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Research Article

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING REVERSE PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR SIMULTANEOUS ESTIMATION OF ATENOLOL, HYDROCHLOROTHIAZIDE AND LOSARTAN IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

Objective: The objective of present work was to develop and validate a simple, fast, precise, selective and accurate reverse phase high-performance liquid chromatography method for the simultaneous determination atenolol (ATN), hydrochlorothiazide (HCTZ) and losartan (LOS) in a pharmaceutical dosage form.

Methods: The separation of these three drugs was achieved on an SHISHEDO C_{18}, 250 mm × 4.6 mm, 5 µ size column with a mobile phase consisting of acetonitrile and 0.5% orthophosphoric acid (30:70 v/v) at a flow rate of 1 ml/minute and UV detection at 224 nm.

Results: The retention times were observed to be 2.242, 3.963 and 6.733 minutes for ATN, HCTZ and LOS, respectively. Linearity was found to be 4-12 µg/ml, 4-12 µg/ml, 1-3 µg/ml for ATN, HCTZ and LOS, respectively. The method was statistically validated for linearity, recovery, the limit of detection (LOD), limit of quantification (LOQ), accuracy and precision. The stress testing of the drugs individually and their mixture is carried out under acidic, alkaline, oxidation, photo-stability and thermal degradation conditions and its degradation products is well-resolved from the analyte peaks.

Conclusion: This method was successfully validated for accuracy, precision, and linearity, LOD and LOQ.

Keywords: Atenolol, Hydrochlorothiazide, Losartan, Reverse phase high-performance liquid chromatography.

INTRODUCTION

Atenolol (ATN), 4-(2-hydroxy-3-[(1-methylethyl) amino] propoxy] benzeneacetamide, (Fig. 1) is an antihypertensive, antianginal, and antiarrhythmic [1].

Hydrochlorothiazide (HCTZ) is chemically 6-chloro-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulfonamide1,1-dioxide (Fig. 2). It is the prototype of the thiazide group and antihypertensive drug [2]. Losartan (LOS) potassium 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, (Fig. 3), a strong antihypertensive agent [3].

Detailed literature survey revealed analytical methods such as spectrophotometric [4-6], high-performance thin-layer chromatographic [7-10] are available for the estimation of these drugs individually or in combination with other drugs. However, very few reverse phase high-performance liquid chromatography (RP-HPLC) methods [11-26] are available for the simultaneous estimation of these drugs. Hence, we tried to develop simple RP-HPLC methods for the simultaneous estimation of these drugs.

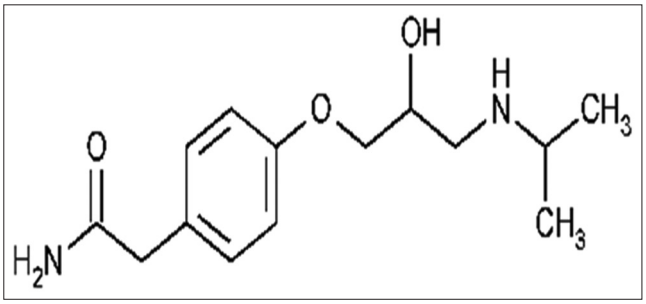

Fig. 1: Chemical structure of atenolol

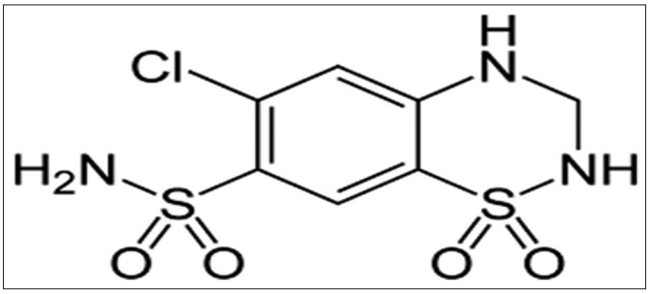

Fig. 2: Chemical structure of hydrochlorothiazide

METHODS

Chemicals and reagents

ATN, HCTZ and LOS working standards were procured from Dr. Reddys Laboratories Ltd., Hyderabad. Commercially, available Repolol-H tablets were purchased from the local pharmacy. HPLC grade acetonitrile and methanol were purchased from Merck Specialities Pvt. Ltd., Mumbai. HPLC grade water was purchased from Thermo Fisher Scientifics Ltd., Mumbai. Orthophosphoric acid, hydrochloric acid, sodium hydroxide pellets purified and hydrogen peroxide 30% of AR grade were procured from Merck Specialties Pvt. Ltd., Mumbai.

