

## SIMULTANEOUS ESTIMATION OF LOSARTAN, HYDROCHLOROTHIAZIDE AND ATENOLOL FROM SOLID DOSAGE FORM BY RP-HPLC

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### ABSTRACT

**Objective:** The objective of the present work was to develop and validate a simple, fast, precise, selective and accurate RP-HPLC method for the simultaneous determination of Losartan, Hydrochlorothiazide and Atenolol from bulk and formulation.

**Methods:** The separation of these three drugs was achieved on a Hypersil Gold column (250 mm X 4.6 mm, 5  $\mu$ ) as stationary phase with a mobile phase consisting of methanol: water in the ratio of 95:5% v/v at a flow rate of 0.8 mL/min and UV detection at 225 nm.

**Results:** The retention times were observed to be 2.633, 3.883 and 7.783 minutes for Losartan, Hydrochlorothiazide and Atenolol, respectively. The method was statistically validated for linearity, recovery, limit of detection, limit of quantification, accuracy and precision.

**Conclusion:** The method was successfully applied for analysis of combined dose tablet formulation containing Losartan, Hydrochlorothiazide and Atenolol.

**Keywords:** Atenolol, hydrochlorothiazide, Losartan, reverse phase high performance liquid chromatography.

### INTRODUCTION

Losartan potassium (LOS) is an angiotensin II receptor antagonist and chemically it is 2-n-butyl-4-chloro-5-hydroxymethyl-1-[2'-(1H-tetrazol-5-yl) (biphenyl-4-yl) methyl] imidazole, a strong antihypertensive agent (Fig.1). Losartan was developed by DuPont-Merck laboratories as a potent non-peptide angiotensin II receptor (type AT<sub>1</sub>) antagonist for hypertension treatment [1]. It is administered in its active form and is partially converted into an active metabolite, which is responsible for the drug's prolonged

pharmacological effect. The therapeutic efficacy of losartan, as well as its renal and antihypertensive effects, seems to be similar to those of angiotensin converting enzyme (ACE) inhibitors. Hydrochlorothiazide (HCTZ) is chemically 6-chloro-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulfonamide 1,1-dioxide (Fig.2). It is the prototype of the thiazide group and antihypertensive drug [2].

Atenolol (ATN), 4-(2-hydroxy-3- [(1-methylethyl) amino] propoxy] benzeneacetamide, (Fig.3) is an antihypertensive, antianginal, and antiarrhythmic [3]. Atenolol is official in the IP, BP, and USP [4-6].

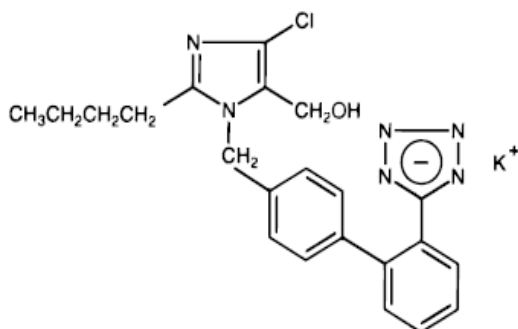


Fig. 1: Chemical structure of Losartan potassium

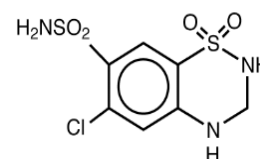


Fig. 2: Chemical structure of HCTZ

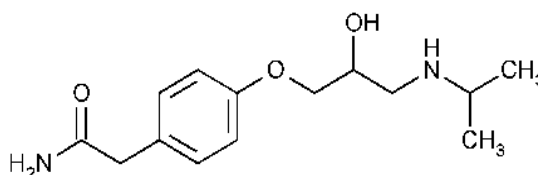


Fig. 3: Chemical structure of Atenolol

A literature survey revealed that spectrophotometric, chromatographic methods have been reported for determination of LOS [7-12], HCTZ [13-14] and ATN [15-20] in single and

multicomponent pharmaceutical formulations or from biological fluids. However, there were few HPLC methods for simultaneous estimation of LOS, HCTZ and ATN reported.

