Research Article

A Sensitive Validated Stability Indicating RP-HPLC Method for Simultaneous Estimation of Losartan, Ramipril and Hydrochlorthiazide in Bulk and Tablet Dosage Form with Forced Degradation Studies

Ganipisetty Lakshmi Aswini*[1], D. Dachinamoorthy[2], J.V.L.N. Seshagiri Rao[3]
[1]School of Pharmaceutical Sciences, Kakinada, Andhra Pradesh, India.
[2]QIS College of Pharmacy, Ongole, Andhra Pradesh, India.
[3]Srinivasa Rao College of Pharmacy, PM Palem, Vishakhapatnam, Andhra Pradesh, India.
***Corresponding author's E-mail:** ganipisettyaswini@gmail.com

Accepted on: 07-02-2015; **Finalized on:** 31-03-2015.

ABSTRACT

An accurate reproducible and efficient isocratic reversed-phase high-performance liquid chromatographic (RP-HPLC) method has been developed and validated for the simultaneous estimation of Losartan, Ramipril and Hydrochlorothiazide. All the drugs were separated on an Altima 150 mm x 4.6 mm, 5µ Column. The mobile phase, optimized through an experimental design, was a 65:35 (v/v) mixture of buffer and acetonitrile, pumped at a flow rate of 1 ml/min. UV detection was performed at 210 nm. The retention time of Losartan, Ramipril and Hydrochlorothiazide was found to be 7.33, 6.04 and 2.53 min respectively. The method was validated in the sample concentration ranges of 40-240µg/ml for losartan, 1-6 µg/ml for Ramipril and 10-60µg/ml for hydrochlorothiazide. The method demonstrated to be robust, resisting to small deliberate changes in pH and flow rate of the mobile phase. The LOD values were 0.10µg/ml, 0.07 µg/ml and 0.13 µg/ml, while the LOQ values were 0.31µg/ml,0.20 µg/ml and 0.39µg/ml for Losartan, Ramipril and Hydrochlorothiazide.

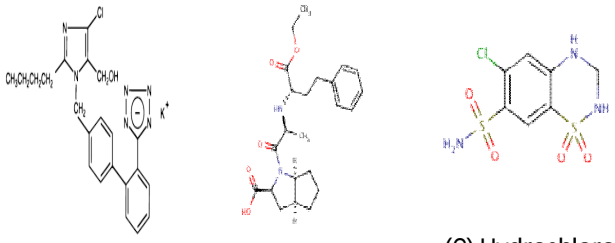
Keywords: RP-HPLC, Losartan, Ramipril and Hydrochlorothiazide, Tablet dosage form.

INTRODUCTION

Losartan is an angiotensin II receptor antagonist drug used mainly to treat high blood pressure (hypertension). Losartan was the first angiotensin II antagonist to be marketed. It is a non-peptide molecule, is chemically described as 2-butyl-4-chloro-1-[p-(o-1H-tetrazol-5ylphenyl) benzyl] imidazole-5-methanol monopotassium. Ramipril is a prodrug belonging to the angiotensin-converting enzyme (ACE) inhibitor and is used in the treatment of hypertension, congestive heart failure, nephropathy, and to reduce the rate of death, myocardial infarction and stroke in individuals at high risk of cardiovascular events.

Chemically it is (2S, 3aS, 6aS)-1-[(2S)-2-{[(2S)-1-ethoxy-1-oxo-4-phenylbutan-2-yl] amino} propanol]-octahydrocyclopenta[b]pyrrole-2-carboxylic acid. Hydrochlorothiazide is a thiazide diuretic often considered the prototypical member of this class. It reduces the reabsorption of electrolytes from the renal tubules. This results in increased excretion of water and electrolytes, including sodium, potassium, chloride, and magnesium. It has been used in the treatment of several disorders including edema, hypertension, diabetes insipidus, and hypoparathyroidism. It is chemically 6-chloro-1,1-dioxo-3,4-dihydro-2H-1$l^{6},2,4-benzothiadiazine-7-sulfonamide. Literature survey reveals High Performance Liquid Chromatographic (HPLC) for determination of Losartan, Ramipril and hydrochlorothiazi de combination are not official in Pharmacopeias of USP and BP.And their determination is official as single compound in Pharmacopeias.