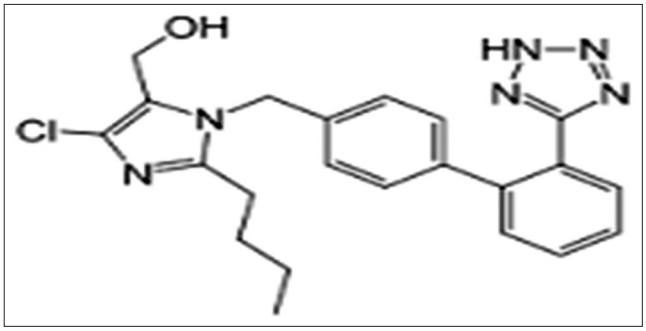

Fig. 3: Chemical structure of losartan

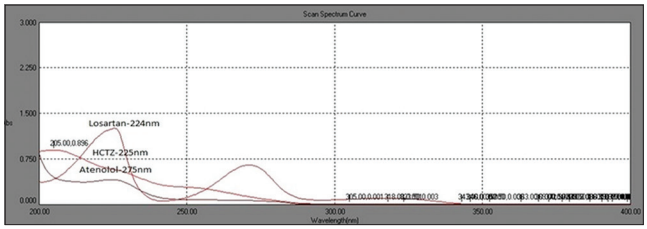

Fig. 4: Overlay spectrum of atenolol, hydrochlorothiazide and losartan

Instrumentation and analytical conditions

RP-HPLC method was performed on the HPLC system (Shimadzu) consisting of binary gradient pump and UV detector (LC-20AD) was employed for analysis and rheodyne injector with 20 µl fixed loop was used for this study (Fig. 4).

Preparation of solutions

Preparation of standard solutions

Standard stock solution of ATN, HCTZ and LOS were prepared by transferring accurately weighed ATN (10 mg), HCTZ (10 mg) and LOS (10 mg) to a 10 ml volumetric flask separately, dissolved and diluted to a mark with the solvent consisting of acetinitrile: water in the ratio of 50:50 v/v, to obtain a standard solution of ATN (1000 µg/ml) HCTZ (1000 µg/ml) and LOS (1000 µg/ml). From these solutions, standard stock solutions were prepared in a 10 ml volumetric flasks and made up to the volume with mobile phase (Orthophosphoric acid:acetonitrile 50:50 v/v) to get the concentration of 100 µg/ml of ATN, 100 µg/ml of HCTZ and 100 µg/ml of LOS.

Preparation of the sample solutions

About 20 tablets were taken and their average weight was calculated, tablets were crushed to fine powder and dose equivalent to 10 mg of ATN, HCTZ and LOS were taken into 10 ml volumetric flask and diluted up to the mark with the solvent consisting of acetonitrile: water in the ratio of 50:50 v/v, to obtain a concentration of 1000 µg/ml of ATN, HCTZ, LOS. 1 ml of the above solution were taken in a 10 ml volumetric flasks and diluted to 10 ml with mobile phase (0.5% orthophosphoric acid: acetonitrile, 50:50 v/v) to obtain a concentration of 100 µg/ml of ATN, HCTZ and LOS. From 100 µg/ml concentrations of LOS pipette out 1 ml into 10 ml volumetric flask and makeup to the final volume using mobile phase to attain a concentration of 10 µg/ml. From 100 µg/ml solution of ATN, HCTZ pipette out 0.8 ml of ATN, 0.8 ml of HCTZ and from 10 µg/ml of LOS pipette out 2 ml into 10 ml volumetric flask and make up to the final volume with mobile phase to attain concentration of 8 µg/ml of ATN, 8 µg/ml of HCTZ and 2 µg/ml of LOS.

Optimized analytical methods

Shiseido C18 column (250 mm × 4.6 mm I.D) was used as stationary phase. ATN, HCTZ, and LOS were eluted isocratically with a flow rate of 1.0 ml/minute using a mobile phase consisting of 0.5% orthophosphoric acid in water and acetonitrile in a proportion of 70:30 v/v, respectively. The wavelength of the UV detector was set at 224 nm. The prepared mobile phase was filtered through 0.45 µm membrane filter (Millipore) and sonicated before use.

Method validation

The developed method was validated according to International Conference on Harmonization guidelines for validation of analytical procedures. Validation was done as per ICH guidelines Q2 (R1). The developed method was validated with respect to parameters such as linearity, Limit of detection (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 retention time, theoretical plates number (N) and tailing factors (T).

Specificity

It is the ability to assess unequivocally the analyte in the presence of impurities, degradation, and matrix. To determine this 20 µl of blank, standard and placebo solutions were injected separately in triplicate and respective chromatograms were recorded under the optimized conditions.

