

# Simultaneous estimation of Losartan and Atenolol by UV Spectrophotometric Method

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**Abstract -** Losar-beta is available for the treatment of hypertension. It contains losartan potassium (LS; 50mg) and atenolol (AT; 50mg). In the present study, simple, rapid, precise and accurate method for the simultaneous estimation of these drugs have been developed and validated by UV spectrophotometry. The method was validated with respect to its linearity, limit of quantitation (LOQ), limit of detection (LOD), precision and accuracy. In this method the LS and AT were scanned with water: methanol (80:20) as solvent and  $\lambda_{max}$  were found to be at 232 and 275 nm for LS and AT respectively. For LS ( $A_1 = 0.0054 C_x + 0.0634 C_y$ ) and AT ( $A_2 = 0.0028 C_x + 0.0128 C_y$ ). The equations were developed by Vierodt's method. The LOD was found to be 0.380  $\mu\text{g/mL}$  for LS and 0.860  $\mu\text{g/mL}$  for AT. LOQ was 1.160  $\mu\text{g/mL}$  for LS and 2.600  $\mu\text{g/mL}$  for AT. The %RSD for day to day precision was found to be 0.0060 for LS and 0.0200 for AT. Percentage recovery was found to be  $99.32 \pm 0.08$  for LS and  $99.54 \pm 0.12$  for AT. The linearity was found to be in the concentration range of 5-50  $\mu\text{g/mL}$  for LS and AT. Statistical analysis proves that, the method is repeatable and selective for the analysis of LS and AT. The result of recovery studies for tablet was found to be nearly 100% showing no interference due to excipients. This method for simultaneous estimation of LS and AT is quite accurate, precise, economic, simple and rapid, hence can be employed for routine analysis in quality control laboratories.

**Keyword –** losartan, atenolol, UV spectrophotometry, method validation, simultaneous estimation.

## 1. INTRODUCTION

Losar-beta is available for the treatment of hypertension. It contains losartan potassium (LS; 50mg) and atenolol (AT; 50mg). Losartan is a angiotensin II receptor antagonist and atenolol is  $\beta_1$  receptor antagonist. These drugs are more effective in combination therapy as compared to monotherapy [1, 2]. The combinations of these drugs are marketed under various brand names as tablet dosage form. Literature reveals that very few spectrophotometric methods are available for the simultaneous estimation for these combinations, which are expensive also. Hence, it was thought that a simultaneous estimation for these combinations can be carried out to make the methods more cost effective [3, 4].

If samples contains two absorbing drugs x and y each of which has absorption maxima at  $\lambda_1$  and  $\lambda_2$ . It may be possible to determine both drugs by simultaneous equation [5, 6]. The following criteria may be applied.

The information required is:

The absorptivities of x at  $\lambda_1$  and  $\lambda_2$ ,  $a_{x1}$  and  $a_{x2}$  respectively.

The absorptivities of y at  $\lambda_1$  and  $\lambda_2$ ,  $a_{y1}$  and  $a_{y2}$  respectively.

The absorbances of the diluted sample at  $\lambda_1$  and  $\lambda_2$ ,  $A_1$  and  $A_2$  respectively.

Let  $c_x$  and  $c_y$  be the concentrations of x and y respectively in the diluted sample.

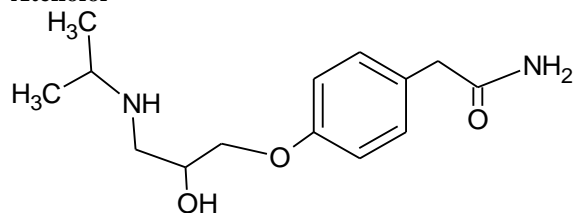
Two equations are constructed based upon the fact that at  $\lambda_1$  and  $\lambda_2$  the absorbance of the mixture is the sum of the individual absorbances of x and y.

