IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
December 2020 Vol.:20, Issue:1

Novel RP-HPLC Method for Simultaneous Analysis of Chlorthalidone and Telmisartan from Combined Dosage Form

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Amar F. Sabhadinde[1], Wrushali A. Panchale[1], Jagdish V. Manwar[2,*], Ravindrakumar L. Bakal[1]

[1] IBSS's Dr. Rajendra Gode Institute of Pharmacy, Mardi Road, Amravati-444 602, MS, India [2] IBSS's Dr. Rajendra Gode College of Pharmacy, Mardi Road, Amravati-444 602, MS, India

Submitted: 12 November 2020
Revised: 02 December 2020
Accepted: 22 December 2020

HUMAN JOURNALS
www.ijppr.humanjournals.com

Keywords: Chlorthalidone, Telmisartan, RP-HPLC,combined dosage form

ABSTRACT

Novel RP-HPLC method was developed for the simultaneous estimation of chlorthalidone and telmisartanin bulk drug and combined dosage form. The separation was achieved by Grace column (4.6 mm I.D × 250 mm) C18 column with mobile phase consist of acetonitrile and potassium phosphate buffer (pH 2.5) in the ratio 45:55 v/vat 0.7 mL/min flow rate.The detection was carried out at 235 nm. The retention time of chlorthalidone and telmisartanwas found to be 3.41 min and 6.05 min, respectively.Linear response obtained for chlorthalidonewere in the range 10-60 µg/ml (r^2 = 0.999) and for telmisartanin the range 10-50 µg/ml (r^2 = 0.999). The relative standard deviation in the tablets was found less than 2% for six replicates. The method was validated according to the ICH guidelines for linearity, precision, accuracy, ruggedness and robustness.

INTRODUCTION:

Chlorthalidone **(Fig.1)** is chemically (RS)-2Chloro-5-(1-hydroxy-3-ox-2,3-dihydro-1H-isoindole-1-yl) benzene-1-sulfonamide.[1]It is official in Indian Pharmacopoeia.[2] It is a diuretic used to treat high blood pressure, swelling including that due to heart failure, liver failure, and nephrotic syndrome, diabetic, and renal tubularacidosis.[3-5]

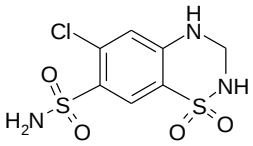

Figure No. 1: Structure of Chlorthalidone

Telmisartan **(Fig. 2)** is chemically 2-(4-{[4-methyl-6(1-methyl-1H-1,3-benzodiazol-2-yl)-2-propyl-1H-1,3benzodiazole-1-yl]methyl}phenyl)benzoic acid.[6] It is official in Indian Pharmacopoeia.[2] Angiotensin receptor blocker and used treatment of high blood pressure. It provides protective benefits against the vascular and renal damage caused by diabetes and cardiovascular disease.[7]

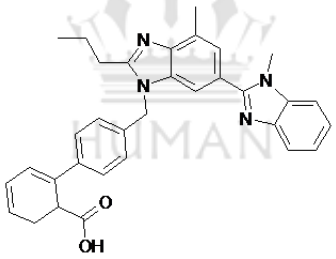

Figure No. 2: Structure of Telmisartan

There is a number of analytical methods for the determination of various drugs from bulk and various formulations like tablets, capsules, injections, etc. These methods include UV-spectrophotometry, HPLC, UPLC, Gas chromatography, etc.[8-26] Literature survey revealed that there are various methods reported for determination of chlorthalidone and telmisartan in alone and in combination with other drugs.[27-30] There is also a report of UV-spectrophotometry, HPLC and HPTLC method for the analysis of the same combination from tablet formulation.[31-35] Nevertheless, none of the above methods covered validation as per ICH guideline. Hence, attempts were made to develop novel RP-HPLC method which covered all validation parameters as per ICH guidelines.

MATERIALS AND METHODS:

Instrumentation

Chromatography was performed with YounglineAcme9000 (Autochro-3000 software) system coupled with UV 730 detector. Chromatographic separation was carried isocratically at room temperature with a Grace C_{18} (250mmX 4.6mm, 5µm) column.

Reagents and Chemicals

All chemicals and reagents used in the method were of HPLC grade. Prior to use, mobile phase and other solvents were filtered through 0.45 µm Whatman filter paper. Standard bulk drugs chlorthalidone and telmisartan were provided as gift samples by Indo Gulf Pharmaceutical Company, Mumbai (India). Marketed tablet formulation (CTD-T^{TM}) were purchased from local market. The contents reported on label were of12.5 mg chlorthalidone and 80 mg telmisartan.