Linearity

The calibration curves were generated with concentrations of the standard solutions of 4-12 µg/ml, 4-12 µg/ml and 1-3 µg/ml for ATN, HCTZ and LOS respectively. Linearity was assessed by regression analysis, which was calculated by the least square regression method.

Accuracy

To check the degree of accuracy of recovery studies were performed in triplicate by the standard addition method at 50%, 100%, and 150% levels.

Precision

The precision was verified by analyzing the samples at different time intervals of the same day (intra-day precision) as well as on different days (inter-day precision).

LOD and limit of quantification (LOQ)

LOD and LOQ were calculated by using the values of slopes and intercepts of the calibration curves for three drugs.

Robustness

Robustness was established by analysis of samples under deliberately changed chromatographic conditions. The flow rate of the mobile phase was changed from 0.9 ml/minute to 1.0 ml/minute and 1.1 ml/minute. The ratio of the organic phase was modified by 2%, i.e. 28%, 30%, 32%. The effect of retention time and peak parameter was studied.

Assay of pharmaceutical dosage form

20 µl of each standard and sample solution were injected and from the peak areas of ATN, HCTZ and LOS amount of each drug in samples was computed.

Stability studies

Degradation studies were conducted in sample solutions containing 8 µg/ml of ATN, 8 µg/ml of HCTZ and 2 µg/ml of LOS.

Acidic degradation

1 ml of 0.1 M HCL were added individually to the final drug solution in different volumetric flasks, and they were refluxed for 1 hr at 60°C. After 1 hr, these solutions were injected under optimized chromatographic conditions.

Alkaline degradation

1 ml of 0.1 M NaOH were added individually to the final drug solution in different volumetric flasks and they were refluxed for 1 hr at 60°C. After 1 hr, these solutions were injected under optimized chromatographic

conditions.

Oxidative degradation
1 ml of 3% H_2O_2 were added individually to the final drug solution in different volumetric flasks and they were refluxed for 1 hr at 60°C. After 1 hr, these solutions were injected under optimized chromatographic conditions.

Photolytic degradation
The final drug solution was maintained at room temperature and exposed to sunlight for 8 hrs. After 8 hrs, these solutions were injected under optimized chromatographic conditions.

Thermal degradation
The final drug solution was maintained at a temperature of 60°C for 6 hrs. After 6 hrs, this solution was injected under optimized chromatographic conditions.

RESULTS AND DISCUSSION

Method development
The HPLC procedure was optimized for simultaneous determination of ATN, HCTZ and LOS. Good resolution of both the components was obtained with 0.5% orthophosphoric acid: Acetonitrile at ratio 70: 30 v/v. The flow rate of 1 ml/minute was optimum. UV detection was made at 224 nm. At this wavelength ATN, HCTZ, and LOS can be quantified. Hence, 224 nm determined empirically has been found to be optimum. The average retention times for ATN, HCTZ and LOS were found to be 2.242, 3.963, and 6.733 minutes, respectively.

System suitability
20 µl of working standard solution containing 8 µg/ml of ATN, 8 µg/ml of HCTZ and 2 µg/ml of LOS was prepared and injected into the system under optimized chromatographic conditions. Chromatograms were recorded and studied for discrete system suitability parameters such as tailing factor, theoretical plates, resolution and peak area, peak heights were also studies. Six different working standard solutions were injected to study this parameter, and all the suitability parameters were considered to be within the limits. The system suitability parameters were given in Table 1.

Specificity
The HPLC chromatograms were recorded for blank and the sample under optimized analytical conditions, compared them with that of standard solution and found no additional peaks. The two peaks were completely separated in HPLC chromatogram, and the resolution was found to be more than 2. Even in the presence of excipients of the sample no interfering peaks were considered in HPLC chromatogram (Fig. 5).

Linearity
For HPLC method, the calibration curves of ATN, HCTZ and LOS were constructed in the concentration range of 4-12 µg/ml, 4-12 µg/ml and 1-3 µg/ml of ATN, HCTZ and LOS respectively. The plots obtained from linear regression and residuals analysis are given below. The linearity parameters were presented in Table 2 (Figs. 6-8).

Table 1: System suitability parameters of ATN, HCTZ and LOS

Parameter	ATN*	HCTZ*	LOS*
Retention time	2.242	3.963	6.733
Number of theoretical plates	3538.5	6069.3	11689.17
Tailing factor	1.22	1.23	1.18

*Average of six readings. ATN: Atenolol, HCTZ: Hydrochlorothiazide, LOS: Losartan

Accuracy
The accuracy of the proposed method was determined, recovery studies were performed in mentioned levels and recorded (Table 3), obtained results was found to be within the limits of 98-102%, indicating an agreement between the true value and found value.