Various analytical methods have been reported for the assay of Losartan, Ramipril and hydrochlorothiazidealone or in combination with other antihypertensive agents in pharmaceutical formulations. They include UV-VIS spectroscopy[1-2], high performance liquid chromatography[3-20], high performance thin layer chromatography[21] and LC - MS/MS[22].



(A) Losartan (B) Ramipril (C) Hydrochloro thiazide

Figure 1: The Chemical Structures of Losartan (A), Ramipril (B) and Hydrochlorothiazide (C).

No methods are available for their simultaneous determination, however, it is essential to develop a suitable analytical method for simultaneous estimation of Losartan, Ramipril and hydrochlorothiazide in bulk and in pharmaceutical preparations, because HPLC methods have been widely used for routine quality control assessment of drugs, because of their accuracy, repeatability, selectivity, sensitivity and specificity. We have developed a simple, accurate method of Losartan, Ramipril and hydrochlorothiazide in pharmaceutical dosage forms. Because analytical methods must be validated before use by the pharmaceutical industry, the

proposed HPLC-UV detection method was validated in accordance with International conference on Harmonization (ICH).

MATERIALS AND METHODS

Chemicals and Reagents

Pharmaceutically pure samples of Losartan, Ramipril and hydrochlorothiazide were obtained as a gift samples from Dr. Reddy's, Hyderabad used as such without further purification. A combination of Losartan, Ramipril and Hydrochlorothiazide 50/1.25/12.5 mg in tablet formulations (Loram-H) was procured from Indian market, HPLC grade methanol, Acetonitrile, water and triethylammonium phosphate buffer (AR grade) purchased from Merck Chemicals India Pvt. Limited, Mumbai, India.

Instrumentation and Chromatographic Conditions

Analysis was performed with a Waters 2695 separation module equipped with Empower-2 software and loop of injection capacity of 80µL, and waters-PDA detector set at 210 nm. Compounds were separated on an Altima 150mm x 4.6 mmi. d, column (5µm particle size) under reversed phase partition conditions. The mobile phase was 0.1%OPA Buffer and Acetonitrile in the ratio of 65:35% v/v. The flow rate was 1ml/min and the run time was 10 minutes. Samples were injected using Rheodyne injector with 10 µL loop and detection was carried out at 210 nm. Before analysis mobile phase were degassed by the use of a sonicator (Ultrasonic Cleaner, Power Sonic 420) and filtered through a 0.45µ nylon filter. The identity of the compounds was established by comparing the retention times of compounds in the sample solution with those in standard solutions. Chromatography was performed in column temperature maintained at 30±5 °C.

The UV spectrum of losartan, Ramipril and hydrochlorothiazide selecting the working wavelength of detection was taken using a shimadzu UV-1800, With UV Probe software UV-Visible spectrophotometer (shimadzu, Kyoto, Japan). All Weighing were done on Shimadzu balance (Model AY-120).

Preparation of Standard Stock Solutions

Preparation of Standard Stock Solution – I

Weight and transfer about 50 mg of losartan working standard or reference standard in to a 25ml volumetric flask, add about 10 ml of diluent and sonicate for 30 min to dissolve the material completely and make up the volume with diluent and mix well.

Preparation of Standard Stock Solution – II

Weight and transfer about 5 mg of ramipril working standard or reference standard in to a 100 ml volumetric flask, add about 20 ml of Diluent and sonicate for 30 minutes to dissolve the material completely and make up the volume with diluent and mix well.

Preparation of Standard Stock Solution – III

Weight and transfer about 12.5 mg of hydrochlorothiazide working standard or reference standard in to a 25 ml volumetric flask, add about 10 ml of Diluent and sonicate for 30 minutes to dissolve the material completely and make up the volume with diluent and mix well.

Preparation of Standard Solution

Pipette out 0.8 ml of standard stock solution –I, II & III in to 10 mL volumetric flask and diluted up to the volume with diluent. (160 µg/ml of Losartan, 4µg/ml Ramipril and 40µg/ml HCTZ)

Procedure for Analysis of Tablet Formulation

5 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 1 tablet was transferred into a 50ml volumetric flask, 30ml of diluent added and sonicated for 30 min, with intermittent vigorous shaking and stir with the aid of magnetic stirrer, further the volume was made upto volume with diluent, mix and allow the sample solution to settle down. Dilute 1.6 ml of supernatant solution to 10 ml with diluent and mix. Filter the solution through the 0.45N nylon filter. After setting the chromatographic conditions and stabilizing the instrument to obtain a steady baseline, the tablet sample solutions were injected, chromatogram was obtained and the peak areas were recorded. The injections were repeated six times and the amount of each drug present per tablet was estimated from the respective calibration curves.