At  $\lambda_1$   $A_1 = a_{x1} b c_x + a_{y1} b c_y$

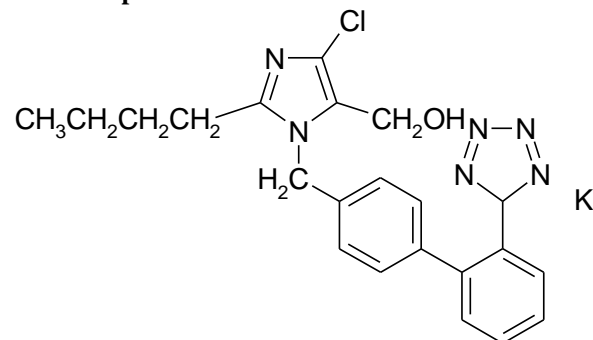
At  $\lambda_2$   $A_2 = a_{x2} b c_x + a_{y2} b c_y$

For the measurements in 1cm cells,  $b = 1$

### Atenolol



### Losartan potassium



## 2. EXPERIMENTAL

### Preparation of calibration curve:

The stock solutions of 10  $\mu\text{g/mL}$  concentration were prepared in methanol and water (20:80 v/v). For atenolol three absorption maxima were observed at value of 225 nm, 275nm and 322nm. The  $\lambda_{max}$  275 nm was used for this study. For losartan potassium an absorption maxima observed at 232nm was used.

Working standard solution (100  $\mu\text{g/mL}$ ) was made from stock solution (S) by suitably diluting with methanol. Aliquots (0.5, 1.0, 1.5..... 5.0 mL) were taken from

this working standard solution and suitably diluted with methanol to give a concentration range of 5 to 50 µg/mL. For atenolol and losartan the absorbance were recorded at 275 nm and 232 nm respectively against a reagent blank and calibration curve was plotted as shown in Fig. 1 and 2.

