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DEVELOPMENT OF A NEW VALIDATED MULTICOMPONENT UV-VISIBLE SPECTROPHOTOMETRIC METHOD FOR AN AGED OLD ANTIHYPERTENSIVE COMBINATION LOSARTAN POTASSIUM AND ATENOLOL

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ABSTRACT

A specific and validated UV spectrophotometric method was developed using Shimadzu model 1700 UV Spectrophotometer for the simultaneous estimation of losartan potassium and atenolol both in bulk drugs as well as pharmaceutical formulations. This method was established using water as the solvent and multicomponent analysis was performed for the better validation parameters which were not reported earlier. The r^2 value estimated was 0.999 and percentage recovery was in the range of 99.97 to 100.05 for both of the drugs. Validation experiments performed to demonstrate system suitability, specificity, precision, linearity, accuracy, inter day assay, intraday assay, robustness, ruggedness, LOD, and LOQ. While estimating the commercial formulation there was no interference of excipients and other additives. Hence this method can be used for routine analysis of losartan potassium and atenolol simultaneously in bulk and their formulations.

Keywords: Losartan, Atenolol, Multicomponent Method, Simultaneous Estimation

Introduction: Hypertension can be considered as the major risk factor in case of ischemic and hemorrhagic stroke, chronic kidney disease, heart failure, myocardial infarction, cognitive decline and premature death. β_1 receptor blocking can be considered as prophylactic treatment. On the other hand, blocking of angiotensin 1 receptor may result into marked fall in blood pressure. However, combination of drugs is chosen on the basis of complementary mechanisms of actions thus provide synergistic effects on raising blood pressure. Combination therapy has advantages like reduction in the risk of development of CHD (coronary heart disease), stroke and better patient compliance. One of the age-old combinations available over the market is losartan potassium and atenolol.

Atenolol (ATN), designated chemically as (RS)-4-(2-hidroxy-3-isopropylaminopropoxy) phenylacetamide is commercially available as a racemic mixture (Figure 1), it is found in the form of tablets, oral solution, and sterile solution for injectable. Atenolol (ATN) is a β1-selective (cardio selective) β-adrenergic receptor-blocking agent without membrane-stabilizing or intrinsic sympathomimetic (partial agonist) activities. ATN is also used to treat myocardial infarction (heart

attack), arrhythmias (rhythm disorders), angina (chest pains), and disorders arising from decreased circulation and vascular constriction, including migraine.[1]

Losartan (I, 2-n-butyl-4-chloro-1-[p-(o-1H-tetrazol- 5- phenyl) benzyl]-imidazole-5 methanol monopotassium salt) (LOS) is a highly selective, orally active, non-peptide angiotensin II receptor antagonist indicated for the treatment of hypertension. It has a more potent active metabolite EXP3174 (II, 2-n-butyl-4-chloro-1-[2-(1H-tetrazol-5 yl) biphenyl- 4-yl) methyl] imidazole-5-carboxyl acid) (Figure 2). [2]

The determination of losartan has been carried out in tablets by HPLC, capillary electrophoresis and super-critical fluid chromatography [3,4] in urine by gas chromatography- mass spectrometry [5] and, simultaneously with its active metabolite in biological fluids, by HPLC. [6-11] Similarly atenolol determination has also been carried out in human plasma by HPLC-fluorescence detector [12], by GC-MS after derivatization using N-methyl-N-(trimethylsilyl)trifluoroacetamide [13] and in human urine by HPLC-amperometric detection. [14]