Analysis of LOS, HCTZ and ATN has been carried out by gradient HPLC with flow rate of 1.5 mL/min [21] while the proposed method employs isocratic elution and hence is simpler. Other methods reported for analysis of LOS, HCTZ and ATN required more buffer solution and pH adjustment [22]. The proposed method is relatively better or comparable in terms of sensitivity, accuracy, and precision to the methods reported for analysis of LOS, HCTZ and ATN. Rapid simultaneous estimation of LOS, HCTZ and ATN using a simple isocratic HPLC system with high sensitivity of estimation indicates easy application for analysis.

## MATERIALS AND METHODS

### Chemicals and Reagents

Losartan (LOS), Hydrochlorothiazide (HCTZ) and Atenolol (ATN) were kindly supplied by Emcure Pharmaceuticals Ltd, Pune, India. Marketed sample of LOS, HCTZ and ATN (Repalol-H) in their

combined tablet dosage form. Each tablet contained 50 mg of LOS, 12.5 mg of HCTZ and 50 mg of ATN. For HPLC work double distilled water was prepared in the laboratory. Methanol used was of HPLC grade and were purchased from Merck Chemicals, Mumbai, India.

### Instrumentation

The HPLC system consisted of Intelligent HPLC pump model (Jasco PU 1580). The solutions were injected into the chromatograph through a Rheodyne valve, with a 20  $\mu$ L loop with auto sampler (AS 1555). The detector consisted of a UV/ VIS (Jasco UV 1575). Data was integrated using Jasco Borwin version 1.5, LC-Net II/ADC system. Hypersil Gold C18 column (250 mm  $\times$  4.6 mm i.d., 5  $\mu$ ) as a stationary phase and methanol: water (95:5% v/v) as mobile phase was used. The mobile phase flow rate was 0.8 mL/min a detection wavelength of 225 nm was selected for analysis (Fig.4). An ultrasonic bath was used to remove the air from the mobile phases, operating at ambient temperature.

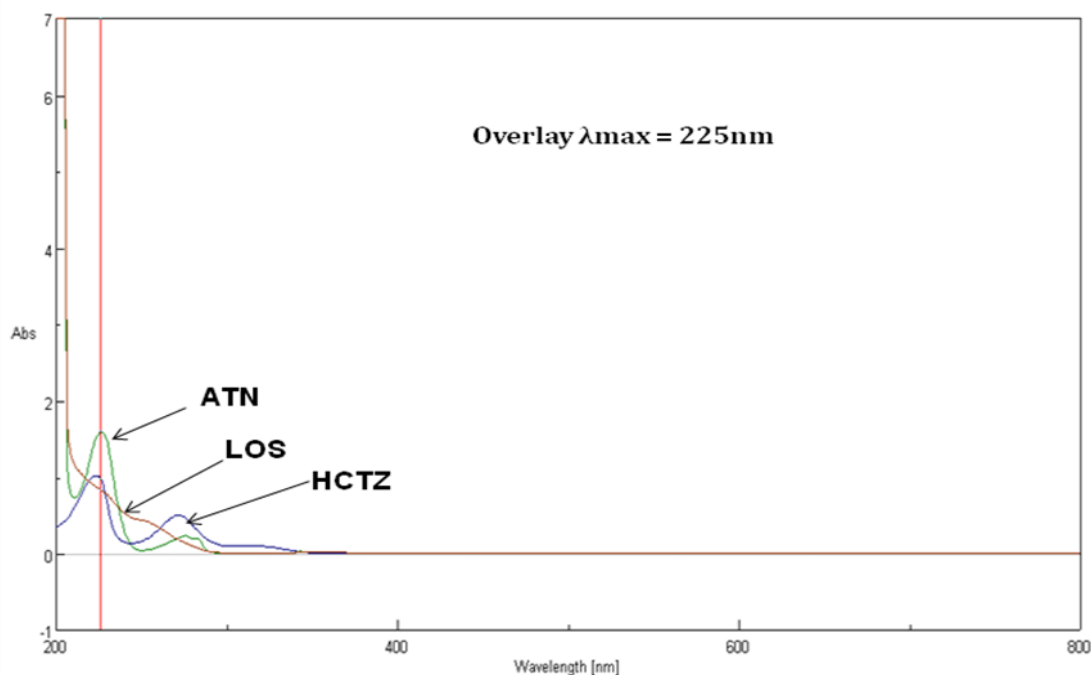


Fig. 4: Overlay Spectrum for LOS, HCTZ and ATN ( $\lambda_{\text{max}} = 225\text{nm}$ )