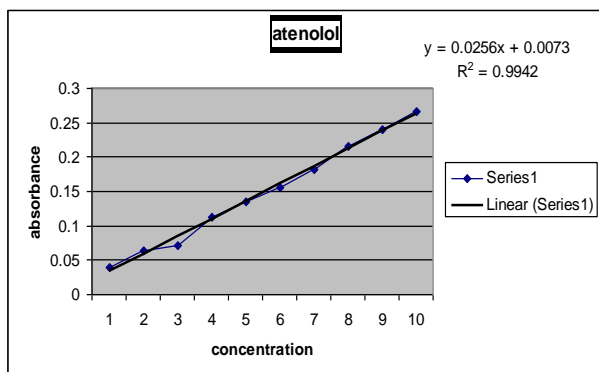


Fig. 1: Calibration curve of atenolol at 275 nm

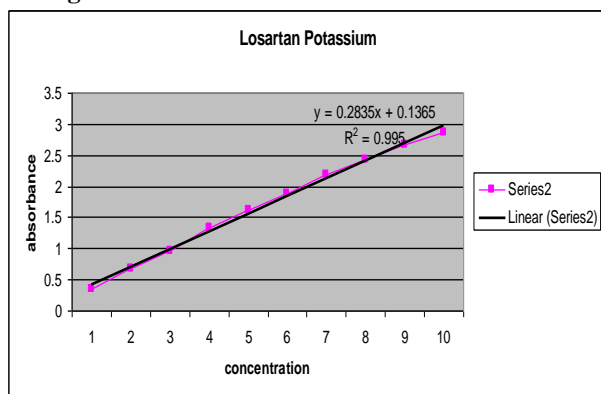


Fig. 2: Calibration curve of losartan potassium at 232 nm

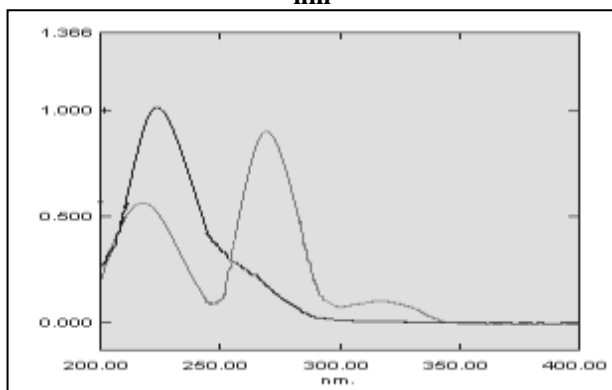


Fig. 3: Overlay UV spectra of atenolol & losartan potassium

### Optical characteristics

The optical characteristics such as absorption maxima, Beer's law limit, correlation coefficient (r), slope (m), intercept (c), molar absorptivity and Sandell's sensitivity were calculated and the results are shown in Table 1. The absorption coefficient data of atenolol and losartan are given in Table 2 and 3 respectively.

Table 1: Optical characteristics

Parameters	Values	
	Atenolol	Losartan potassium
$\lambda_{max}$ (nm)	275	232
Beer's Law Limit (µg/mL)	5-60	5-70
Molar absorptivity (L/mol.cm)*	$0.0150 \times 10^4$	$0.2681 \times 10^4$
Sandell's sensitivity (µg cm <sup>-2</sup> /0.001)*	47.25	6.67
Regression equation (y = mx + c)	y=0.0256x+0.0073	y=0.283x+0.1365
Slope (m)	0.0256	0.283
Intercept (c)	0.0073	0.1365
Correlation coefficient (r)	0.9942	0.995

Table 2: Absorption coefficient data of atenolol

Conc. (µg/mL)	275 nm		232 nm	
	Abs.	$E_{1cm}^{1\%}$	Abs.	$E_{1cm}^{1\%}$
5	0.0387	7.74	0.0151	3.02
10	0.0635	6.35	0.0247	2.47
15	0.0712	4.75	0.0341	2.27
20	0.1132	5.66	0.0503	2.51
25	0.1355	5.42	0.0675	2.70
30	0.1549	5.16	0.0676	2.25
35	0.1818	5.19	0.0725	2.071
40	0.2158	5.39	0.0839	2.098
45	0.2396	5.32	0.0966	2.14
50	0.2654	5.31	0.1957	3.91
	(Mean) $a_{x1} = 5.393$		(Mean) $a_{x2} = 2.816$	

### Development of simultaneous equation

For atenolol and losartan potassium Vierordt's equations were developed for simultaneous estimation by using the following set of equations:

$$\text{At 275nm} \quad A1 = ax1 \ bCx + ay1 \ bCy$$

$$\text{At 232nm} \quad A2 = ax2 \ bCx + ay2 \ bCy$$

Cx and Cy = Concentration of losartan potassium and atenolol respectively in µg/mL.

A1 and A2 = absorbance at 275 nm and 232 nm respectively.

ax1 and ax2 = absorption coefficient of losartan potassium at 275 nm and 232 nm respectively.

ay1 and ay2 = absorption coefficient of atenolol at 275 nm and 232 nm respectively.

b = 1 (for measurement in 1 cm. Cells).

Substituting the values of ax1, ax2, ay1 and ay2 equation can be rearranged as:

$$A_1 = 0.0054 C_x + 0.0634 C_y$$

$$A_2 = 0.0028 C_x + 0.012 C_y$$

**Table 3: Absorption coefficient data of losartan potassium**

Concentration (µg/mL)	232 nm		275 nm	
	Abs.	$E_{1cm}^{1\%}$	Abs.	$E_{1cm}^{1\%}$
5	0.3678	68.84	0.0687	13.74
10	0.5425	66.78	0.1161	11.61
15	0.6687	64.85	0.1765	11.76
20	0.8396	66.28	0.2207	11.04
25	1.0856	64.84	0.2914	11.66
30	1.3417	62.75	0.3593	11.98
35	1.7672	62.46	0.3855	11.01
40	1.9890	60.67	0.4637	11.59
45	2.1351	59.10	0.5105	11.34
50	2.3005	57.36	0.5627	11.25
	<b>(Mean) <math>a_{Y1} =</math></b>		<b>(Mean) <math>a_{Y2} =</math></b>	
	<b>63.39</b>		<b>12.80</b>	

