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Pharmaceutical Sciences **RESEARCH ARTICLE……!!!**

CHEMOMETRIC ASSISTED METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF PHARMACEUTICALS

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KEYWORDS:

Losartan Potassium, Atenolol**,** PLS, PCR, Validation.

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ABSTRACT

This presented work is based on application of two multivariate calibration methods for simultaneous UV-Vis spectrophotometric determination of active substances in combined pharmaceutical formulation composed of Atenolol (AT) and Losartan Potassium (LP). The methods used were Principal Component Regression (PCR) and Partial Least Square (PLS). The Spectra of both AT and LP were recorded at concentrations within their linear range 2.0-12.0 µg/ml. 36 set of mixtures were used for calibration and 08 set of mixtures were used for validation in the wavelength range of 200 to 250 nm with the wavelength interval λ= 0.5 nm in water. The methods were validated as per International Conference on Harmonization Q2 (R1) (ICH) guidelines. These methods were successfully applied for determination of drugs in pharmaceutical formulation (tablet) with no interference of the excipients as indicated by the recovery study results. The proposed methods are simple, rapid and can be easily used as an alternative analysis tool in the quality control as well as in process control of drugs and formulation.

1. INTRODUCTION:

Atenolol (AT) [2-4-{2-hydroxy-3-(propan-2-yl amino) propoxyl} phenyl acetamide] Fig. 1 a and Losartan Potassium (LP) [2-Butyl-4-chloro-1-2-(1H-etrazol-5-yl) (1,1'-biphenyl)-4-yl) methyl) -1H-imidazole -5-methanol] Fig. 1 b [2]. Chemometric is the science of extracting information from chemical systems. Multivariate calibration methods (e.g., multiple linear regression (MLR), principle component regression (PCR) and partial least squares (PLS) utilizing spectrophotometric data are the important chemometric approach for determination of mixtures including drugs combination [13].As there are no reports on chemometric analysis of these drugs, this work was undertaken which presents simple, accurate and reproducible multivariate spectrophotometric methods for simultaneous determination of AT and LP in tablet dosage form.

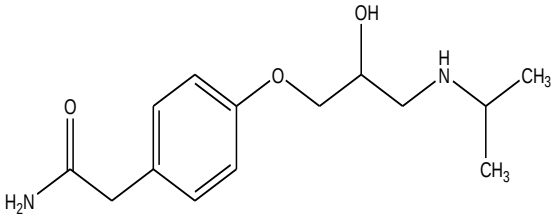


Fig. 1 (a) Structural formula of Atenolol

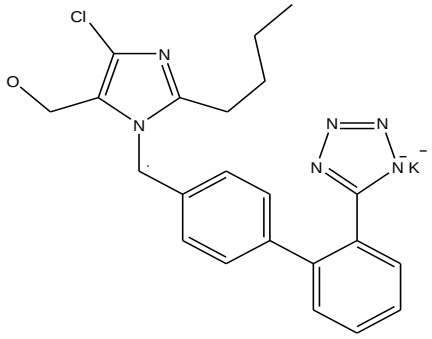


Fig. 1 (b) Structural formula of Losartan potassium

2. MATERIALS AND METHODS:

2.1. Instrumentation

Double beam UV- Vis spectrophotometer (Jasco V-730) with matched pair of 1cm quartz cells were used to record spectra of all solutions. The spectra were recorded at spectral band width of 2.0 nm, scanning speed 100 nm/min and data pitch 0.5 nm. Unscrambler X (10.3) (64-bit) trial version and Microsoft Excel 2013 were used for model generation and application of chemometric.