Several methods have been reported for the simultaneous estimation of the two drugs in bulk as well as formulations using UV spectrophotometer. Satyanarayana *et al.* 2006 reported an artificial neural networking method to determine ATN and LOS in tablets using Levenberg-Marquardt algorithm. [15] Sathe *et al*. 2007 developed simultaneous analysis of losartan potassium, atenolol and hydrochlorothiazide in bulk and in tablets by high performance thin-layer chromatography with UV absorption densitometry. [16] Few methods have been reported for the determination of LOS and ATN along with hydrochlorothiazide. [17,18] Shah *et al.* 2010 developed a UV spectrophotometric method for the content uniformity study of 5 marketed tablets containing LOS and ATN. [19] Diwedi *et al*. 2012 published simultaneous estimation of atenolol and losartan potassium by high performance liquid chromatography and UV spectrophotometric method where the linearity range was 5-50 µg/ml and r^2 value was close to 0.995 for both drugs.[20] Lalitha *et al.* 2013 developed the method using Q-analysis and RSD values of atenolol and losartan potassium were found to be less than 2%. LOD and LOQ values for atenolol and were found to be 0.74µg/ml and 2.45µg/ml at 275 nm and LOD and LOQ values for losartan potassium were found to be 0.72 and 1.78 µg/ml at 282 nm, respectively. [21] Singh *et al.* 2014 used Vierordt's simultaneous equation method and % R.S.D. were found to be less than 2 % .[22]

No methods have yet been reported using multicomponent analysis method and therefore present study is having scientific rationale and importance. In addition to that using water as the solvent simplifies the analysis method resulting into cost effectiveness.

Materials And Methods:

Reference standards of LOS (purity > 99.75) and ATN (purity > 99.80) were procured from Centaur Pharmaceuticals Pvt. Ltd. (Mumbai, India). HPLC grade ultra pure water was prepared by Milli-Q® system (Millipore, Milford, MA, USA). Sample aliquots were filtered through a 0.22

μm Nylon- 66 filters (Agilent Technologies, Inc., CA, USA). Shimadzu UV-1700 is a double beam high speed scanning spectrophotometer with advanced quantitative software was used for the UV spectrophotometric analysis. The compact body of UV-1700 incorporates a high-performance monochromatic display therefore permits high speed absorption spectrophotometric, data processing and data presentation without addition of any costly option.

Preparation of Standard Solutions:

About 10 mg of losartan potassium and atenolol were separately weighed and transferred to calibrated, clean and dried 100mL volumetric flasks containing small volume of water. Then the final volume was made up to the mark with water. This yields standard solutions of losartan potassium & atenolol of 100μg/ml. For preparation of calibration curve appropriate aliquots from the standard stock solutions of atenolol and losartan potassium were used to prepare two different sets of dilutions Series A and B stock solutions as follows. Series A consisted of different concentration of atenolol (5-90 μg/ml). Aliquot from the stock solution of atenolol (5-90 μg/ml) was pipetted out in to a series of 10 ml volumetric flask and diluted with water to get final concentration in range of 5-30 μg/ml (5,10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85 and 90 ml). Series B consisted of varying concentrations of losartan potassium (5-30 μg/ml). Appropriate volume of the stock solution of losartan potassium (100 μg/ml) was transferred into a series of 10 ml volumetric flask and the volume was adjusted to the mark with water to get final concentration in range of 5-30 μg/ml (5,10,15,20,25,30 and 35 ml).

Spectrophotometric Conditions:

The standard drug solutions were scanned between in the UV spectrophotometer at a range of 400-200 nm using spectrum mode. The losartan potassium & atenolol showed absorption maxima at 206nm and 225nm respectively. These absorption maxima values were taken for multicomponent analysis.

Validation Parameters

Linearity

The linearity of calibration curves in pure solution was checked over the concentration ranges of about 5-90 μg/ml for atenolol and 5-35 μg/ml losartan potassium (Figure 3 and 4). The represented data was shown in below Table 1. The calibration curve were constructed by plotting drug concentration versus the absorbance values of Absorption ratio spectrum 225 nm for atenolol and 206 nm for losartan potassium. The concentration of individual drugs present in the mixture was determined from the calibration curves in quantification mode.