### Standard Stock Solutions and Sample solution

Accurately weighed LOS (50 mg), HCTZ (12.5 mg) and ATN (50 mg) were transferred to 25 mL volumetric flask and dissolved in, and then diluted to the mark with methanol. The stock solution was further diluted with methanol to obtain a solution of LOS (2  $\mu$ g/ml), HCTZ (0.5  $\mu$ g/ml) and ATN (2  $\mu$ g/ml), respectively.

To determine the content of LOS, HCTZ and ATN simultaneously in tablets (label claim: LOS (50 mg), HCTZ (12.5 mg) and ATN (50 mg) per tablet), twenty tablets were weighed, their mean weight determined and they were finely powdered and powder equivalent to 50 mg of LOS, 12.5 mg of HCTZ, and 50 mg of ATN was weighed. Then equivalent weight of the drug was transferred into a 25 mL volumetric flask containing 10 mL methanol, sonicated for 10 min and diluted to 25 mL with methanol to obtain solution of LOS (2 mg/ml), HCTZ (0.5 mg/ml) and ATN (2 mg/ml), respectively. The mixture was filtered using whatmann filter. The solution was further diluted with methanol to obtain a solution of LOS (2  $\mu$ g/ml), HCTZ (0.5  $\mu$ g/ml) and ATN (2  $\mu$ g/ml), respectively.

### Validation of the method

Validation was done as per ICH guideline Q2 (R1) [23]. The developed method was validated with respect to parameters

such as linearity, LOD and LOQ, precision, accuracy and specificity

### System suitability

The system suitability of the HPLC method was determined by making six replicate injections from freshly prepared standard solutions and analyzing each solute for their resolution ( $R_s$ ), retention time, theoretical plates number ( $N$ ) and tailing factors ( $T$ ).

### Specificity:

The specificity of the method was ascertained by analysis of drug standards and samples. The mobile phase resolved all the drugs very efficiently, as shown in Fig. 5. The identities of the peak for LOS, HCTZ and ATN were confirmed by comparing the  $R_t$  with those of standards.

### Linearity

Linearity is generally evaluated by visual inspection of a plot of signals as a function of analyte concentration or content. For determining linearity, calibration curves were plotted over a concentration range of 2–12  $\mu$ g/mL for LOS, 0.5–3  $\mu$ g/mL for HCTZ and 2–12  $\mu$ g/mL for ATN, respectively. A 20  $\mu$ L of sample solution was injected into the chromatographic system using fixed volume

loop injector. Chromatograms were recorded. All measurements were repeated three times for each concentration and calibration

curve was constructed by plotting the peak areas of analyte versus the corresponding drug concentration.