Degradation Study

The drug content was employed for acidic, alkaline, and oxidant media and also for thermal and photolytic stress conditions. After the degradation treatments were completed, the stress content solutions were allowed to equilibrate to room temperature and diluted with diluent to attain 160 µg/mL Losartan, 4 µg/mL ramipril and 40 µg/mL hydrochlorothiazide concentration 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample. Specific degradation conditions were described as follows.

Acidic Degradation Condition

To 1 ml of stock s solution Losartan and Ramipril and HCTZ, 1ml of 2N Hydrochloric acid was added and refluxed for 30 mins at 60°C.

Alkali Degradation Condition

To 1 ml of stock solution Losartan and Ramipril and HCTZ, 1 ml of 2N sodium hydroxide was added and refluxed for 30 mins at 60°C.

Oxidative Degradation Condition

To 1 ml of stock solution of Losartan and Ramipril and HCTZ, 1 ml of 20% hydrogen peroxide (H_2O_2) was added separately. The solutionswere kept for 30 min at 60°C.

Thermal Degradation Condition

The standard drug solution was placed in oven at 105 °C for 6h to study dry heat degradation.

Photolytic Degradation Condition

The photochemical stability of the drug was also studied by exposing the 300 µg/ml & 10µg/ml & 25µg/ml solution to UV Light by keeping the beaker in UV Chamber for 7days or 200 Watt hours/m^2 in photo stability chamber.

RESULTS AND DISCUSSION

Method Development

Several tests were performed in order to get satisfactory separation-resolution Losartan, Ramipril and hydrochlorothiazide in different mobile phases with various ratios of buffers and organic phases by using different columns. The ideal mobile phase was found to be a Buffer and Acetonitrile. This mobile phase used gave a very satisfactory and good resolution of Losartan, Ramipril and hydrochlorothiazide. Increasing or decreasing pH of mobile phase by ± 0.2 did not show significant change in retention time of each analyte. The retention time of Losartan Ramipril and hydrochlorothiazide on the analytical column was evaluated at a flow rate of 1 ml/min. The injection volume was 10 µL. The retention time of standard and sample for Losartan, Ramipril and hydrochlorothiazide were satisfactory with good resolution. This work was focused on optimization of the conditions for the simple and rapid as well as low cost effective analysis including a selection of the proper column or mobile phase to obtain satisfactory results. Solvent type, solvent strength (volume fraction of organic solvent(s) in the mobile phase and pH of the buffer solution), detection wavelength, and flow rate were varied to determine the chromatographic conditions giving the best separation. The mobile phase conditions were optimized so there was no interference from solvent and excipients. Finalized chromatographic conditions were mentioned on below Table 1.

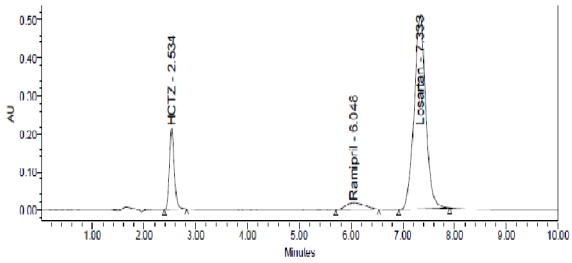


Figure 2: Optimized chromatograms for Losartan, Ramipril and hydrochlorothiazide

Table 1: Finalized Chromatographic Conditions

Flow rate:1 ml/min	**Wave length**: 210 nm	**Injection Volume**: 10 µL
Column temperature: 30 °C	**Sample temperature**: Ambient	**Run time**:10 minutes
Mobile phase: 0.1% OPA Buffer and Acetonitrile in the ratio of 65:35		
Column: Altima 150mm x 4.6 mm, 5µ		

To inject the standards on above finalized chromatographic conditions and their results was mentioned on below Table 2.