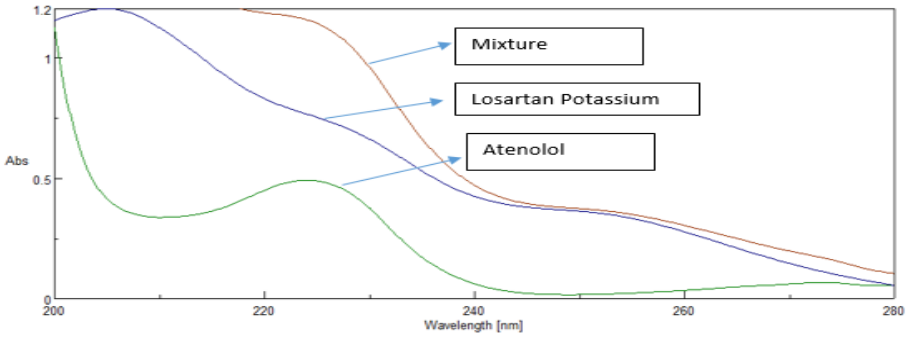
2.2. Material and Reagents

Atenolol and Losartan CEP grade were supplied from Kopran Ltd. (Mahad India). HPLC grade Acetronitrile (ACN) and methanol were procured from S.D.Fine chemical (Mumbai, India) and all other chemical were parched from Poona chemical lab reagent (Pune, India) Losar beta tablets

manufactured by Ltd containing Atenolol IP 50 mg and Losartan potassium IP 50 mg were procured from local pharmacy shop.

2.3 One component calibration

To find linear concentration of each drug, one component calibration was performed. Linear dynamic ranges were studied in the concentration range of 2.0-12.0µg/ml for both AT and LP. Absorbance values were recorded at λ_{max} of each drug (275 nm for AT and 205nm for LP) against Water as blank. Linear dynamic range for each compound was determined by least-square linear regression of concentration and the corresponding absorbance. Fig. 2 represents overlain spectra of AT and LP and their mixture.



2.4 Preparation of standard stock solution

Stock sol of AT and LP were prepared by dissolving the 10 mg of standard drug in 10 ml of water. The concentration of AT & LP were 1000 µg/ml from which further 1 ml was pipette out and 10 ml to achieve final conc. of 100 µg/ml of AT & LP respectively. Finally 1 ml of stock solutions here further diluted 10 ml with distilled water to get 10µg ml $^{-1}$ sol.

2.5. Preparation of working stock solution

Working standard solutions were prepared from standard stock solution of 100 µg/ml by appropriate dilution with Water to obtain final concentration of 2, 4, 6, 8, 10 and 12 µg/ml for both AT and LP.

2.6. Construction of calibration and validation set

A total set of 36 mixtures were prepared by combining working standard of AT and LP in their linear concentration range of 2.0-12.0 µg/ml (Table I). From these 28 mixtures were used for calibration set and 08 mixtures were used for validation set by random selection. The absorbance spectra were recorded in range of 200- 250 nm with 0.5 nm interval. The spectra were saved as ASCII (.txt) format which were further extracted in MS-Excel as required by Unscrambler software for model generation. The PCR and PLS models were developed utilizing absorption data using Unscrambler software. Selection of proper number of latent variables for development of model was necessary to obtain good prediction. Leave-one-out (LOO) cross validation method was

used to obtain necessary number of latent variables (LVs), as shown in Fig. 3 and calculated using formula [14],

$$RMSECV = \sqrt{\sum \frac{(Cact - Cpre)^2}{Ic}}$$

Where,
RMSECV= Root mean square error of cross validation
Cact= actual concentration of calibration set
Cpre= predicted concentration of validation set
Ic= Total number of samples in calibration set