Preparation of mixed standard stock solution

Mixed standard stock solution was prepared in water having a concentration 320 µg/mL of chlorthalidone and 50 µg/mL of telmisartan.

Selection of wavelength

Standard solution of chlorthalidone (320µg/mL) and telmisartan (50 µg/mL) were scanned individually between the range 200 to 400 nm in spectrum mode. From the spectra, 235 nm wavelength was selected for further analysis as both the drugs showed significant absorption and maximum sensitivity.

Experimental condition

The chromatographic condition for experimental work was selected by using different mobile phases alone and in combination, at different flow rates, at ambient temperature. The mobile phase consists of acetonitrile and potassium phosphate buffer pH 2.5 (45:55 v/v) was proved to be more effective. Flow rate 0.7mL/min and wavelength 235nm were selected. At the selected condition, we got better resolution with Gaussian shape peaks.

System Suitability Parameters

System suitability parameters were checked according to USP by using a standard mixture containing chlorthalidone (32 µg/mL) and telmisartan (5 µg/mL).About 20 µL of the solution was injected into the chromatographic conditions and results were recorded.

Tablet Formulation Assay

The average weight of 20 tablets was determined and was then crushed to fine powder. Average powder equivalent to 32 mg of chlorthalidone (also contain 5mg of telmisartan) was weighed accurately and was transferred to 100 ml volumetric flask. To this 20 ml of methanol was added and shaken for 30 min and sonicated for 10 min. Final volume was added up to 100 ml with same solvent. The solution was filtered the Whatman filter paper.10 ml of above solution was diluted to 100 ml with methanol. They contained 32µg/ml of chlorthalidone and 5 µg/ml of telmisartan. The solution was injected into the system and the concentration of each drug was calculated from the respective regression equation and prepared for individual drug-using AUC.

Validation of Method[36-38]

Validation of proposed method was carried out in terms of recovery and precision, linearity and range, limit of detection (LOD), limit of quantitation (LOQ) and robustness.

Linearity & range,

To study the linearity of drugs, a series of dilutions were prepared from mixed standard stock solution containing chlorthalidone (8-48 µg/mL) and telmisartan (2.5-12.5 µg/mL). The calibration graph was plotted as concentration versus peak area response. From linearity graph, concentration selected for containing chlorthalidone and telmisartan were 32µg/mL and 5 µg/mL, respectively.

Accuracy

Accuracy was determined by performing a recovery study using the standard addition method. It was determined at 80%, 100%, and 120% concentration level. The results were expressed in percentage.

Precision

The precision of the method was measured by replicate injections of a mixed standard solution containing chlorthalidone (32µg/mL) and telmisartan (5 µg/mL). Results were expressed in terms of %RSD calculated from the measurement of AUC.

Robustness

Robustness of the method was determined by making changes in the chromatographic conditions, such as slight change in mobile phase composition, change in wavelength, and change in the flow rate was varied by ± 0.1 mL/min, intra-day and inter-day variation. The percent content in preanalysed formulation were determined.

RESULTS AND DISCUSSION:

Based on the literature survey and use of marketed formulation, combination of chlorthalidone and telmisartan were selected for the method development. RP-HPLC method was selected because of its advantages. Solvent methanol was used as it dissolved both the drugs. Wavelength for detection selected was 235 nm because at this wavelength both the drug showed higher sensitivity. The method was validated as per ICH guidelines.[33-34]Linearity and range was studied using the series of dilution of each drug solution. From this, concentration for chlorthalidone and telmisartan were selected32µg/mL (for chlorthalidone) and 5 µg/mL (telmisartan) respectively. The LOD and LOQ were determined by diluting known concentrations of standard drug until the mean responses were approximately 3 or 10 times the standard deviation of the responses of the blank for six replicate determinations. The signal/noise ratios 3:1 and 10:1 were taken as the LOD and LOQ, respectively. The limit of detection and limit of quantitation of chlorthalidone and telmisartan were determined by analysing results of the linearity study. Results of the linearity study are given in **Table 1** and for linearity, see **Fig. 3 & 4**.