Precision

The precision of the proposed method was ascertained by actual determination of six replicates of fixed concentration of the drugs within the Beer's range and finding out the absorbance by the proposed method. From the absorbance, Mean, Standard deviation and %RSD were calculated. Different parameters included for precision study were repeatability, intraday and interday precision. Study results are tabulated in Table 2.

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Accuracy

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of bulk samples within the linearity range and added to the pre-analyzed formulation of concentration 20 µg/ml and from that percentage recovery values were calculated. The values are shown in Table 3.

Ruggedness

Ruggedness is the degree of reproducibility of the results obtained under a verity of conditions. These conditions included different analysts and different instruments etc. In this method different analysts was considered. The data was subjected to statistical analysis and the results are expressed in mean, standard deviation and %RSD in Table 4, 6.

Robustness

Robustness of the method was studied by deliberate variations of the analytical parameter such as solvent composition. The data was then subjected to statistical analysis and the results are expressed in mean, standard deviation and %RSD in Table 5, 7.

Detection Limit and Quantification Limit

Calibration curves were plotted by using concentration in the expected detection limit range (0.1-5 µg/ml) for each drug. The standard deviation of y-intercept of regression line were determined and substituted in the following equation for the determination of detection limit and quantification limits.

Detection limit = 3.3 σ/s and

Quantification limit = 10 σ/s

Where, σ is the standard deviation of y-intercept of regression line and s is the slope of the calibration curve. The calculated LOD value for LOS and ATN were 0.178 µg/ml and 0.2 µg/ml. LOQ values determined were 0.54 µg/ml for LOS and 0.6 µg/ml for ATN.

Analysis of Formulation Using Multicomponent Method:

For analysis of commercial formulations of tablets, 20 REPALOL tablets were accurately weighed and finely powdered. The amount of powder equivalent to 50 mg of ATN and 50 mg of LOS were weighed and transferred into 100 ml volumetric flask followed by volume make up with water. The solution was filtered through Whatman filter paper No. 41. From the above solution suitable aliquot was prepared as per Table 8. The absorbance of sample solutions were measured at both selected wavelengths. The result is shown in Figure 5 and Table 9 and 10.

Results And Discussion

Developed multicomponent method of analysis was validated by analyzing the laboratory prepared samples where the obtained results were satisfactory. The percentage mean was found to be 99.98, the S.D. was 1.122 and the %RSD was found to be 1.122 for LOS. Similarly percentage

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mean was found to be 100.18, the S.D. was 1.02 and the %RSD was found to be 1.018 for ATN. These results were also well supported by the ruggedness and robustness studies. This indicates that the method developed is accurate and precise. The results obtained for the analysis of formulation was studied and it was found that the % mean were 99.65 and 99.68 for losartan potassium and atenolol respectively where the S.D. were found to be 0.177 and 0.266 respectively. This suggests that the results are accurate and precise and this method is also suitable for the estimation purpose of these drugs in formulations. Hence, it can be concluded that the developed simultaneous multicomponent method for the analysis of losartan potassium and atenolol is rapid, simple, economical, accurate and precise and can be used in the industries for the routine analysis of these drugs.

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TABLE 1: VALIDATION PARAMETERS

Parameters	ATN	LOS
Wavelength (nm)	225	206
Beer's Law Limit (µg/ml)	5-90	5-35
Regression Equation (y=a+bx)	y=0.034x+0.023	y=0.1x+0.016
Slope (b)	0.023	0.016
Intercept (a)	0.034	0.1
Correlation Coefficient (r^2)	0.999	0.999
LOD (µg/ml)	0.2	0.178
LOQ (µg/ml)	0.6	0.540
Precision (%RSD n=6)		
Inter-day	0.184	0.324
Intra-day	0.497	0.544
Accuracy (%RSD n=9)	99.98-100.03	99.97-100.05