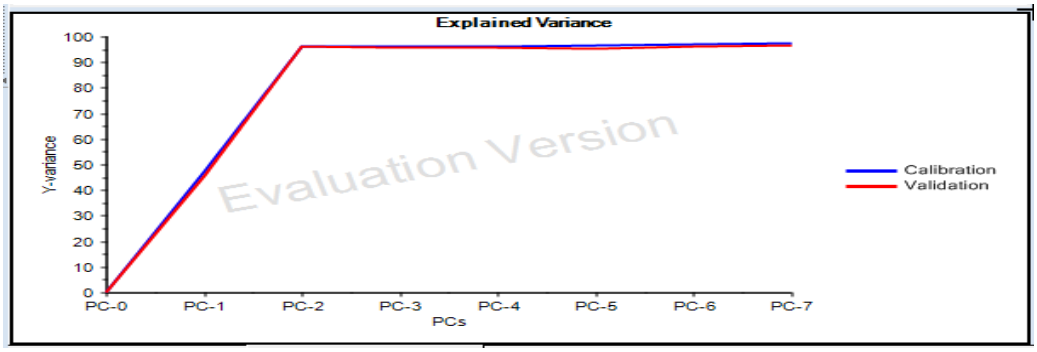


Figure.3: Explained Variance describing number of optimum PCs (Principle Components)

After the PCR and PLS models have been constructed, it was found that the optimum number of LVs were two factors for both PCR and PLS. For validation of generated models, concentration in validation set was predicted by using proposed PCR and PLS models (Table II). The validation of all methods was performed as per ICHQ2 (R1) [15].

Table 1: Composition of calibration and validation sets
*Mix no. 1-36 calibration set

MIX. NO	AT (µg/ml)	LP (µg/ml)	MIX. NO	AT (µg/ml)	LP (µg/ml)	MIX. NO	AT (µg/ml)	LP (µg/ml)
1	2	2	13	2	6	25	2	10
2	4	2	14	4	6	26	4	10
3	6	2	15	6	6	27	6	10
4	8	2	16	8	6	28	8	10
5	10	2	17	10	6	29	10	10
6	12	2	18	12	6	30	12	10
7	2	4	19	2	8	31	2	12
8	4	4	20	4	8	32	4	12
9	6	4	21	6	8	33	6	12
10	8	4	22	8	8	34	8	12
11	10	4	23	10	8	35	10	12
12	12	4	24	12	8	36	12	12

*Mix no. 2-6 validation set

Table 2: Predicted results for UV validation set by PCR and PLS method.

METHOD		PCR				PLS			
AT	LP	AT		LP		AT		LP	
Actual (µg/ml)		Predicted	% R*	Predicted	% R*	Predicted	% R*	Predicted	% R*
2	2	1.8544	101.0	1.8813	94.06	1.8443	92.21	1.8712	93.56
2	2	2.02133	95.92	2.0420	99.66	2.0131	100.6	2.0319	101.5
2	2	1.9185	103.2	1.9933	104.6	1.9085	95.95	1.9832	99.8
4	4	4.1295	101.1	4.1866	101.4	4.1195	102.9	4.1765	104.4
4	4	4.0430	100.2	4.0647	100.4	4.0428	101.1	4.0546	101.3
4	4	4.0036	100.2	4.0169	99.57	4.0033	100.2	4.0165	100.4
6	6	5.9421	99.31	5.9744	99.57	5.9421	99.03	5.9743	99.57
6	6	5.9630	99.38	5.9947	99.9	5.9528	99.2	5.9946	99.91

2.7. Assay of marketed preparation.

20 tablets of Losar Beta were accurately weighed and finely powdered. Tablet powder equivalent to 10 mg of drug (equivalent to label claim) was taken and transferred to 10 ml volumetric flask and was diluted to 10 ml with Water. The solution was sonicated for 10 minutes. This solution was then filtered with help of whatman filter paper no. 41. 1 ml of filtrate solution was diluted to 10 ml with water. Further 0.4 ml of this solution was diluted to 10 ml with water to get final concentration of 10 µg/ml of both AT and LP. The procedure was repeated 6 times for tablet formulation. The results of assay is presented in Table III.