Precision
Precision was calculated as intra-day and inter-day variations for the drugs. Percent relative standard deviations for estimation of ATN, HCTZ

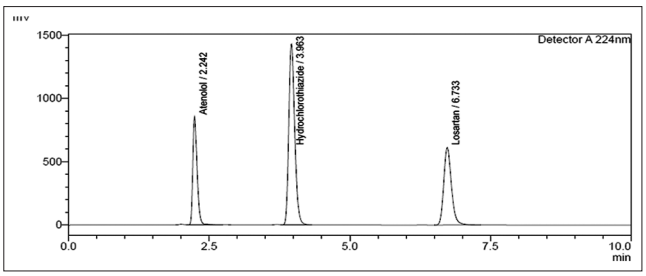

Fig. 5: Chromatogram of well-resolved peaks of atenolol, hydrochlorothiazide and losartan

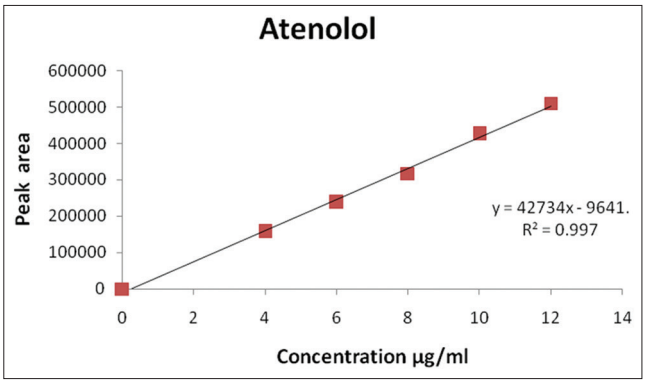

Fig. 6: Linearity graph of atenolol

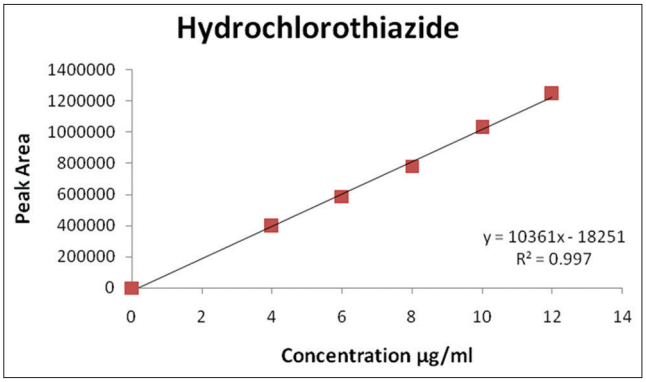

Fig. 7: Linearity graph of hydrochlorothiazide

Table 2: Linearity of ATN, HCTZ and LOS

Parameter	ATN	HCTZ	LOS
Regression equation	Y=42734x-9641.4	Y=103614x-18251	Y=56951x-2887.1
Linearity (µg/ml)	4-12 µg/ml	4-12 µg/ml	1-3 µg/ml
Correlation coefficient (R^2)	0.9970	0.9979	0.9966

ATN: Atenolol, HCTZ: Hydrochlorothiazide, LOS: Losartan

and LOS under intra- and inter-day variations were found to be less than 2 (Table 4).

LOD and LOQ

The LOD and LOQ were calculated according to the 3.3 σ/s and 10 σ/s criteria, respectively; where σ is the standard deviation of the peak area and s is the slope of the corresponding calibration curve. The LOD and LOQ parameters were presented in Table 5.

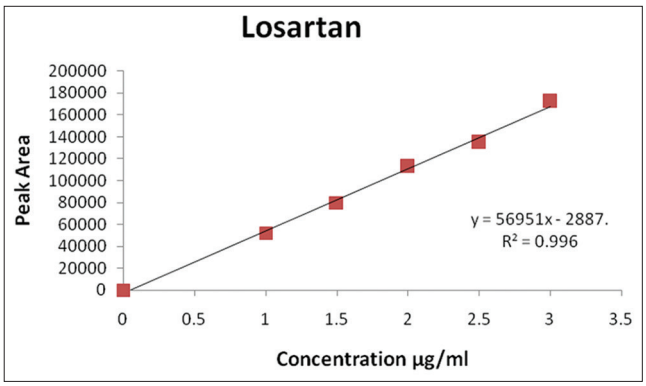

Fig. 8: Linearity graph of losartan

Table 3: Accuracy of ATN, HCTZ and LOS

Drug	Recovery			%RSD*		
	50%	100%	150%	50%	100%	150%
ATN	99.78*	99.92*	99.86*	1.215	1.313	0.996
HCTZ	98.60*	99.63*	101.11*	1.898	1.495	1.036
LOS	99.70*	99.86*	99.78*	0.645	1.198	1.610

