81% and >92.34% respectively for method-I; >100.29% and >98.75% respectively for method-II confirming the accuracy of the proposed methods. From the results we can conclude that method-II is more accurate than method-I which indicates that commonly used excipients in pharmaceutical formulations were not interfering in the same method. The proposed methods are recommended for routine analysis since they are rapid, simple, accurate and also sensitive and specific by no heating and no organic solvent extraction.

KEY WORDS: losartan potassium, atorvastatin calcium, simultaneous equation method, Q-analysis.

1.INTRODUCTION

Losartan potassium (LOS) is a white free-flowing crystalline powder and it is a derivative of imidazole-5-acetic acid attenuated vasoconstriction induced by angiotensin II. LOS is the first orally active antagonist of the AT1-receptor subtype1 and used for the treatment of hypertension (Byyny,1995). Chemically, LOS [Fig. 1] is 2-butyl-4-chloro-1-[p-(o-1H-tetrazol-5-ylphenyl)benzyl]imidazole-5-methanol monopotassium salt (Sica,1995). LOS and its principal active metabolite block the vasoconstrictor and aldosterone-secreting effects of angiotensin II by selectively blocking the binding of angiotensin II to the AT_1 receptor found in many tissues. Atorvastatin (ATR) is a synthetic hydroxyl methyl glutaryl coenzyme-A (HMG-CoA) reductase inhibitor that has been used as a lipid lowering agent (Mohammadi,2007). Chemically, ATR [Fig. 2] is [R-(R*, R*)]-2-(4-flurophenyl)-B, B—dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenyl amino) carbonyl]-1H-pyrrole-1-heptanoic acid (Desager,1996). ATR is a competitive inhibitor of HMG-CoA reductase. This enzyme catalyzes the reduction of 3-hydroxy-3-methylgultaryl coenzyme-A to mevalonate, which is the rate-determining step in hepatic cholesterol synthesis. Because cholesterol synthesis decreases, hepatic cells increase the number of low density lipoproteins (LDL) receptors on the surface of the cells, which in turn increase the amount of LDL uptake by the hepatic cells, and decrease the amount of LDL in the blood (Malinowski,1998; Burnham,2002).

Fig.1.Chemical structure of Losartan

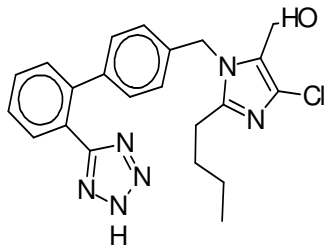
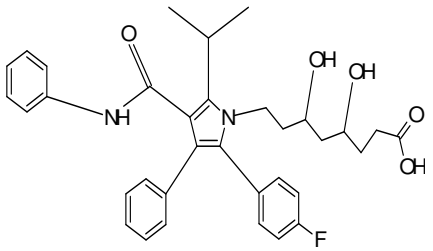


Fig.2. Chemical structure of Atorvastatin



Literature survey revealed that no UV methods are reported for the simultaneous determination of LOS and ATR till date. Methods are available for the quantification of LOS individually and with other combinations other than ATR by HPLC (Yeung,2000; Don,1997; Shivakumar,2007;Vanessa,2005), by UV (Gandhimathi,2002; Patil,2009;

Topale,2003). Methods are available for the quantification of ATR individually and with other combinations other than LOS HPLC (Mohammadi,2007;Sandeep,2006; Lincy,2008;Bahrami,2005), by UV (Sandeep,2006; Lincy,2008). Present study involves development and validation of two spectrophotometric methods for the simultaneous determination of LOS and ATR in pharmaceutical formulations and in drug substances.

2.MATERIALS AND METHODS

2.1. Instrumentation: Spectral runs were made on a double beam UV-Visible spectrophotometer, model-T80+ was employed with wavelength interval of 0.2 nm and automatic wavelength corrections with a pair of 10 mm quartz cells. The software is UVWin5 ver. 5.1.1. PG Instruments, UK.

2.2. Standards and chemicals: ATR gift sample obtained from TheraDose pharma Pvt.Ltd (Mumbai) and LOS was purchased from SL drugs & Pharmaceuticals (Hyderabad, India). Purified water was prepared using a Millipore Milli-Q (Nanopure Diamond, Barnstead thermolyne, USA) water purification system. Acetonitrile, Methanol were purchased from Merck Ltd. (Mumbai, India).