TABLE 2: PRECISION DATA

Serial No.	Sample	Inter-day (%RSD)		Intra-day (%RSD)	
		ATN at 225 nm	LOS at 206 nm	ATN at 225 nm	LOS at 206 nm
1	LQC	1.108	0.853	0.549	0.884
2	MQC	1.048	0.440	0.937	1.973
3	HQC	0.946	0.879	1.048	1.962

TABLE 3: RECOVERY DATA AND ACCURACY STUDY

Serial No.	Name of the drug	Amount of sample (µg/ml)	Recovery level	Amount of drug added (µg/ml)	SD	% Recovery	% RSD
			80%	16	0.02	99.99	0.020
1	ATN	20	100%	20	0.025	100.00	0.024

			120%	24	0.025	100.00	0.024
			80%	16	0.04	100.13	0.0399
2	LOS	20	100%	20	0.017	100.02	0.0169
			120%	24	0.037	100.006	0.0369

TABLE 4: RUGGEDNESS DATA OF LOS

Analyst-1				Analyst-2			
Conc. (µg/ml)	Abs.	Calc. Amt.	Statistical Analysis	Conc. (µg/ml)	Abs.	Calc. Amt.	Statistical Analysis
20	2.011	19.95		20	1.990	19.74	
20	2.022	20.06	Mean=20.17	20	2.053	20.73	Mean=20.37
20	2.044	20.28	S.D=0.172	20	2.011	19.95	S.D=0.435
20	2.059	20.43	%RSD=0.852	20	2.086	20.7	%RSD=0.135
20	2.038	20.22		20	2.052	20.36	
20	2.025	20.09		20	2.091	20.74	

TABLE 5: ROBUSTNESS DATA OF LOS

H_2O + methanol (99:1)				H_2O + methanol (98:2)			
Conc.(µg/ml)	Abs.	Calc. Amt.	Statistical Analysis	Conc. (µg/ml)	Abs.	Calc. Amt.	Statistical Analysis
20	20.33	20.17		20	2.011	19.95	
20	2.072	20.56	Mean=20.22	20	2.022	20.06	Mean=20.17
20	1.981	19.64	S.D=0.405	20	2.044	20.28	S.D=0.172
20	2.011	19.95	%RSD=0.200	20	2.059	20.43	%RSD=0.852
20	2.042	20.26		20	2.038	20.22	
20	2.092	20.76		20	2.025	20.09	

TABLE 6: RUGGEDNESS DATA OF ATN

Analyst-1				Analyst-2			
Conc. (µg/ml)	Abs.	Calc. Amt.	Statistical Analysis	Conc.(µg/ml)	Abs.	Calc. Amt.	Statistical Analysis
20	0.733	20.88		20	0.715	20.35	
20	0.721	20.52	Mean=20.61	20	0.722	20.55	Mean=20.48
20	0.713	20.29	S.D=0.223	20	0.730	20.79	S.D=0.197
20	0.730	20.79	%RSD=1.081	20	0.723	20.58	%RSD=0.961
20	0.719	20.47		20	0.711	20.23	
20	0.728	20.73		20	0.717	20.41	

TABLE 7: ROBUSTNESS DATA OF ATN

Serial No.	Conc. Of drug claimed (µg/ml)	Conc. Of drug after multicomponent analysis (µg/ml)	Percentage of result obtained	Statistical Analysis
SAMPLE-1	5	4.98	99.6	%Mean=99.65 S.D.=0.177 % RSD=0.117
SAMPLE-2	10	9.94	99.4	
SAMPLE-3	15	14.95	99.66	
SAMPLE-4	20	19.92	99.6	
SAMPLE-5	25	24.94	99.76	
SAMPLE-6	30	29.98	99.93	