*Average of three readings. %RSD: % Relative standard deviation, ATN: Atenolol, HCTZ: Hydrochlorothiazide, LOS: Losartan

Table 4: Precision of ATN, HCTZ and LOS

Drug	Concentration (µg/ml)	Intra-day (%RSD)*	Inter-day (%RSD)*
ATN	4	0.25	1.17
	8	1.08	0.45
	12	0.89	0.85
HCTZ	4	0.82	0.15
	8	0.46	0.45
	12	0.82	0.95
LOS	1	1.18	0.75
	2	1.80	0.51
	3	0.18	0.46

*Average of three readings. % RSD: % Relative standard deviation, ATN: Atenolol, HCTZ: Hydrochlorothiazide, LOS: Losartan

Table 5: LOD and LOQ of ATN, HCTZ and LOS

Drug	LOD (µg/ml)	LOQ (µg/ml)
ATN	0.1	0.5
HCTZ	0.05	0.15
LOS	0.04	0.12

ATN: Atenolol, HCTZ: Hydrochlorothiazide, LOS: Losartan

Robustness

For robustness studies, conditions like flow rate and concentration of the organic phase were changed, and method was performed. In all deliberately varied conditions, percent relative standard deviations for peak areas, retention times, theoretical plates and tailing factor were considered to be less than 2% (Table 6).

Assay

Percent of assay was calculated using absorbance's using peak areas of standard and sample. The experimental values obtained for the determination of ATN, HCTZ and LOS in the pharmaceutical formulation were within the claimed limits (Table 7).

Stability studies

The following degradation results were found when ATN, HCTZ and LOS were subjected to:

Acidic degradation

ATN showed good stability in acidic conditions compared to HCTZ and LOS. HCTZ showed more degradation in the 3rd day compared with 1st and 2nd day. Chromatogram of the acidic condition can be seen in Fig. 9 for ATN, HCTZ and LOS.

Alkaline degradation

ATN showed more degradation in basic conditions than HCTZ and LOS. ATN showed more degradation on the 3rd day. Chromatogram of the basic condition can be seen in Fig. 9 for ATN, HCTZ and LOS.

Oxidative degradation

All the three drugs showed good stability in Oxidative condition. Chromatogram of oxidative condition can be seen in Fig. 9 for ATN, HCTZ and LOS.

Photolytic degradation

All the three drugs showed good stability under photolytic conditions with very less degradation. LOS showed more degradation in the 5th day compared to ATN, HCTZ. Chromatogram of photolytic degradation can be seen in Fig. 9 for ATN, HCTZ and LOS.

Thermal degradation

All the three drugs showed good stability under thermal conditions with very less degradation. Chromatogram of the thermal condition can be observed in Fig. 9 for ATN, HCTZ and LOS.

The percent amount of drug degraded after degradation studies and the Rt of degradation products are given in (Table 8). The pattern of degradation of the drugs individually in all the conditions and in different days was well portrayed in the Figs. 10-12. In the proposed HPLC method, different proportions of acetonitrile and orhophosphoric acid (OPA) were tried for selection of the mobile phase. Ultimately, 0.5% OPA in water and acetonitrile in a proportion of 70:30 v/v, respectively, was finalized as

Table 6: Robustness parameters of ATN, HCTZ and LOS

Serial number	Parameter	ATN Rt* (minute)	HCTZ Rt* (minute)	LOS Rt* (minute)
1	Initial flow	2.242	3.963	6.733
2	Flow 0.9 ml/minutes	2.233	3.957	6.979
3	Flow 1.1 ml/minutes	2.221	3.955	6.978
4	Initial oraganic phase conc	2.242	3.963	6.733
5	Organic phase, 2% less	2.191	3.806	7.099
6	Organic phase, 2% more	2.217	3.934	6.268

*Average of three readings. ATN: Atenolol, HCTZ: Hydrochlorothiazide, LOS: Losartan

the mobile phase. The elution order was ATN (Rt=2.242 minutes), HCTZ (Rt=3.963) and LOS (Rt=6.733 minutes), at a flow rate of 1.0 ml/minute.

Table 7: Assay data of marketed formulation

Drug	Amount labeled (mg)	Amount found*	% Assay*
ATN	50	49.02	98.04
HCTZ	50	49.48	98.96
LOS	12.5	12.39	99.12

*Average of three readings. ATN: Atenolol, HCTZ: Hydrochlorothiazide, LOS: Losartan

The chromatogram was recorded at 224 nm. The developed method was validated as per ICH guidelines. Parameters like precision, accuracy, specificity, ruggedness, robustness were done and found to be within the acceptance criteria. LOD and LOQ were determined and the developed method was applied for the determination of assay of REPOLOL-H tablets. The stability of the drugs was examined under different stress conditions such as acidic, basic, peroxide, thermal and under UV conditions.