2.3. Preparation of standard drug solutions: An accurately weighed 10 mg of each of ATR and LOS was dissolved in 10 ml of methanol to obtain a concentration of 1 mg/ml each. From 1 mg/ml solution 1 ml was taken and made to 10 ml with methanol to obtain a concentration of 100µg/ml each. Daily working standard solutions of LOS and ATR was prepared by suitable dilution of the stock solution with methanol.

2.4. Determination of maximum wavelength and Iso-absorptive point: By appropriate dilution of two standard drug solutions with methanol, solutions containing 10 µg /ml of LOS and 10 µg /ml of ATR were scanned separately in the range of 200- 400 nm to determine the wavelength of maximum absorption for both the drugs. LOS and ATR showed absorbance maxima at 207 nm (λ1) and 246 nm (λ 2) respectively. The overlain spectra showed λ max of both drugs and also isoabsorptive points at 237.4 nm (Fig. 3).

2.5. Method- I (Simultaneous equation method) (Becket): Two wavelengths selected for the method are 207 nm and 246 nm that are absorption maxima of LOS and ATR respectively in methanol. The stock solutions of both the drugs were further diluted separately with methanol to get a series of standard solutions of 0.5-20 µg /ml concentrations. The absorbances were measured at the selected wavelengths and absorptivities (A 1%, 1 cm) for both the drugs at both wavelengths were determined as mean of three independent determinations. Concentrations in the sample were obtained by using following equations:

$$Cx = \frac{(A2ay1 - A1ay2)}{(ax2ay1 - ax1ay2)},\ Cy = \frac{(A1ax2 - A2ax1)}{(ax2ay1 - ax1ay2)}$$

Where, A_1 and A_2 are absorbances of mixture at 207nm (λ_1) and 246 nm (λ_2) respectively, ax_1 and ax_2 are absorptivities of LOS at λ_1 and λ_2 respectively and ay_1 and ay_2 are absorptivities of ATR at λ_1 and λ_2 respectively. Cx and Cy are concentrations of LOS and ATR respectively.

2.6. Method-II (Absorption ratio or Q-Analysis method) (Becket): From the overlain spectrum of LOS and ATR, two wavelengths were selected one at 237.4 nm which is the isoabsorptive point for both the drugs and the other at 246 nm which is λ max of ATR. The stock solutions of both the drugs were further diluted separately with methanol to get a series of standard solutions of 0.5-20 µg /ml concentrations. The absorbances of the sample solutions were measured and calculated the absorptivity values at the selected wavelengths for both the drugs. The method employs Q values and the concentrations of drugs in sample solution were determined by using the following formula**,**

Conc. of LOS: $C1 = \frac{Qm - Q1}{Q2 - Q1} x \frac{A}{a}$, Conc. Of ATR: $C2 = \frac{Qm - Q2}{Q1 - Q2} x \frac{A}{a}$

A = Absorbance of sample at isoabsorptive point, a = Absorptivities of LOS and ATR respectively at isoabsorptive point. Q_m, Q_1 and Q_2 are absorbance ratio of mixture, LOS and ATR at Iso-absorptive point to maximum wavelength of one of the component (selected wavelength).

2.7.Method validation: Method was validated accordance to ICH guidelines (ICH,1995;1996), for system suitability, linearity, precision, accuracy, limit of detection and limit of quantification.

2.8.Linearity: The linearity of this method was evaluated by linear regression analysis, which was calculated by least square method and the drug was linear in the concentration range of 0.5-20 µg/ml for both the drugs. Calibration standards were prepared by spiking required volume of working standard (100µg/ml) solution into different 10 ml volumetric flasks and volume made with methanol to yield concentrations of 0.5, 1, 2, 5, 10, 20µg/ml. The resultant absorbances of the drugs were measured. Calibration curve was plotted between absorbance of drug against concentration of the drug. These results show there was an excellent correlation between absorbance and analyte concentration. The linearity results of graph are presented in [Fig. 4, 5&6].

2.9. limits of Detection and Quantification: The limit of detection of an analytical method may be defined as the concentration, which gives rise to instrument signal that is significantly different from the blank(signal to noise ratio 3) and The LOQ is the concentration that can be quantitated reliably with a specified level of accuracy and precision (signal to noise ratio 10). The LOD and LOQ were calculated based on the standard deviation of the response and the slope. The values were shown in [Table.1].

2.10. Intra-day and Inter-day Precision and Accuracy: Precision and accuracy was studied by quality control samples of standard solutions covering low, medium and high concentrations (1.5, 8, 15µg/ml) of linearity range were prepared and measured the absorbance of three replicated samples of each concentration. Intra-day precision was studied by six replicate measurements at three concentration levels in the same day. Inter-day precision was conducted during routine operation of the system over a period of 3 consecutive days. Accuracy of the method was determined by calculating recovery studies. Statistical evaluation revealed that relative standard deviation (RSD) of the drug at different concentration levels for six injections were less than 2. Precision and accuracy data were shown in [Table. 2 and 3].