Table No. 1: Linearity & range, LOD & LOQ

Parameters	Result	
	Chlorthalidone	Telmisartan
Linearity	8-48 µg/ml	2.5-12.5 µg/ml
% RSD*	0.73	1.29
Slope	894.1	15.06
LOD	0.051	0.190
LOQ	0.098	0.003

*Mean of three results

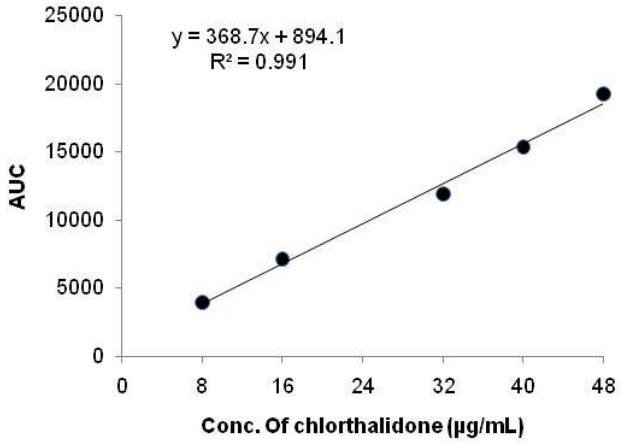

Figure No. 3: Linearity of Chlorthalidone

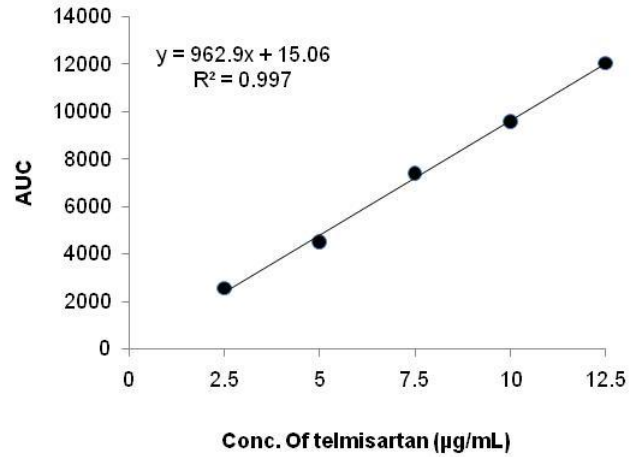

Figure No. 4: Linearity of Telmisartan

The chromatographic condition was selected on trial and error basis. The set of chromatographic conditions suitable for separation were mobile phase ofacetonitrile:potassium phosphate buffer (pH 2.5) (45:55v/v), Flow rate 0.7mL/min at ambient temperature. At the selected set of condition, as chlorthalidone is more polar than telmisartan, it elutes first with retention time 3.41 min followed by less polar drug telmisartan which elutes with retention 6.05 min. Chromatogram of mixed standard solution is show in **Fig. 5**.

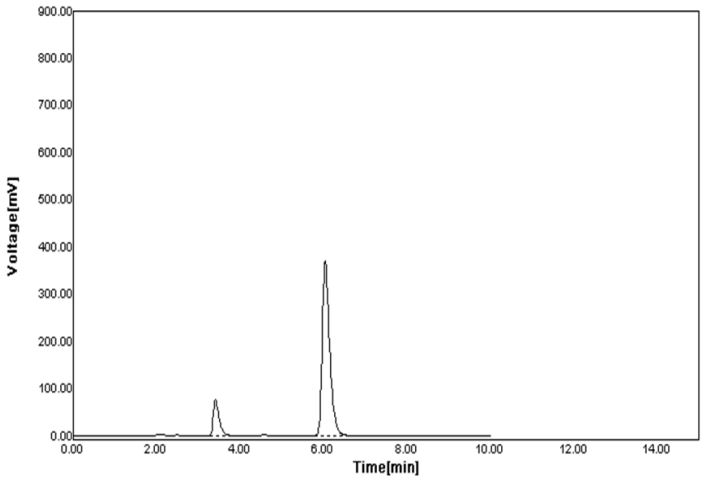

Figure No. 5: HPLC Chromatogram of Chlorthalidone (first peak from left) and telmisartan (second peak)

The precision of the method was determined by checking system suitability parameter by replicating injection of mixed standard solution in the system. The results are expressed % RSD. Accuracy of the method was performed by recovery study by standard addition method at three levels i.e. 80,100,120 %. The percentage recovery for both the drug was closed to 100% w/w for both drugs. Precision was determined by studying system suitability parameters by injecting a standard solution. The results of System Suitability Parameters are shown in **Table 2.**

Table No. 2: Result of System Suitability Parameters.