H_2O + methanol (99:1)				H_2O + methanol (98:2)			
Conc. (µg/ml)	Abs.	Calc. Amt.	Statistical Analysis	Conc. (µg/ml)	Abs.	Calc. Amt.	Statistical Analysis
20	0.719	20.47	Mean=20.42	20	0.732	20.85	Mean=20.54
20	0.722	20.55	S.D=0.1158	20	0.728	20.73	S.D=0.226
20	0.718	20.44	%RSD=0.567	20	0.718	20.44	%RSD=1.1
20	0.715	20.35		20	0.717	20.41	
20	0.711	20.23		20	0.711	20.23	
20	0.720	20.5		20	0.723	20.58	

TABLE 8: ALIQUOT PREPARATION CHART

Serial No.	LOS Conc.(µg/ml)	ATN Conc.(µg/ml)
SAMPLE-1	5	5
SAMPLE-2	10	10
SAMPLE-3	15	15
SAMPLE-4	20	20
SAMPLE-5	25	25
SAMPLE-6	30	30

TABLE 9: ANALYSIS OF LOS IN FORMULATION

TABLE 10: ANALYSIS OF ATN IN FORMULATION

Serial No.	Conc. Of drug claimed (µg/ml)	Conc. Of drug after multicomponent analysis(µg/ml)	Percentage of result obtained	Statistical analysis
SAMPLE-1	5	4.96	99.20	
SAMPLE-2	10	9.97	99.7	

SAMPLE-3	15	14.94	99.6	%Mean=99.68
SAMPLE-4	20	19.97	99.85	S.D.=0.266
SAMPLE-5	25	24.97	99.84	%RSD=0.266
SAMPLE-6	30	29.98	99.93	