### 3. RESULTS

#### Estimation from tablets

The powdered tablets (powder equivalent to 50 mg atenolol and 50 mg losartan potassium) were taken in 100 mL of conical flasks separately. These were extracted with 4 X 20 mL portion of water and methanol (80:20), and filtrate was taken in 100 mL volumetric flasks and the volumes were made up to 100 mL with water and methanol (80:20). Aliquots of a definite concentration were further suitably diluted to give the concentration in the range of 5-50 µg/mL. The drug content in the tablets was calculated [5, 6, 7]. The experiments were repeated six times to check its reproducibility and the results are shown in Table 4, 5 and 6.

#### Method Validation

##### Accuracy

The % recovery was found to be 99.54±0.12 for atenolol and % recovery was found to be 99.32±0.08 for losartan . Results of % recovery found are shown in Table 7 and 8.

##### Linearity

It was linear in the range of 5µg/mL-50µg/mL for both atenolol and losartan potassium at 275 and 232 nm respectively.

**Table 4: Estimation of drugs from Losar-beta tablet**

Abs.	Conc. found (µg/mL)	Amt. found/ tablet (mg)	Amt. Claimed/ tablet(mg)
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275 nm	232 nm	AT (C <sub>X</sub> )	LS (C <sub>Y</sub> )	X	Y	X	Y
0.012	0.019	9.97	10.01	49.88	50.05	50.0	50.0
0.012	0.019	10.0	10.0	50.04	50.03	50.0	50.0
0.012	0.019	10.0	9.98	50.04	49.9	50.0	50.0
0.012	0.019	10.0	9.97	50.03	49.882	50.0	50.0
0.012	0.019	10.0	10.0	50.04	50.04	50.0	50.0
0.012	0.019	9.99	9.99	49.98	49.960	50.0	50.0

**Table 5: Statistical results of Losar-beta tablets**

Parameters	Atenolol	Losartan
Standard deviation	0.0671	0.0328
Coefficient of variation	0.0997	0.0007
Standard error of mean	0.0274	0.0134
Percentage range of error (within 95% confidence limits)	50.1305 ± 0.0537	49.98 ± 0.0262

**Table 6: Compilations of results of statistical analysis of commercial formulations of Atenolol and losartan potassium**

Component	Label claim	Amt. found	S.D.	% RSD	SE
AT	50	50.13 ± 0.054	0.0671	0.1338	0.0274
LS	50	49.98 ± 0.026	0.0328	0.0659	0.0134

Values represents average of six determinations

**Table 7: Results of drug recovery studies of atenolol**

Level of standard addition (%)	Amount found in three replicate			% recovery ± SD
90	49.82	49.98	49.86	99.88 ± 0.12
100	49.48	49.78	49.88	99.34 ± 0.28
110	49.28	49.98	49.94	99.65 ± 0.32

**Table 8: Results of drug recovery studies of losartan potassium**

Level of standard addition (%)	Amount found in three replicate			% recovery ± SD
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90	49.68	49.58	49.82	99.48±0.18
100	49.47	49.65	49.88	98.32±0.23
110	49.64	49.86	49.98	99.65±0.18