Table III: Assay result for AT and LP in tablet (Losar Beta) by proposed methods

UV METHOD		PCR		PLS	
AT	LP	AT	LP	AT	LP
Actual (µg/ml)		% R	% R	% R	% R
10	10	101.04	101.1	101.02	100.5
10	10	100.07	100.08	100.8	100.8
10	10	100.05	101.1	100.9	101.1
10	10	100.8	100.09	101.2	100.8
10	10	100.06	100.05	100.2	101.8
10	10	101.5	101.2	100.5	101.1
Mean		100.46	100.60	100.77	100.8
SD		0.639	0.581	0.364	0.241

2.8. Accuracy study

The accuracy study was carried out at three levels 50 %, 100 % and 150 % of assay concentration. Calculated amount of AT and LP from standard solutions were spiked into sample solution and scanned in range of 200-250 nm. Concentrations were predicted by using developed PCR and PLS models. Accuracy data is presented in Table IV and Table V.

Table No. IV Accuracy:

Level %	Total Conc. µg/ml	AT				LP			
		% R		% RSD		% R		% RSD	
		PCR	PLS	PCR	PLS	PCR	PLS	PCR	PLS
50 %	5	97.6	97.6	0.65	0.72	97.82	97.66	0.51	0.45
		107.4	107.4			97.66	97.58		
		98.6	98.6			96.68	96.68		
100 %	10	93.9	93.9	0.10	0.11	96.54	96.41	1.14	0.81
		103.6	103.6			98.32	97.31		
		103.3	100.3			99.24	98.35		
150 %	15	101.7	101.7	0.18	0.18	101.0	102.0	0.61	0.49
		100.9	100.9			102.1	102.1		
		97.3	97.3			100.6	101.1		

Table V: Precision results obtained using developed PCR and PLS models.

METHOD		UV							
Amount taken µg/ml		AT				LP			
		% R		% RSD		% R		% RSD	
AT	LP	PCR	PLS	PCR	PLS	PCR	PLS	PCR	PLS
4	4	98.7	98.2	1.75	0.94	98	99.7	1.28	1.46
		102	100.5			101	102.7		
		99.2	99.7			98.7	99.5		
6	6	100.2	99.9	0.62	0.96	100.2	94.6	0.62	0.87
		101.1	101.4			101.2	101.8		
		100.1	101.3			99.8	100.8		
8	8	101.2	101.6	1.40	1.31	100.6	101.8	0.86	0.89
		100.6	100.7			101.5	101.3		
		98	98.5			99.7	100.1		

2.10. LOD and LOQ:

LOD and LOQ were calculated as 3.3 σ/S and 10 σ/S, respectively; where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot.

3. RESULTS AND DISCUSSION:

Out of 36 mixtures, 28 set of mixtures were used for calibration and 08 set of mixtures were used for validation. The models were tried to develop with varying Δ λ. The best results were obtained with the wavelengths intervals λ= 0.5 nm in methanol. The developed method found to be accurate as results are close to 100 % and precise with % RSD less than Summary of results is presented in Table VI.

Table VI: Summary of results of UV for AT and LP

UV results		Atenolol		Losartan	
Sr.no	Parameter	PCR	PLS	PCR	PLS
1	Range (µg/ml)	2-12		2-12	
2	Wavelength	200-250		200-250	
3	Data interval	0.5		0.5	
4	Factor/PC-2	2		2	
5	Assay (%)	100.46	100.60	100.77	100.8
6	Accuracy	100.47	100.14	100.14	98.79
7	Precision	98.88	99.50	98.79	100.7
8	LOD	2.23		2.22	
9	LOQ	6.76		6.75	
10	R^2	0.998		0.995	

4. CONCLUSION:

A study of the use of UV spectrophotometric in combination with PLS and PCR for the simultaneous determination of Atenolol in (AT) & Losartan Potassium (LP) in a binary mixture has been accomplished. The results obtained confirmed the suitability of the proposed method for simple, accurate and precise analysis of AT and LP in pharmaceutical preparations. The proposed methods do not need separation of AT and LP before analysis. In addition, the proposed methods can be applied for analysis of drugs in quality control lab as well as for in process quality control.

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