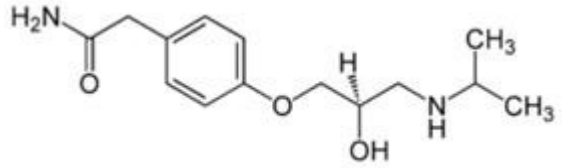

Figure 1: Atenolol

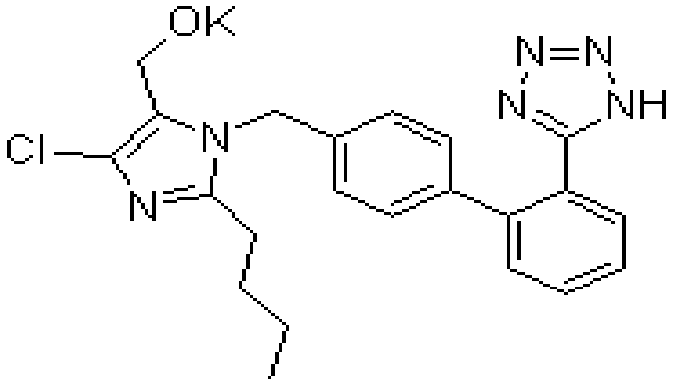

Figure 2: Losartan Potassium

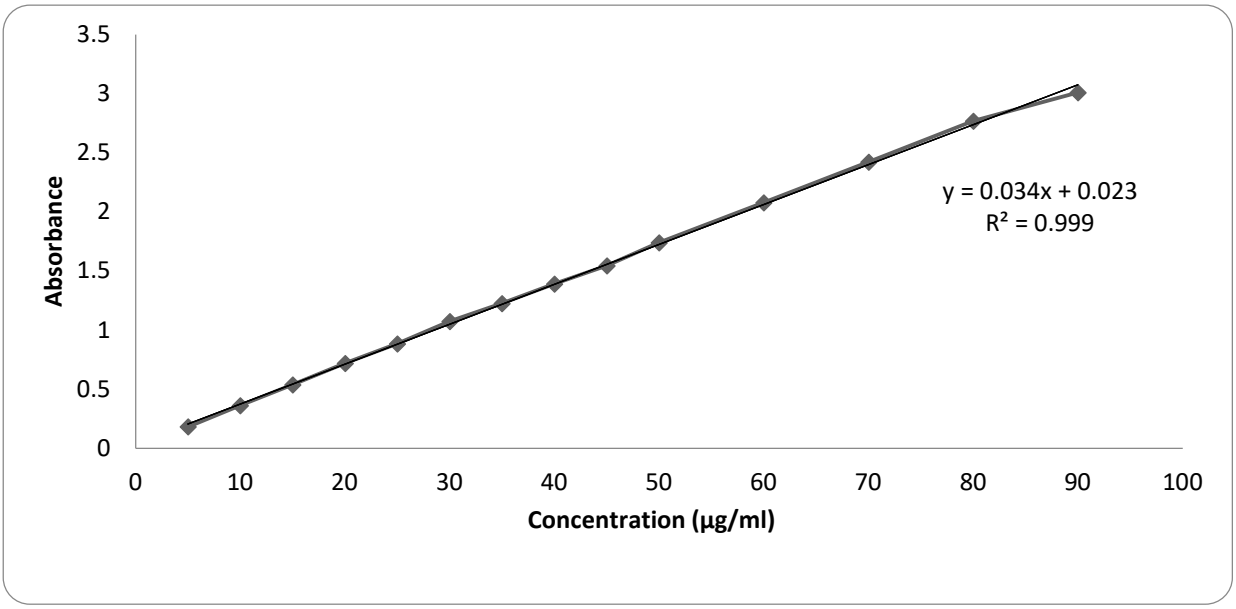

Figure 3: Calibration Curve of ATN

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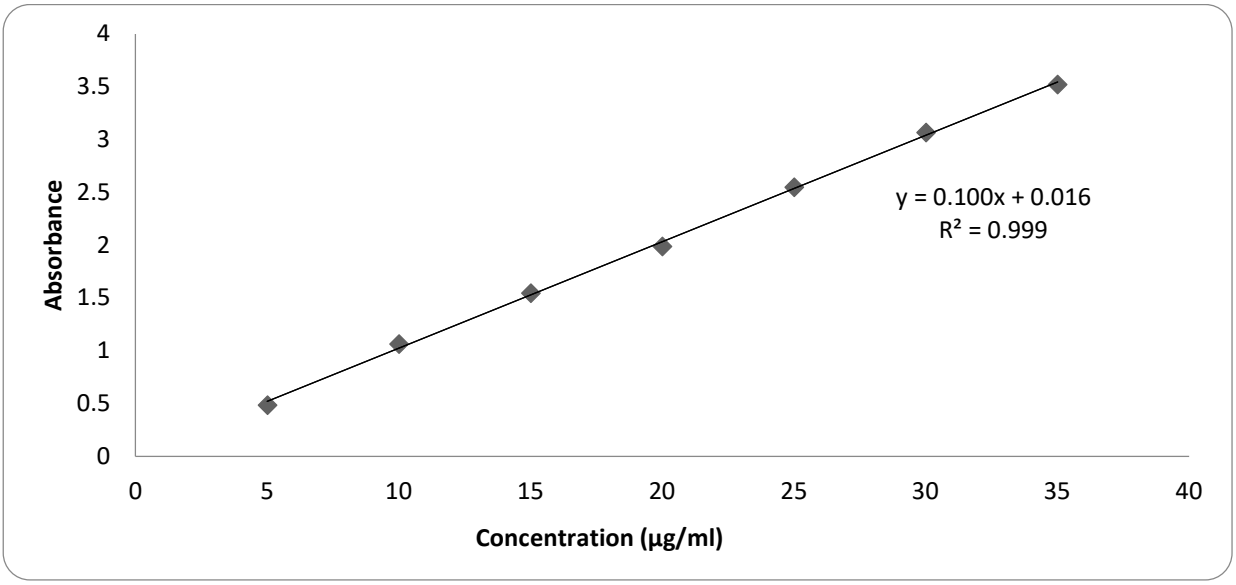