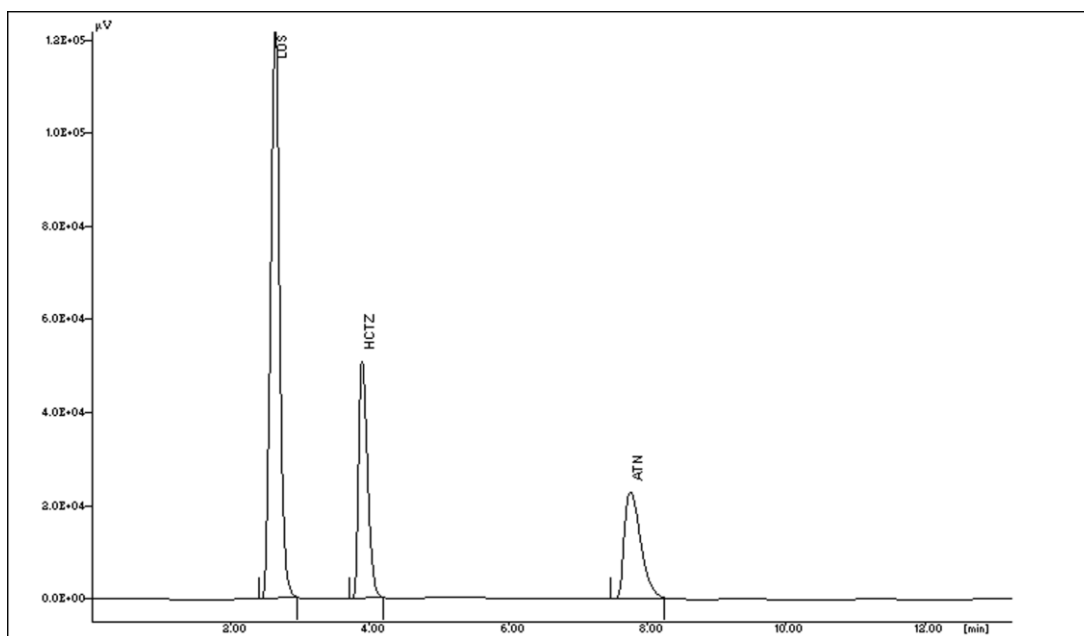


Fig. 5: Chromatogram of LOS, HCTZ and ATN

#### Limit of detection and limit of quantitation

The LOD and LOQ were calculated according to the  $3.3\sigma/s$  and  $10\sigma/s$  criteria, respectively; where  $\sigma$  is the standard deviation of the peak area and  $s$  is the slope of the corresponding calibration curve.

#### Precision

The precision of the proposed method was assessed as repeatability and intermediate precision by preparing three different sample solutions at low, medium and high concentrations, which were freshly prepared and analyzed daily. These experiments were repeated 3 different days over a period of a week to evaluate day-to-day variability (intermediate precision).

#### Accuracy

To check the accuracy of the developed method and to study the interference of formulation additives, analytical recovery experiments were carried out by standard addition method, at 80, 100 and 120% level. The experiment was conducted in triplicate. Percentage recovery and relative standard deviation were calculated.

#### Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

### RESULTS AND DISCUSSION

#### Method development

The HPLC procedure was optimized for simultaneous determination of LOS, HCTZ and ATN. Good resolution of both the components was obtained with methanol: water at ratio 95: 5 v/v. The flow rate of 0.8 mL/min was optimum. UV detection was made at 225 nm. At this wavelength LOS, HCTZ and ATN can be quantified. Hence, 225 nm determined empirically has been found to be optimum. The average retention times for LOS, HCTZ and ATN was found to be 2.633, 3.883 and 7.783 min, respectively.

#### System suitability

To ascertain its effectiveness, system suitability tests were carried out on freshly prepared stock solutions. The parameters obtained are shown in Table 1.

Table 1: System Suitability Parameters of RP-HPLC for Tablet Analysis

Parameter	LOS	HCTZ	ATN
Resolution (Rs)	0.00	6.508	14.485
Retention Time in min	2.633	3.883	7.783
Theoretical plates number (N)	2989.38	6236.65	8684.15
Tailing Factor	1.175	1.420	1.247

#### Linearity

Linear regression data for the calibration plots revealed good linear relationships between response and concentration over the ranges 2–12  $\mu\text{g/mL}$  for LOS, 0.5–3  $\mu\text{g/mL}$  for HCTZ and 2–12  $\mu\text{g/mL}$  for ATN, respectively. The linear regression equations were  $Y = 52287X + 120009$  ( $r^2 = 0.9993$ ) for LOS,  $Y = 126353X + 6931$  ( $r^2 = 0.9992$ ) for HCTZ and  $Y = 28808X + 18283$  ( $r^2 = 0.9996$ ). The plots obtained from linear regression and residuals analysis are given in Fig 6a and 6b for LOS, 7a and 7b for HCTZ and 8a and 8b for ATN, respectively.

#### Limits of Detection and Quantitation

The limits of detection and quantitation, calculated as described above, were 0.6  $\mu\text{g/mL}$  and 1.8  $\mu\text{g/mL}$  respectively, for LOS, 0.2  $\mu\text{g/mL}$  and 0.5  $\mu\text{g/mL}$  for HCTZ and 0.8  $\mu\text{g/mL}$  and 2  $\mu\text{g/mL}$  for ATN. This indicates the method is sufficiently sensitive.