2.11.Sample preparation and analysis of pharmaceutical dosage forms: 20 Tablets (Brand Name: Zivast-L Forte, FDC Limited, Mumbai) were weighed, and an accurately weighed sample of powdered tablets equivalent to 50mg of LOS and 10mg of ATR [equivalent to one tablet]. For analysis of drug, a standard addition method was used. An accurately weighted 40 mg of pure ATR was added to finely powdered samples to bring the ratio of LOS and ATR to 1:1. Quantity of powder equivalent to 10 mg of LOS and 10 mg of ATR was weighed and dissolved in 60 mL of methanol and sonicated for 10 minutes. This solution was filtered through Whatmann No.1 filter paper. The residue on the filter paper washed with 10mL methanol three times with equal volume of methanol. Then the solution was transferred into a 100ml volumetric flask and made up the volume with methanol. The solution obtained was diluted with the methanol so as to obtain a concentration in the range of linearity previously determined. All determinations were carried out in five replicates. In Method-I, the concentration of both LOS and ATR were determined by measuring the absorbance of the sample at 207 nm and 246 nm. Values were substituted in the respective formula to obtain concentrations. For Method-II, the concentration of both LOS and ATR were determined by measuring absorbance of the sample at 237.4 nm and 246 nm and values were substituted in the respective formula to obtain concentrations. Results of tablet analysis are shown in [Table 4].

3.RESULTS AND DISCUSSION

The developed method shown from the results good correlation between the absorbance and concentration of the drugs under prescribed conditions and also the recoveries were found to be 91.81% -98.80% for LOS and 91.94%-101.54% for ATR from method I and 96.67-106.23% for LOS and 92.07%-106.58% for ATR from method II. The drug recovered from dosage form were found to be >93.78% for LOS and >92.1% for ATR from method-I and >98.62% for LOS and >97.91% for ATR from method-II. From the results method-II is more accurate than method-I. This indicates that commonly used excipients in pharmaceutical formulation were not interfering in the proposed method. The differences of less than 2.0 % for both intra- and inter-day data reflect the precision of the method. The observation of % RSD less than 2.0 for both intra- and inter-day measurements also indicates high degree of precision. In the present study, a linearity range of 0.5-20 µg /ml; this linearity range covers all the strengths of LOS and ATR. Hence these methods can be applied for quantifying the low levels of LOS and ATR in bulk and pharmaceutical dosage form.

4.CONCLUSION

It can be seen from the results and discussion presented above; the proposed method has good sensitivity and Precise. Results of the analysis of pharmaceutical formulations reveal that the proposed method is suitable for their simultaneous determination with virtually no interference of usual additive present in pharmaceutical formulations. The most striking feature of this method is its simplicity and rapidity, non- requiring, consuming sample preparations such as extraction of solvents, heating, degassing which are needed for HPLC procedure. These are new and novel methods and can be employed for routine analysis in quality control analysis. The described methods give accurate and precise results for determination of ATR and LOS mixture in marketed formulation.

Fig. 3. Overlain spectra of losartan and atorvastatin

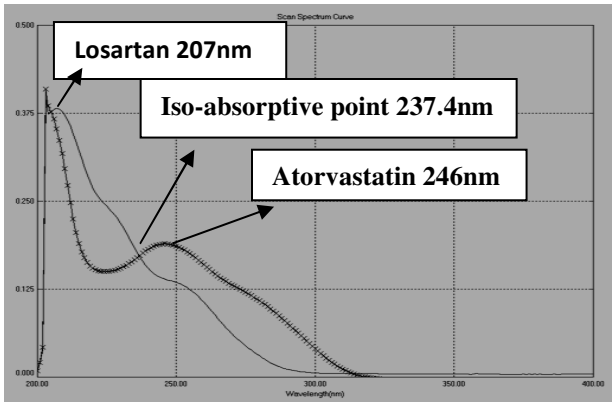


Fig. 4. Linearity graph of losartan potassium

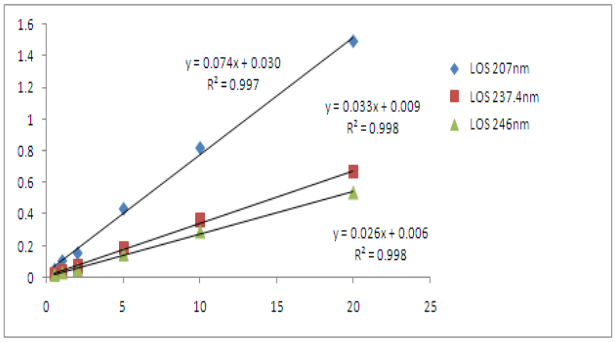


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Fig. 5. Linearity graph of atorvastatin calcium