Figure 4: Calibration Curve of LOS

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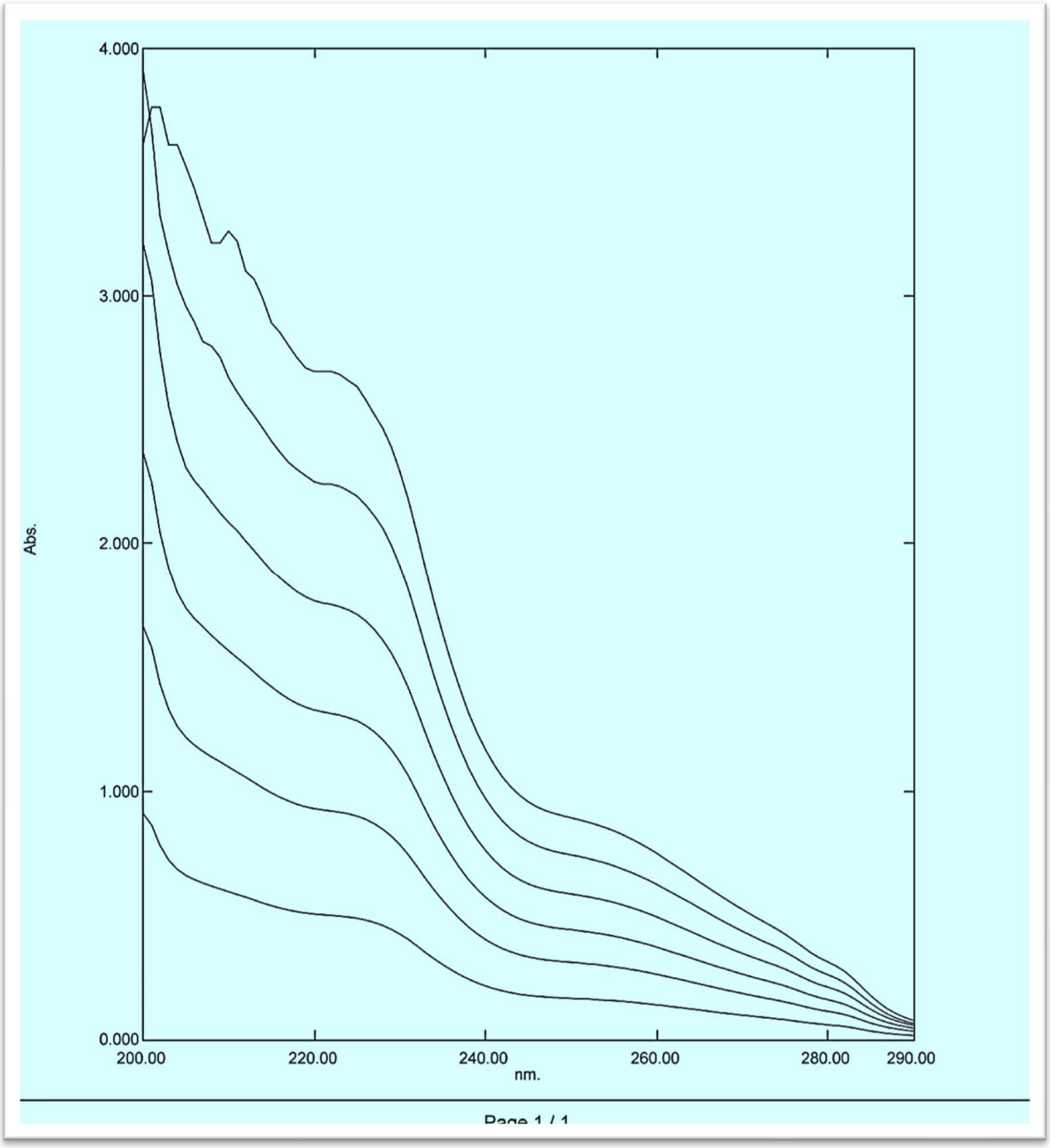

Figure 5: Absorbance Curves of Losartan Potassium & Atenolol in Formulation

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