#### Precision

The precision of the method was expressed as relative standard deviation (RSD, %). The results listed in Table 2 reveal the high precision of the method.

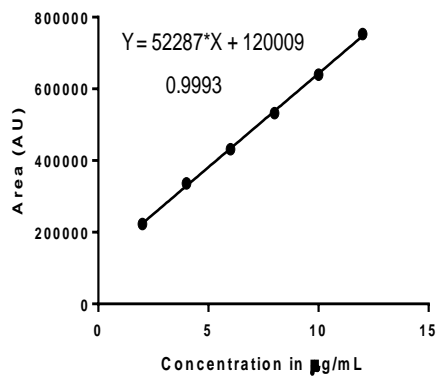


Fig. 6a: linear regression for Losartan

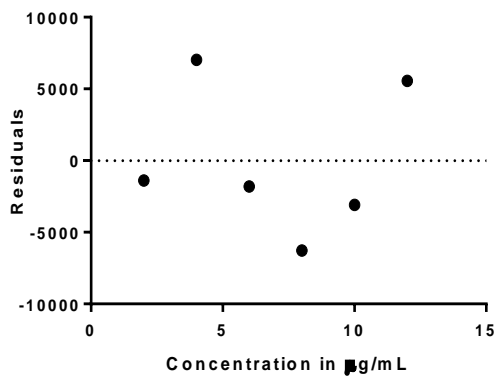


Fig. 6b: Residuals plot for Losartan

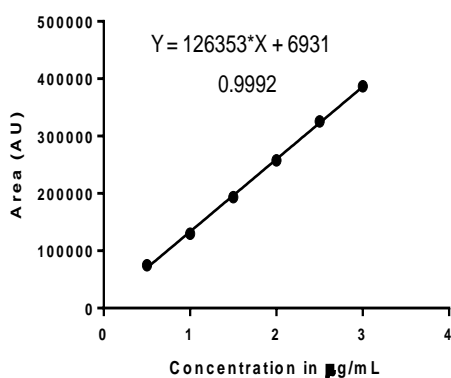


Fig. 7a: linear regression for HCTZ

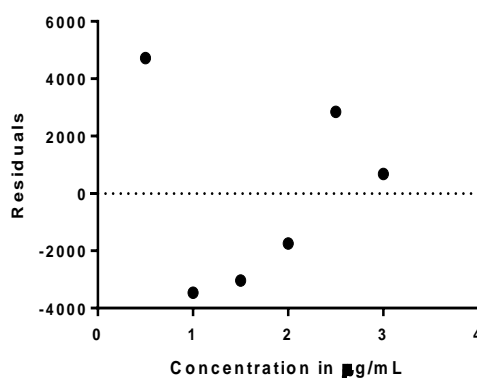


Fig. 7b: Residuals plot for HCTZ

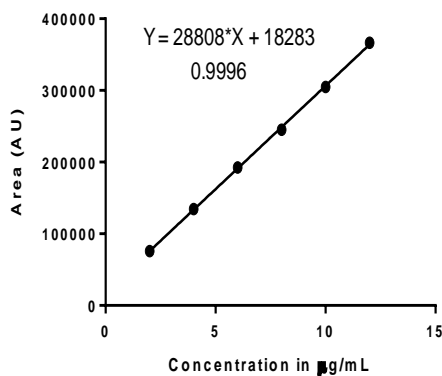


Fig. 8a: linear regression for ATN

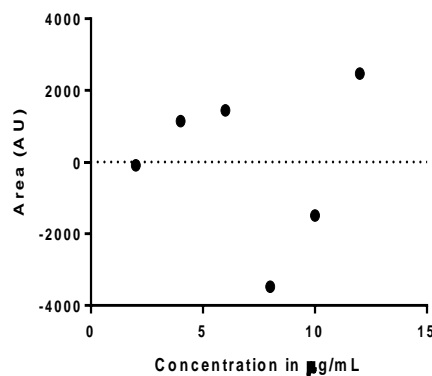


Fig. 8b: Residuals plot for ATN

**Accuracy**

The difference between theoretical added amount and practically achieved amount is called accuracy of analytical method. Accuracy was determined at three levels 80%, 100% and 120% of the target concentration in triplicate. The results are presented in Table 3.