Sr. No.	System Suitability Parameters	Result*	
		Chlorthalidone	Telmisartan
1	Retention Time	3.466	3.75
2	Area	1577.133	10082.20
3	Theoretical Plate Number	2553.97	2305.72
4	Tailing Factor	1.40	1.37

*Mean of five results

The capacity of developed method was checked by performed robustness study. The conditions were changed deliberately like change in mobile phase composition (± 1), flow rate (± 0.1), and wavelength (± 1), Intraday and inter-day variation and percent contents in formulation were estimated. The mean of inter-day precision was found to be 1.18 and 1.04 for telmisartan and chlorthalidone, respectively. The results showed that the developed method remain unaffected**.** The results of robustness are given in **Table 3.**

Table No. 3: Results of robustness study

Drug	Factor	Level	% Content	% RSD
Chlorthalidone	Mobile phase composition *(A : B)**	44:56	100.99	0.36
		46:54	99.25	1.26
	Wavelength	234 nm	99.74	1.19
		236 nm	99.21	0.57
	Flow rate	0.6 ml	98.98	0.16
		0.8 ml	99.08	0.97
	Intra-day	-	100.05	0.39
	Inter-day	-	99.06	1.87
Telmisartan	Mobile phase composition *(A : B)**	44:56	100.45	0.16
		46:54	98.95	0.17
	Wavelength	234 nm	99.54	0.19
		236 nm	99.85	0.77
	Flow rate	0.6 ml	99.76	0.13
		0.8 ml	101.03	0.15
	Intra-day	-	99.18	0.80
	Inter-day	-	100.38	1.66

* *A*- Acetonitrile, *B*- Potassium Phosphate Buffer (pH 2.5), V/V.

CONCLUSION:

Novel RP-HPLC method for the simultaneous analysis of chlorthalidone and telmisartan from the combined dosage form is simple, accurate, and precise. It does not get affected upon smaller variation in experimental condition. Thus, it is used for routine quality control analysis of bulk drugs and marketed tablet formulation.

REFERENCES:

1. Budawari S. The Merck Index. 13th Edn. Merck& Co. Inc. Whitehouse station. NJ 2003.
2. Indian Pharmacopoeia. Indian Pharmacopoeia Commission, Ghaziabad; Government of India, Ministry of Health and Family Welfare. 2020.
3. Cooney D, Milfred-LaForest S, Rahman M. Diuretics for hypertension: Hydrochlorothiazide or chlorthalidone? Cleve Clin J Med. 2015 Aug;82(8):527-33. doi: 10.3949/ccjm.82a.14091. PMID: 26270432.
4. Hripcsak G, Suchard MA, Shea S, Chen R, You SC, Pratt N, Madigan D, Krumholz HM, Ryan PB, Schuemie MJ. Comparison of Cardiovascular and Safety Outcomes of Chlorthalidonevs Hydrochlorothiazide to Treat Hypertension. JAMA Intern Med. 2020 Apr 1;180(4):542-551. doi: 10.1001/jamainternmed.2019.7454. PMID: 32065600; PMCID: PMC7042845.
5. Roush GC, Buddharaju V, Ernst ME, Holford TR. Chlorthalidone: mechanisms of action and effect on cardiovascular events. CurrHypertens Rep. 2013 Oct;15(5):514-21. doi: 10.1007/s11906-013-0372-1. PMID: 23839110.
6. Kumar G.V., Murthy T.E.G.K. and Rao K.R.S., Validated RP-HPLC method for the estimation of telmisartan in serum samples, International Journal Of Research In Pharmacy And Chemistry, 2011,1(3),703-706.
7. Jump Philippe, Gosse. A Review of Telmisartan in the Treatment of Hypertension: Blood Pressure Control in the Early Morning Hours. Vasc Health Risk Manag. 2006. 2 (3): 195–201. doi:10.2147/vhrm.2006.2.3.195. PMC 1993985. PMID 17326326.
8. Bakal RL, Manwar JV, Sahare AY, Bhajipale NS, Manikrao AM. Spectrophotometric estimation of amitriptyline HCl and chlordiazepoxide in pharmaceutical dosage form. Indian Journal of Pharmaceutical Education and Research. 2008; 42: 23–26.
9. Pophalkar PB, Wakade RB, Hole SU, Kadam CY, Suroshe RS. Panchale WA. Development and Evaluation of Ondansetron Medicated Jelly. World Journal of Pharmaceutical Research. 2018; 7(19): 1252-1263.
10. Suroshe RS, Wakade RB, Panchale WA, Sakhare AD, Rathod RR, Pophalkar PB. Development and Characterization of Osmotic Drug Delivery System of Model Drug. World Journal of Pharmaceutical Research. 2018; 7(18): 1158-1171.
11. Kadam CY, Bobade NN, Pophalkar PB, Hole SU, Suroshe RS, Panchale WA. Design and *In vitro*Characterization of Phase Transition System using Rivastigmine Tartrate for Nasal Drug Delivery System. World Journal of Pharmaceutical Research. 2018; 8(1): 815-829.
12. Bakal RL, Manikrao AM, Sahare AY, Manwar JV. Spectrophotometric estimation of amitriptyline HCL and chlordiazepoxide in tablet dosage form, International Journal of Biological and Chemical Sciences. 2007; 5(1): 360–364.
13. Manwar JV, Nagargoje BU, Gurumukhi VC, et al. Application of simultaneous equation method for thedetermination of azithromycin and cefixime trihydrate in tablet formulation, Research Journal of Pharmacy andTechnology. 2017;10(1): 108-112.
14. Manwar J, Kumbhar DD, Bakal RL, Baviskar SR, Manmode RS. Response surface based co-optimization of release kinetics and mucoadhesive strength for an oral mucoadhesive tablet of cefiximetrihydrate. Bulletin of Faculty of Pharmacy. Cairo University. 2016; 54: 227–235. http://dx.doi.org/10.1016/j.bfopcu.2016.06.004
15. Manwar J, Mahadik K, Paradkar A. Plackett–Burman design: A statistical method for the optimization of fermentation process for the yeast *Saccharomyces cerevisiae*isolated from the flowers of *Woodfordiafruticosa*. Fermentation Technology. 2013; 2: 109. http://dx.doi.org/10.4172/2167-7972.1000109.