CONCLUSION

The proposed RP-HPLC were developed and validated as per ICH guidelines. The standard deviation and % RSD calculated for the

Table 8: Degradation data of ATN, HCTZ and LOS in different conditions

Day	Condition	Mean±SD*		
		% Degradation of ATN	% Degradation of HCTZ	% Degradation of LOS
1st day	Acid	10.17±2.09	8.59±2.08	16.46±14.3
	Base	12.34±2.25	2.77±1.77	2.58±1.03
	Oxidative	2.65±1.78	2.58±1.12	1.64±0.70
	Photolytic	5.82±2.05	7.25±2.10	8.57±2.22
	Thermal	2.95±1.81	16.65±1.98	14.5±2.20
3rd day	Acid	16.1±2.60	21.53±1.90	29.75±2.55
	Base	24.55±2.19	10.36±1.94	14.76±2.11
	Oxidative	10.93±2.72	4.20±2.25	2.79±1.44
	Photolytic	8.68±2.50	9.83±1.90	10.2±1.45
	Thermal	4.30±2.005	29.74±1.79	17.46±2.25
5th day	Acid	43.22±2.44	48.22±2.61	32.57±1.85
	Base	40.69±2.16	14.50±1.74	16.63±2.20
	Oxidative	33.35±2.47	5.45±2.24	3.59±1.87
	Photolytic	11.21±2.65	10.25±2.21	12.82±2.62
	Thermal	6.52±1.92	32.46±1.88	32.66±2.05

*Average of three determinations (each condition), SD: Standard deviation, ATN: Atenolol, HCTZ: Hydrochlorothiazide, LOS: Losartan

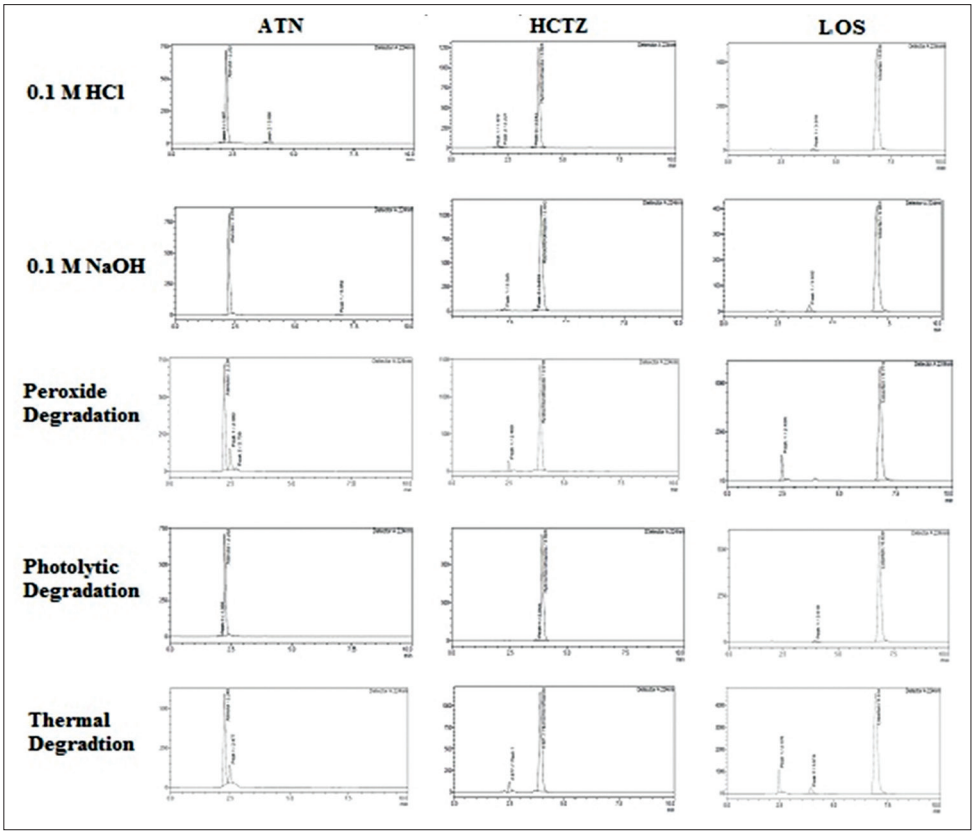

Fig. 9: Chromatograms of atenolol, hydrochlorothiazide and losartan in different stress conditions