Table 2: Results from system suitability study of Losartan, Ramipril and hydrochlorothiazide

System Suitability Parameters	Results			Acceptance Criteria
	Losartan	Ramipril	Hydrochlorothiazide	
Retention time	7.33	6.04	2.53	
%RSD for area of Losartan, Ramipril and Hydrochlorothiazide for five replicate injections of standard solution	0.30	0.71	0.38	NMT 2.0
Tailing factor for Losartan, Ramipril and hydrochlorothiazide peak	1.03	1.25	1.17	NMT 2.0
Theoretical plates for Losartan, Ramipril and hydrochlorothiazide	5261	2214	3867	NLT 2000

Table 3: Precision studies

S. No	% Assay(n=6)					
	Losartan		Ramipril		Hydrochlorothiazide	
	Intraday precision	Interday precision	Intraday precision	Interday precision	Intraday precision	Interday precision
1	99.9	99.6	100.2	99.9	98.4	98.4
2	100.6	99.4	100.8	100.4	100.1	100.8
3	100.4	99.6	99.6	99.7	99.9	100.0
4	100.2	99.6	99.1	98.7	101.1	100.9
5	99.8	100.2	98.5	98.8	98.1	99.4
6	100.9	100.2	100.7	100.4	101.3	100.5
Mean	100.3	99.8	99.8	99.6	99.8	100.0
%RSD	0.39	0.34	0.92	0.75	1.33	0.96
Over all % RSD (n=12)	0.45		0.8		1.1	

Method Validation

The method was validated for specificity, linearity, accuracy, intra-day and inter-day precision and robustness, in accordance with ICH guidelines.

Linearity

Aliquots 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2ml of stock solution of Working standard solution Losartan, Ramipril and hydrochlorothiazide were transferred in a series of 10 mL volumetric flasks for 25, 50, 75, 100, 125 and150% levels.

Finally the volume was made up to the mark with the diluent. Two replicates per concentration were injected and chromatograms were recorded. The peak area ratios of Losartan, Ramipril and hydrochlorothiazide were calculated and respective calibration curves were plotted of response against concentration of each drug. Calibration curves for Losartan, Ramipril and hydrochlorothiazide were plotted separately of response against respective concentration of Losartan, Ramipril and hydrochlorothiazide.

The slope and intercept value for calibration curve were y = 50765x + 8608.7 (R^2 = 0.9993) for losartan, y =11163x + 679.16 (R^2 = 0.9997) for Ramipril and y = 36330x + 2148.2 (R^2 = 0.9998) for hydrochlorothiazide, where Y represents the peak area of analyte and X represents analyte concentration.

The results are satisfactory, because there is a significant correlation between response factor and concentration of drugs within the concentration range.

Precision

Precision of the method was confirmed by the repeated analysis of formulation for six times. The % RSD values were found to be satisfactory. The low % RSD values indicated that drugs showed good agreement with the label claim ensures the precision of the method.

Intraday and Interday precision was determined by preparing six (n=6) replicate samples and analyzed on same day for intraday and on different days for interday precision. (Table3). The peak areas were recorded and Relative standard deviation (RSD) was calculated for both series of analyses. The %RSD of intraday precision was 0.39, 0.92 and 1.33 for Losartan, Ramipril and hydrochlorothiazide, respectively. The %RSD of interday precision was 0.34, 0.75 and 0.96 for Losartan, Ramipril and hydrochlorothiazide respectively and overall %RSD for Losartan, Ramipril and hydrochlorothiazide are 0.45, 0.8 and 1.1 respectively (Table3).

Accuracy

To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 50%, 100% and 150%. The percentages of recoveries were calculated, results of which are represented in Table 4.

Table 4: Recovery studies of Losartan, Ramipril and hydrochlorothiazide

Product name		Level of % Recovery		
		50	100	150
Losartan	% Mean Recovery*	100.2	100.0	99.5
	% R.S.D*	1.1	1.0	1.0
Ramipril	% Mean Recovery*	100.1	100.1	99.8
	% R.S.D*	0.6	1.2	1.6
Hydrochlorothiazide	% Mean Recovery*	99.1	99.5	99.6
	% R.S.D*	0.9	0.9	1.1

**Avg. & R.S.D. of three determinations for 50, 100 & 150% concentration levels*

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.

Robustness

As defined by ICH, the robustness of an analytical procedure describes to its capability to remain unaffected by small and deliberate variations in method parameters. Robustness was performed to injected the standard and samples by small variation in the chromatographic conditions and found to be unaffected by small variations like ± 2% variation in volume of mobile phase composition with respect to acetonitrile, ± 0.2 mL/min in flow rate of mobile phase, ± 0.5 variation in pH, different type of filters and ± 5 column temperature variation. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed is robust.