16. Manwar JV, Mahadik KR, Sathiyanarayanan L, Paradkar AR, Patil SV. Comparative antioxidant potential of *Withaniasomnifera* based herbal formulation prepared by traditional and non-traditional fermentation processes. Integr Med Res. 2013; 2, 56-61. http://dx.doi.org/10.1016/j.imr.2013.04.002
17. Manwar JV, Sonawane BV, Patil SV, Takle SP. Rapid RP-HPLC method for estimation of zidovudine from tablet dosage form, Der ChemicaSinica 2 (2011) 152–156.
18. Panchale WA, Suroshe RS, Rathod MS, and Pandhare YL. Chromatographic analysis of famotidine, paracetamol and ibuprofen from tablet formulation. Research Journal of Pharmacy and Technology, 2019:12: 231-263.
19. Panchale WA, Gulhane CA, Manwar JV, Bakal RL. Simultaneous estimation of salbutamol sulphate and ambroxol HCl from their combined dosage form by UV-Vis spectroscopy using simultaneous equation method. GSCBiological and Pharmaceutical Sciences. 2020; 13(03): 127-134. https://doi.org/10.30574/gscbps.2020.13.3.0397
20. Panchale WA, Badukle NA, Sabhadinde AF, Bakal RL, Manwar JV. Concurrent analysis of ambroxol HCl and salbutamol sulphate from tablet formulation by RP-HPLC. GSCBiological and Pharmaceutical Sciences. 2020; 13(03). 13(03): 197-202. https://doi.org/10.30574/gscbps.2020.13.3.0404
21. Manwar JV, Mahadik KR, Paradkar AR, Takle SP, Sathiyanarayanan L, Patil SV. Determination of withanolides from the roots and herbal formulation of *Withaniasomnifera*by HPLC using DAD and ELSD detector. Der Pharmacia Sinica. 2012; 3: 41–46.
22. Manmode RS, Dhamankar AK, Manwar JV, and Laddha SS. Stability indicating HPLC method for simultaneous determination of methocarbamol and nimesulide from tablet matrix. Der Chemica Sinica.2011; 2: 81–85.
23. Manwar JV, Vispute SS, Kumbhar DD, Manmode RS, Bakal RL, Jadhao RG, Jogdand SD. Response surface based optimization of system variables for liquid chromatographic analysis of candesartan cilexetil. Journal of Taibah University for Science. 2017; 11: 159–172. http://dx.doi.org/10.1016/j.jtusci.2016.02.004
24. Gulhane CA, Khadabadi SS, Atram SC. Analytical method development and validation for simultaneous estimation of some drugs in pharmaceutical dosage form. Asian Journal of Pharmaceutical Analysis. 2019; 9(3):107-112.
25. Manwar JV, Patil SS, Bhalerao CA, Mandpe SR, Kumbhar DD. Experimental Design Approach for Chromatographic Determination of Ketorolac Tromethamine from Bulk Drug and Tablet Formulation. Global Journal of Pharmacy & Pharmaceutical Sciences. 2017; 3(2): 555609. http://doi.org/10.19080/GJPPS.2017.03.555609
26. Manwar J, Mahadik K, Paradkar A, Patil S, Sathiyanarayanan L, Manmode R.Gas chromatography method for the determination of non-ethanol volatile compounds in herbal formulation. International Journal of Analytical and Bioanalytical Chemistry. 2013; 3(1): 12-17.
27. Chavhan V, Lawande R, Salunke J, Ghante M, Jagtap S. UV spectrophotometric method development and validation for telmisartan in bulk and tablet dosage form. Asian J Pharm Clin Res, Vol 6 Issue 4, 2013, 19-21.
28. Reddy BH, Spandana B, D. Mounika, Sindhu Devi, Anusha Dacha, K.Vanitha Prakash. Simultaneous Estimation of Telmisartan, Chlorthalidone and Cilnidipine by Absorbance Correction Method Using UV Spectrophotometry, Indo Am. J. P. Sci, 2018; 05(03).
29. SawaleV, Dangre D, Dhabarde D. Development and validation of RP-HPLC method for the simulteneous estimation of olmesartanmedoxomil and chlorthalidone in tablet dosage form. International Journal of Pharmacy and Pharmaceutical Sciences. 2015, 7(5):266-269.
30. Sujana K, GowriSankar D, BalaSouri O, Swathi RG Stability indicating RP HPLC method for the determination of telmisartan in pure and pharmaceutical formulation. International Journal of Pharmacy and Pharmaceutical Sciences 2011; 3(2):164-167.
31. Sahoo, S; Mishra, SK and Panda, PK, HPLC Method Development for Simultaneous Estimation of Telmisartan and Chlorthalidone in Tablet Dosage Form. Int. J. Pharmaceut. Res. Sch. 2012.4, 1-5.