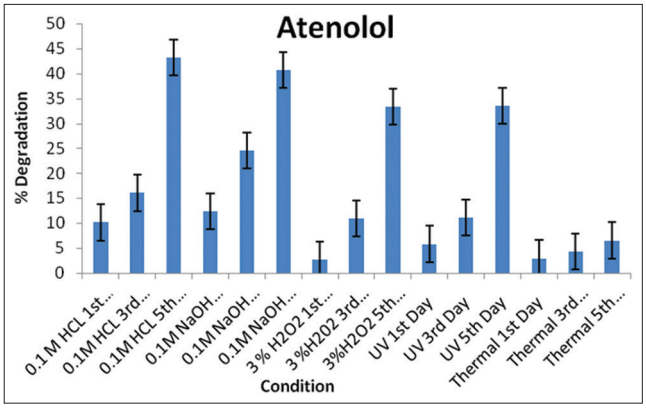

Fig. 10: Degradation pattern of atenolol in different stress conditions

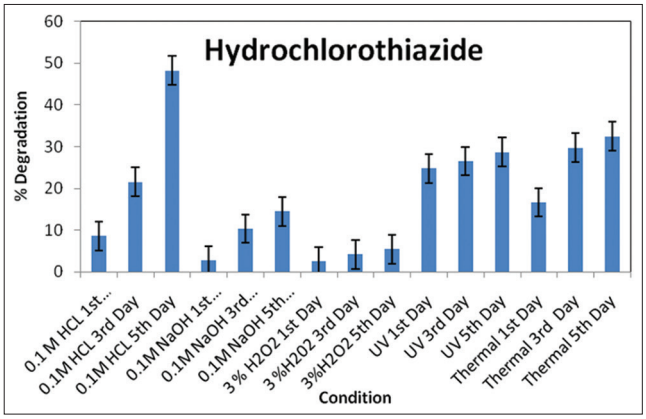

Fig. 11: Degradation pattern of hydrochlorothiazide in different stress conditions

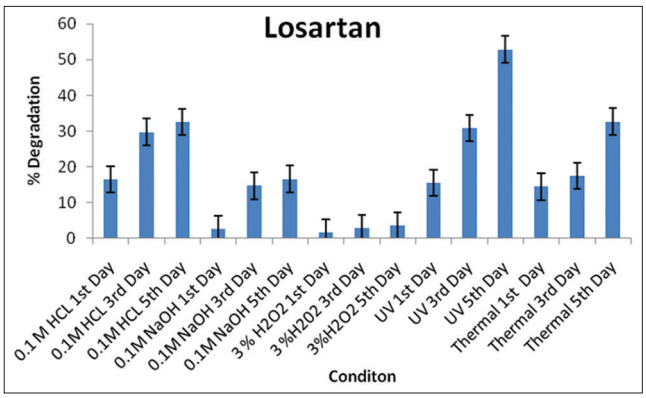

Fig. 12: Degradation pattern of losartan in different stress conditions

proposed methods are subtle, indicating a high degree of precision of the methods. The results of the recovery studies performed show the high degree of accuracy of the proposed methods. The RP-HPLC method could selectively quantify ATN, HCTZ, and LOS in the presence of its degradation products hence; it can be employed as a stability indicating method. From the found experimental data it can be concluded that the developed stability indicating chromatographic methods are accurate, precise and selective and can be employed successfully for the estimation of ATN, HCTZ and LOS in pharmaceutical dosage form.