Specificity

Specificity was tested against standard compounds and against potential interferences. Specificity was determined by comparing the responses of standard and sample solution. No interference was detected at the retention times of both Losartan, Ramipril and hydrochlorothiazide in sample solution.

Forced Degradation studies

Forced degradation studies were performed to demonstrate the stability of the sample Degradation studies were carried out under conditions of hydrolysis, dry heat, oxidation, UV light and photolysis. The degradation study indicated that Losartan, Ramipril and hydrochlorothiazide was susceptible to acid, base, oxidation, photo, thermal and neutral degradation. Typical chromatograms of stressed samples are shown in Figs.6-10. In all degradations the drug degrades as observed by the decreased area in the peak of the drug when compared with peak area of the same concentration of the non-degraded drug, without giving any additional degradation peaks. Both the drugs showed no degradation at 0 h, in all the degradation conditions. In

that percent degradation was calculated by comparing the areas of the degraded peaks in each degradation condition with the corresponding areas of the peaks of both the drugs under non-degradation condition. It also showed retention time of degraded products which were observed in different degradation conditions for both drugs.

Table 5: Summary of validation parameters of proposed RP-HPLC method

Parameters	Losartan	Ramipril	Hydrochlorothiazide
Linearity range (µg/mL)	40-240	1-6	10-60
Correlation co-efficient	0.9993	0.9997	0.9998
LOD [a] (µg/mL)	0.10	0.07	0.13
LOQ [b] (µg/mL)	0.31	0.20	0.39
Accuracy (% Recovery)	99.5 - 100.2	99.8 - 100.1	99.1 - 99.6
Precision (% RSD)[c]			
Intraday (n[d]= 6)	0.39	0.92	1.33
Interday (n[d]= 6)	0.34	0.75	0.96

[a] *LOD = Limit of detection.*

[b] *LOQ =Limit of quantitation.*

[c] *RSD = Relative standard deviation.*

[d] *n = Number of determination*

Table 6: Forced Degradation Studies

S. No.	Injection	% Assay	% Degradation	Purity Angle	Purity Threshold
1	Acid Degradation	96.4	6.06	0.133	0.246
2	Base Degradation	97.93	8.34	0.140	0.285
3	Peroxide	94.64	5.04	0.270	0.512
4	Thermal Degradation	98.14	9.05	0.208	0.552
5	UV Degradation	99.08	4.42	0.222	0.372

CONCLUSION

The validated RP-HPLC method employed here proved to be simple, fast, accurate, precise and robust, thus can be used for routine analysis of Losartan, Ramipril and hydrochlorothiazide in combined tablet dosage form.

Acknowledgements: The authors express their gratitude to Dr. Reddys Pharmaceuticals Ltd. (Hyderabad, India) for the gift sample of pure Losartan, Ramipril and hydrochlorothiazide.