32. SurwaseBH, TapkirAS,Jadhav SB, ChaudhariPD. Development and validation of RP-HPLC method for simultaneous estimation of telmisartan and clorthalidone in bulk and tablet dosage form. Scholar research library 2013, 5 (1):149-154.
33. ParmarKE, MehtaRS, PatelND. Development and validation of HPTLC method for simultaneous determination of telmisartan and chlorthalidone in bulk and pharmaceutical dosage form. Int J Pharm PharmSci, 2013, 5, 2, 420-425.
34. ChaudharyBR, DaveJB. Spectrophotometric method development and validation for simultaneous estimation of telmisartan and chlorthalidone in dosage form by second derivative method. Pharma Science Monitor. 2019, 10(3), 1-12.
35. ParmarKE, PatelKD. Stability indicating RP-HPLC Method for Simultaneous Determination of Telmisartan and Chlorthalidone in Bulk and Pharmaceutical Dosage Form. Int. J. Pharm Tech Res.2013,5(4), 1728-1735.
36. ICH validation of analytical procedures: text and, methodology Q2(R1), 2005.
37. ICH, International Convention on Quality for the PharmaceuticalIndustry, Toronto, Canada, 2002, September.
38. Snyder LR, Kirkland JJ, Glajch JL. Practical HPLC Method Development, 2nd ed., Wiley-Interscience, New York, 1997.

Image *Author -1*	***Amar F. Sabhadinde*** *Assistant Professor* *IBSS's Dr. Rajendra Gode Institute of Pharmacy, Mardi Road, Amravati-444 602, MS, India.*
Image *Author -2*	***Wrushali A. Panchale*** *Assistant Professor* *IBSS's Dr. Rajendra Gode Institute of Pharmacy, Mardi Road, Amravati-444 602, MS, India.*
Image *Author -3*	***Jagdish V. Manwar*** *Principal* *IBSS's Dr. Rajendra Gode* ***College*** *of Pharmacy, Mardi Road, Amravati-444 602, MS, India.*
Image *Author -4*	***Ravindra L. Bakal*** *Principal* *IBSS's Dr. Rajendra Gode* ***Institute*** *of Pharmacy, Mardi Road, Amravati-444 602, MS, India.*