### Precision

#### Intermediate precision: day to day

The % RSD for atenolol was 0.0200 and for losartan potassium it was 0.0060

**Table 9: Intermediate precision: day-to-day**

Atenolol			Losartan		
Conc. (µg/mL)	Day-1	Day-2	Conc. (µg/mL)	Day-1	Day-2
5	50.28	50.18	5	50.22	50.42
10	50.18	50.12	10	49.86	50.28
20	50.02	49.98	20	50.06	49.89
<b>Mean</b>	50.16	50.093	<b>Mean</b>	50.05	50.20
<b>Mean</b>		50.13	<b>Mean</b>		50.12
<b>S.D.</b>		0.05	<b>S.D.</b>		0.11
<b>%RSD</b>		0.094	<b>%RSD</b>		0.212

#### Intermediate precision: analyst to analyst

The % RSD for atenolol 0.0036 and for losartan potassium it was 0.0034

**Table 10: Intermediate precision: analyst-to-analyst**

Atenolol			Losartan		
Conc. (µg/mL)	A-1	A-2	Conc. (µg/mL)	A-1	A-2
5	50.32	50.12	5	50.18	50.28
10	50.10	49.78	10	49.82	49.84
20	50.20	50.26	20	50.08	49.98
<b>Mean</b>	50.21	50.05	<b>Mean</b>	50.02	50.03
<b>Mean</b>		50.13	<b>Mean</b>		50.03
<b>S.D.</b>		0.100	<b>S.D.</b>		0.002
<b>%RSD</b>		0.100	<b>%RSD</b>		0.003

A-1 = Analyst1, A-2 =Analyst 2

#### Limit of Detection (LOD)

Based on the standard deviation of the response and slope  
The detection limit may expressed as:

$$LOD = 3.3 \sigma / S$$

Where  $\sigma$  = the standard deviation of the response

S = the slope of the calibration curve.

For atenolol LOD was found to be 0.860 µg/mL and for losartan potassium it was 0.380 µg/mL.

#### Limit of Quantitation (LOQ)

Based on the standard deviation of the response and the slope

The quantitation limit may be expressed as:

$$LOQ = 10 \sigma / S$$

For atenolol LOQ was found to be 2.600 µg/mL and for losartan potassium it was 1.160 µg/mL.

#### Stability

Solution containing 10 µg/mL of the atenolol and 5 µg/mL losartan of Nebistar SA was analyzed by UV spectrophotometry method at 1, 2, 3, 5, 24, 48, 72, hours after preparation. The behavior of the analyte remained unchanged up to 4 days. All the measurements were made at room temperature (27-28°C). Tablet analysis % recovery was found to be 99.54 ± 0.12 for AT and 99.32 ± 0.08 for LS.

The results of validation are summarizes in Table 11.

**Table 11: Validation data for the developed UV spectroscopic method**

Validation Parameters	Losar-Beta	
	AT	LS
<b>Linearity (r2)</b>	0.9997	0.9995
<b>Analyst variation</b>	0.1000	0.0700
<b>Inter day Variation</b>	0.0200	0.0060
<b>Accuracy (%SD) (%found)</b>	0.12	0.08
	99.54	99.32
<b>LOD</b>	0.860	0.380
<b>LOQ</b>	2.600	1.160

## 4. CONCLUSION

The UV spectrophotometric method developed is simple, precise, rapid, selective and economical for the simultaneous estimation of atenolol and losartan potassium in solid dosage form. It can also be used for the analysis of these drugs in biological fluids and in quality control laboratories.

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### AUTHOR'S PROFILE



Dr. Asmita Gajbhiye has about 15 years experience of research and teaching experience at both UG and PG levels. She is a well renowned scientist who has published more than 25 papers in journals of international and national repute and presented more than 50 papers in the various conferences/ seminars and symposia at national and international level. She has successfully completed the various research projects at PG and Ph. D. level. She has also received the best presentation awards at national level. Her research projects have been appreciated at international level during presentation of research papers. She has delivered invited lectures and chaired many sessions in several National and International conferences and symposia in India and abroad. Presently, she is working as Associate Professor in Department of Pharmaceutical Sciences, Dr. H.S. Gour Central University, Sagar, MP.