REFERENCES

1. AnandaThangadurai M, Mnusamy Jambulingam, Dhanapal Kamalakannan, Ramachandran Sundaraganapathy and Chennakesavulu Jothimanivannan, Spectrophotometric method development of ramipril and hydrochlorthiazide in bulk and marketed formulation by vierordt's method, International research journal of pharmacy, 3, 2012, 208-211.
2. Bankey S., Tapadiya G. G., Saboo S.S., Bindaiya S., Deepthi Jain, Khadbaddi S.S. Simultaneous determination of ramipril hydrochlorthiazide and telmisartan by spectrophotometry. International journal of chem tech research, 2, 2009, 183-188.
3. Ashutosh Kumar S., Manidipa Debnath, Seshagiri Rao J. V. L. N. Simultaneous estimation of losartan potassium, ramipril and hydrochlorothiazide in bulk as well as in pharmaceutical formulation by rp-hplc, IAJPR, 3, 2013, 3871-3894.
4. Tengli, Anandkumar R.; Neeraj, Soni, Gurupadayya B. M., Chandan R. S.RP-HPLC Method for Simultaneous Estimation of Hydrochlorothiazide, Ramipril and Telmisartan in Tablet Dosage Form, Journal of Pharmacy Research, 5, 2012, 1290.
5. Shravan Banke, Shailendra Bindaiya, Ritesh Patel. Simultaneous determination of ramipril, hydrochlorothiazide and telmisartan by rp-hplc. International Journal of Pharmaceutical and Medical Sciences, 1, 2012, 9–5.
6. Pankaj Kumara, Shubhanjali Shuklab, B. B. Subudhia, Ashok Laxmanrao Ganurea. RP-HPLC Method for the Simultaneous Determination of Ramipril and Hydrochlorothiazide in Tablet Dosage Form.
7. Kurade V.P., Pai MG., Gude R. RP-HPLC estimation of ramipril and telmisartan in tablets. Ijps, 2, 2009, 148-151.
8. Kalyan Kumar B., Santhosh Kumar T., Shravan Kumar A., Venkateshwar Rao P. Development and Validation of RP-HPLC Method for Simultaneous Estimation of Ramipril, Telmisartan and Hydrochlorothiazide in Pharmaceutical Dosage Forms, Journal of Pharmacy Research, 4, 2011.
9. Mandlekar R., Patil P., Phoujdar M., Mulgund S. Simultaneous rp-hplc determination of ramipril, hydrochlorothiazide and telmisartan in the bulk drug and in tablet. Inventi Rapid: Pharm Analysis & Quality Assurance.
10. Lakshmi K.S. and lakshmisiva subramanian. A stability indicating hplc method for the simultaneous determination of valsartan and ramipril in binary combination. J. Chil. Chem. Soc, 55, 2010, 223-226.
11. Chintan V. Patel, Amit P. Khandhar, Anandi D. Captain, Kalpesh T. Patel. Validated absorption factor spectrophotometric and reversed-phase high-performance liquid chromatographic methods for the determination of ramipril and olmesartan medoxomil in pharmaceutical formulations. 2, 2007.
12. Manjulatha Y.B., Gowrisankar D. Stability indicating rp-hplc for determination of ramipril in pure and pharmaceutical formulation, Asian journal of pharmaceutical and clinical research, 6, 2014, 158-161.
13. Khan M, Rizwan, Shaikanis, Thaku A. K. Simultaneous determination and method development for assay of losartan potassium and hydrochlorthiazide drugs in solid dosage form byRP-HPLC. IJPSR, 2(1), 2012, 42-45.
14. Praveen S. Rajput, Amanjotkaur, Navdeepkaur Gill, Karan Mittal, Subrahmanya Sarma Ganti. Simultaneous estimation of ramipril and amlodipine in bulk and tablet dosage form by RP-HPLC method. Journal of applied pharmaceutical sciences, 2, 2012, 160-165.

15. Maste M. M., Karekar M.C., Kadain N and Bhat A. R. Development of RP-HPLC method for simultaneous estimation of amlodipine and ramipril in tablet dosage form. Asian J. of Research Chem, 4, 2011, 1210-1213.

16. Mahesh M., Kumaran R., Jayaveera K. N. Isocratic RP-HPLC-UV method development and validation for the simultaneous estimation of hydrochlorthiazide and ramipril in tablet dosage form and bulk drug. International journal of current pharmaceutical research, 3, 2011, 119-123.

17. Narasimha Rao Nalluri, Rajavel P., Venkateswararao P., Sudhakarbabu AMS. Analytical method development and validation of ramipril and telmisartan in pharmaceutical dosage form by using RP-HPLC method. International journal of biological and pharmaceutical research, 3, 2012, 784-788.

18. Parthiban C., Bhagavanraju, Sudhakar M. A validated RP-HPLC method for simultaneous estimation of ramipril and valsartan in pharmaceutical dosage form, RJPBCS, 3, 2012, 198-205.

19. Sunil Jawla, Jejalakshmi K., Krishnamurthy T., Kumar Y. Development and validation of simultaneous HPLC method for estimation of telmisartan and ramipril in pharmaceutical formulation, International Journal of Pharma Tech Research, 2, 2010, 1625-1633.

20. Jothieswari D., Priya D., Brito Raj S., Monambal E., Wasim Raju S. Design and RP-HPLC method for the simultaneous determination of valsartan and hydrochlorthiazide in bulk and pharmaceutical formulation. IJNTPS, 1, 2011, 18-22.

21. Simultaneous determination and validation of telmisartan and ramipril in pharmaceutical dosage form by rp – hplc and hptlc.

22. Manna L., Valvo L., Alimonti S. A liquid chromatographic Ion-pairing method for simultaneous determination of benazepril hydrochloride, fosinopril sodium, ramipril and hydrochlorothiazide in pharmaceutical formulations, Chromatographia, 53, 2001, S271-S275.

Source of Support: Nil, **Conflict of Interest:** None.