REFERENCES

1. Data Base of Atenolol, Compilation Prepared by Drug Bank. Available from: http://www.Drug bank.ca/drugs/DB00335.
2. Data Base of Hydrochlorothiazide, Compilation Prepared by Drug Bank. Available from: http://www.Drug bank.ca/drugs/DB00999.
3. Data Base of Losartan, Compilation Prepared by Drug Bank. Available from: http://www.Drugbank.ca/drugs/db00678.
4. Erk N. Application of first derivative UV-spectrophotometry and ratio derivatives spectrophotometry for simultaneous determination of candesartan cilexetil and hydrochlorothiazide. Pharmazie 2003;58:796-800.
5. Bhatia NM, Desai RB, Jadhav SD. Simultaneous estimation of losartan potassium and hydrochlorothiazide from tablets by first order derivative spectroscopy. Int J Pharm Pharm Sci 2013;5(1):464-6.
6. Bari S, Sathe S, Jain P, Surana S. Spectrophotometric method for simultaneous estimation of atenolol in combination with losartan potassium and hydrochlorothiazide in bulk and tablet formulation. J Pharm Bioallied Sci 2010;2(4):372-5.
7. Selvadurai M, Meyyanathan SN. Sensitive and accurate estimation of losartan potassium formulation by high-performance thin-layer chromatography. Pharm Methods 2011;2(2):95-8.
8. Kolsure AK, Chavan BB, Chabukswar AR, Kuchekar BS. Development and validation of HPTLC method for simultaneous estimation of atorvastatin calcium and losrtan potassium in combined dosage form. J Med Pharm Innov 2014;1(4):58-61.
9. Lakshmi KS, Lakshmi S. Simultaneous analysis of losartan potassium, amlodipine besylate and hydrochlorothiazide in bulk and in tablets by high-performance thin layer chromatography with UV-Absorption densitometry. J Anal Methods Chem 2012;4(2):85-91.
10. Sathe SR, Bari SB. Simultaneous analysis of losartan, atenolol and hydrochlorothiazide in bulk and in tablets by HP-TLC with UV absorption densiometry. Acta Chromatogr 2007;19(2):53-62.
11. Chaudhari V, Hussian S, Ubale M. A newer validated and stability indicating HPLC method for estimation of atenolol and hydrochlorothiazide in bulk and dosage form. Chem J 2013;1:93-101.
12. Ravisankar P, Kand Pavan G. Development and validated RP-HPLC method for simultaneous determination of atenolol and hydrochlorothiazide in tablet dosage form. Int J Pharm Chem Biol Sci 2015;5(3):665-75.
13. Mhaske RA Sahasrabudhe S, Mhaske AA. RP-HPLC method for simultaneous determination of irbesartan, losartan, hydrochlorothiazide and chlorthalidone-application to commercially available drug products. Int J Pharm Sci Res 2012;3(4):1116-23.
14. Rani VP, Patil KR, Sangshetti JN, Yeole RD, Shinde DB. Stability indicating LC method for simultaneous determination of irbesartan and hydrochlorothiazide in pharamaceutical preparations. J Chromatogr Sci 2010;39(4):91-101.
15. Gavini R, Puranik SB, Kumar GV, Sridhar KA. Development and validation of stability indicating RP-HPLC method for simultaneous estimation of amlodipine and losartan in bulk and tablet dosage formulation. Int Res J Pharm 2012;3(11):18-22.
16. Indrani B, Basak S, Maity A, Ghosh SK. Isolation and characterization of acid and base degradation products in atenolol, hydrochlorothiazide and a validated stability indicating HPLC-UV method for their quantification. Int J Adv Pharm Anal 2015;5(2):36-41.
17. Tengali AR, Shivakumar G, Gurupadayya BM. UPLCMS method development and validation of amlodipine, hydrochlorothiazide and losartan in combined tablet dosage form. Am J Anal Chem 2015;6:228-38.
18. Yadav SS, Rao JR. Simultaneous estimation of losartan, hydrochlorothiazide and atenolol from solid dosage form by RP-HPLC. Int J Pharm Pharm Sci 2014;6(1):53-62.
19. Tengali AR, Gurupadayya BM. Method development and validation of tablet dosage form containing losartan, atenolol, hydrochlorothiazide using internal standard by RP-HPLC. J Chromatogr Sep Tech 2013;2(5):78-86.
20. Kavitha J, Muralidharan S. Development and validation of new method for atenolol, hydrochlorothiazide and losartan potassium by RP-HPLC: Its application to routine quality control analysis. Int J ChemTech Res 2010;2(2):15-21.
21. Partiban C, Bhagavan Raju M, Sudhakar M. Simultaneous estimation and validation of atenolol, hydrochlorothiazide and losartan in tablet dosage form by RP-HPLC method. Int J Pharm Indian Res 2011;1(4):62-9.

22. Sharma R, Khanna S, Sharma GP. RP-HPLC method for Simultaneous estimation of atenolol, hydrochlorothiazide and losartan in tablet dosage form. Chem Sci Trans 2013;4(1):58-63.
23. Halvadar Freddy H, Vairal Dharmendra L. RP-HPLC method for Simultaneous estimation of atenolol, hydrochlorothiazide, losartan and valsartan in the pharmaceutical dosage form. Int J Pharm Life Sci 2010;1(5):282-89.
24. Kolhal S, Lokhande R, Sutar R, Surve S. A validated RP-HPLC method for simultaneous determination of multicomponent dosage form containing amlodipine, telmisartan, hydrochlorothiazide, atenolol and losartan. Int J Pharm Sci Rev Res 2014;25(2):154-59.
25. Abdussaleem K, Boopathy D, Perumal P. Analytical method development and validation of losartan potassium and atenolol in combined dosage form by RP-HPLC. Int J Pharm Tech Res 2010;2(1):471-74.
26. del Rosario Brunetto M, Contreras Y, Clavijo S, Torres D, Delgado Y, Ovalles F, *et al.* Determination of losartan, telmisartan, and valsartan by direct injection of human urine into a column-switching liquid chromatographic system with fluorescence detection. J Pharm Biomed Anal 2009;50:194-99.