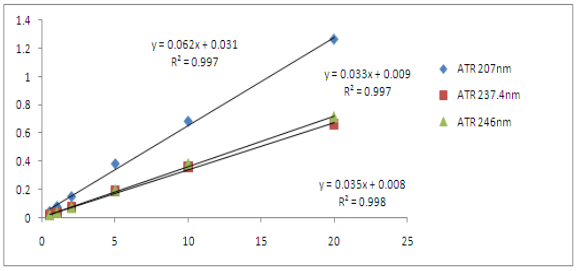

Fig. 6. Linearity graph of Mixture (LOS+ATR)

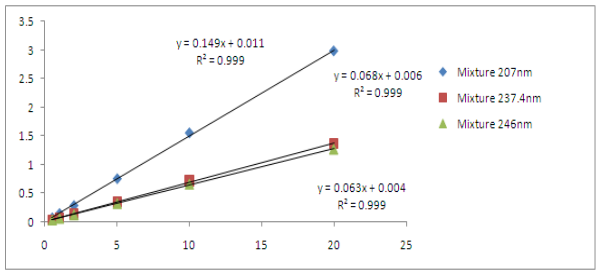

Table.1. Linear regression analysis of calibration curves with their respective absorptivty values

parameter	Method I				Method II			
	LOS		ATR		LOS		ATR	
Wavelength (nm)	207	246	207	246	237.4	246	237.4	246
Beer's law limit (µg/ml)	0.5-20	0.5-20	0.5-20	0.5-20	0.5-20	0.5-20	0.5-20	0.5-20
Molar absorptivity (Lit/mole/cm)	8.6×10^3	2.9×10^3	7.4×10^3	3.8×10^3	3.5×10^3	2.9×10^3	3.5×10^3	3.8×10^3
Correlation coefficient (R^2)	0.997	0.998	0.997	0.997	0.998	0.998	0.998	0.997
Slope(m)	0.074	0.026	0.062	0.035	0.033	0.026	0.033	0.035
Intercept(c)	0.030	0.006	0.031	0.008	0.009	0.006	0.009	0.008
LOD (µg/ml)	0.05	0.14	0.05	0.09	0.11	0.14	0.11	0.09
LOQ (µg/ml)	0.15	0.44	0.16	0.28	0.34	0.44	0.34	0.44

Table.2. Intra-day precision and accuracy of losartan and atorvastatin

Conc. in (µg/ml)	Method- I				Method- II			
	LOS		ATR		LOS		ATR	
	A	P	A	P	A	P	A	P
1.5	93.26±0.60	0.64	96.35±0.43	0.45	103.85±1.62	1.56	102.57±1.40	1.37
8	91.81±1.19	1.29	92.34±1.43	1.35	100.29±1.49	1.48	98.75±1.46	1.48
15	96.13±0.21	0.21	93.36±0.33	0.35	105.57±1.16	1.10	103.95±1.15	1.10

Values expressed Mean±SD, (n=6); standard deviation. A= accuracy, P= precision expressed as % relative

Table.3. Inter-day precision and accuracy of losartan and atorvastatin

Conc. in (µg/ml)	Method -I				Method- II			
	LOS		ATR		LOS		ATR	
	A	P	A	P	A	P	A	P
1.5	93.42±1.21	1.30	96.31±1.29	1.34	105.73±0.93	0.88	104.11±0.92	0.88
8	92.41±0.08	0.09	91.94±0.20	0.22	100.64±0.73	0.72	99.10±0.71	0.72
15	95.73±0.39	0.41	93.85±0.45	0.48	104.98±0.83	0.79	103.37±0.81	0.79

Values expressed Mean±SD, (n=9); standard deviation. A= accuracy, P= precision expressed as % relative

Table.4. Recovery study from formulation of losartan and atorvastatin

Method I				Method II			
LOS		ATR		LOS		ATR	
46.89±0.05	93.78	9.21±0.03	92.1	49.313±0.4	98.62	9.791±0.01	97.91

Values are expressed in Mean ±SD, (n=5); Labeled amount of each tablet was 50mg of LOS and 10mg of ATR. Values given in table was amount of drug recovered from tablet with percentage recovery.

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