**Robustness**

The standard deviation of peak the areas was calculated for each parameter and the % RSD was found to be less than 2 %. The low

values of the % RSD, as shown in Table 4 indicated robustness of the method.

**Sample Analysis**

When the Repalol-H tablets were analysed, sharp and well defined peaks for LOS, HCTZ and ATN were obtained at Rt 2.633, 3.883 and 7.783min, respectively, when scanned at 225 nm. The amount of the label claim measured were 100.35 ± 0.73% for LOS, 99.80 ± 1.16% for HCTZ and 99.16 ± 1.22% for ATN.

Table 2: Precision studies of proposed HPLC method

Concentration ( µg/mL)	Intra-day precision			Inter-day precision		
	Measured Conc. ± SD	(%) RSD	Recovery <sup>a</sup> (%)	Measured Conc. ±SD	(%) RSD	Recovery <sup>a</sup> (%)
<b>Losartan</b>						
4	3.96 ± 0.042	1.06	99.00	3.94 ± 0.043	1.09	98.50
8	7.94 ± 0.087	1.096	99.25	7.92 ± 0.089	1.12	99.00
12	11.96 ± 0.122	1.02	99.66	11.93 ± 0.13	1.09	99.42
<b>Hydrochlorothiazide</b>						
1	0.99 ± 0.011	1.11	99.00	0.98 ± 0.011	1.12	98.00
2	1.97 ± 0.022	1.12	98.50	1.96 ± 0.023	1.17	98.00
3	2.97 ± 0.032	1.08	99.00	2.96 ± 0.036	1.21	98.66
<b>Atenolol</b>						
4	3.98 ± 0.043	1.08	99.50	3.96 ± 0.047	1.19	99.00
8	7.97 ± 0.089	1.12	99.63	7.95 ± 0.096	1.21	99.38
12	11.95 ± 0.14	1.17	99.58	11.92 ± 0.137	1.15	99.33

<sup>a</sup>Mean from three analyses

Table 3: Standard addition techniques for determination of LOS, HCTZ and ATN

Drug	Label claim (mg/tablet)	Amount Added (%)	Total amount (mg)	Amount recovered (mg)	Recovery (%)
LOS	50	80	90	89.55	99.50
		100	100	99.79	99.79
		120	110	109.32	99.38
HCTZ	12.5	80	22.5	22.35	99.33
		100	25	24.93	99.72
		120	27.5	27.35	99.45
ATN	50	80	90	89.42	99.36
		100	100	99.56	99.56
		120	110	109.20	99.27

(n= 3)

Table 4: Robustness evaluation of LOS, HCTZ and ATN

Chromatographic factors	Level	Chromatographic changes in t <sub>R</sub> <sup>a</sup>		
		LOS	HCTZ	ATN
<b>A: Flow rate mL/min.</b>				
0.7	-0.1	2.662	3.892	7.800
0.8	0.0	2.633	3.883	7.783
0.9	+0.1	2.601	3.867	7.750
Mean ± SD		2.632 ± 0.0305	3.883 ± 0.0155	7.778 ± 0.0254
<b>B: % of methanol in the mobile phase (± 5%)</b>				
90	-5.0	2.665	3.897	7.808
95	0.0	2.633	3.883	7.783
100	+5.0	2.600	3.850	7.715
Mean ± SD		2.633 ± 0.0325	3.877 ± 0.0241	7.769 ± 0.048

<sup>a</sup>Mean from three estimates

## CONCLUSION

A simple isocratic RP-HPLC method was developed and validated for the simultaneous quantitative assay of Losartan, Hydrochlorothiazide and Atenolol in tablet dosage form. The validation results reveal that, method is precise, linear, robust and accurate, which proves the reliability of the proposed method. The short runtime and low solvent consumption are advantageous for applying routine quality control analysis.

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