Original Article

Development And Validation Of Stability Indicating Rp-Hplc Method For Determination Of Chlorthalidone

Ramanlal N. Kachave[1], Manoj S. Sonawane[2] and Sushil D. Patil[3*]

[1]Department of Pharmaceutical Chemistry, Amrutvahini College of Pharmacy, Sangamner, Affiliated Savitribai Phule Pune University, Maharashtra, India.
[2]Department of Pharmaceutical Quality Assurance, Amrutvahini College of Pharmacy, Sangamner, Affiliated Savitribai Phule Pune University, Maharashtra, India.
[3*]Department of Pharmaceutical Chemistry, Shree Mahavir Education Society's Mahavir Institute of Pharmacy, Varvandi Dist. Nashik, Maharashtra, India.

*Corresponding author: Sushil D. Patil, Department of Pharmaceutical Chemistry, Shree Mahavir Education Society's Mahavir Institute of Pharmacy, Varvandi Dist. Nashik, Maharashtra, India; Email: sushilpharmamet@gmail.com

Received 15.03.2022 , Revised 21.04.2022 , Accepted 28.05.2022
DOI: 10.47750/pnr.2022.13.S06.583

Abstract

An RP-HPLC stability-indicating method was made and tested to find out how much Chlorthalidone is in both the bulk medicine sold in stores and the individual tablet lots. The chromatographic separation was performed at a flow rate of 1 ml/min at a measurement wavelength of 275 nm on an Agilent C18 column (250 x 4.6 mm, 5 m) using Water and Methanol (30/70 v/v) as the mobile phase. LOD and LOQ (Lower and upper limits of detection) as well as stability tests were performed on the method. The linearity was analyzed between 5 and 25 g/ml, and a correlation value of 0.990 was discovered. Both 0.40g/ml and 1.20g/ml were found to be the analytical and detection limits, respectively. Chlorthalidone was exposed to acidic, alkaline, oxidative, photolytic, and thermal breakdown stress conditions. Chlorthalidone is more affected by acidic conditions than by oxidation. It is less affected by alkaline conditions, heat, and light. The method is simple, reliable, sensitive, and accurate. It can tell the difference between the drug and its broken-down product that forms under different stress conditions. This allows for its application as a stability-indicating technique for locating CHD in both bulk and pharmaceutical dose form.

KEYWORDS: Development, Chlorthalidone, Validation, RP-HPLC, Stability indicating.

INTRODUCTION

(RS)-2-Chloro-5-(1-hydroxy-3-oxo-2, 3-dihydro-1H-isoindol-1-yl) benzene-1-sulfonamide is the scientific name for chlorthalidone [1]. It is a chlorthalidone that is taken by mouth. It blocks the Na+/Cl- symporter in the apical membrane of distal convoluted tubule cells in the kidney, which stops sodium and chloride from being reabsorbed. The Na+/Cl symporters are blocked by thiazide diuretics, such as chlorthalidone. It also stops isoform of carbonic anhydrase in the proximal tubules from working well. It slows down the rate of filtration by globules. Diuretics like chlorthalidone made the person with kidney function worse [2]. It also makes more sodium reach the end of the renal tubule, which indirectly makes the potassium exchange process work better. As a result, the amount of potassium and salt in the blood drops. Chlorthalidone has no effect on how acidic or basic a person is. It causes a drop in blood pressure through

a long-term mechanism. It lowers blood pressure by lowering the flow of the heart and the amount of plasma and extracellular fluid in the body [3]. Chlorthalidone is a drug that is slowly absorbed by the GIT after being taken by mouth. It has a long half-life, and skipping a dose makes it last longer. Most chlorthalidone leaves the body through the kidneys. Stops the reabsorption of sodium and chloride directly at the luminal membrane of the early section of the distal convoluted tubule (DCT) in the kidney. This makes the body make more sodium, chloride, bicarbonate, and potassium, which makes it get rid of more water [4].

Pharmaceutical impurities are unwanted chemicals that stay in active pharmaceutical ingredients (APIs) or drug product formulations. Impurities in drug substances can come from the starting materials, intermediates, chemicals, solvents, catalysts, or by-products of a reaction, or they can be made during the synthesis process. During the process of making a drug product, impurities can also be made because some drug substances are inherently unstable, don't work well with added excipients, or react with packing materials. How safe the final pharmaceutical product is will depend on how much of different impurities are in the drug substances. Because of this, figuring out how to find, measure, qualifies, and control toxins are a very important part of making a new drug [4-6].

The International Conference on Harmonisation (ICH), the U.S. Food and Drug Administration (USFDA), and the European Medicines Agency (EMA) are some of the regulatory bodies that focus on controlling impurities [7]. Also, a number of official compendia, such as the British Pharmacopoeia (BP), the United States Pharmacopoeia (USP), the Japanese Pharmacopoeia (JP), and the European Pharmacopoeia (EP), have limits on impurity levels in APIs and drug formulations. An impurity profile is a full study of all the known and unknown impurities in a batch of API made using a specific controlled production method that keeps an eye on the same impurities throughout the development of a formulation. This helps find out the risk that comes with a drug's harm when it is taken by a patient. So, there have been a lot of pieces written about stability and analytical methods for impurities and products of forced degradation in pharmaceuticals [8-10].

The current study was done to find out how to identify and measure Chlorthalidone in bulk and in pill form. A force degradation study was also done to find out how stable Chlorthalidone is using an HPLC-DAD device [12].

EXPERIMENTAL

Materials: Niksan Pharmaceutical, Ankleshwar, India provided a complimentary sample of chlorthalidone bulk medication. Glacial acetic acid and acetonitrile of HPLC quality were bought from Merck. Ammonium acetate, hydrogen peroxide (6%) and sodium hydroxide (AR grade) were purchased from Loba Chemie. A Milli-Q system produced water with a high degree of purity [13].

Instrumentation: The chromatographic study's WATER 2695 HPLC was equipped with a quaternary pump, a manual injector, and a diode array detector. The system control, process monitoring, and data collection were all done using the Open Lab Software [14].

Chromatographic conditions: Specifically, a 250 mm x 4.6 mm x 5 m Agilent C18 analytical column was used. chromatographic separation and analysis were performed at room temperature. The optimised mobile phase of methanol and water (70/30 v/v) was adjusted to a pH of 4.8 with glacial acetic acid (GAA) and then propelled through the column at a flow rate of 1 ml/min. Before using the mobile phase, it must be ultrasonically degassed and filtered through a 0.45 m nylon membrane filter. The detection wavelength was set to 275 nm, and the injection volume was set to 20 l nm [15].

Standard compound working and stock solutions: A stock solution of chlorthalidone was made by weighing out 10.0 mg, dissolving it in acetonitrile, and adding enough water to fill a volumetric flask to the 10 ml mark. To make the working standard solutions, the stock solution was diluted with the mobile phase in the right way [16].

Force degradation studies: Chlorthalidone force breakdown tests were done in situations of hydrolysis (acid, base, and neutral), oxidation, heat, and light. For all of the solution state force decline tests, 20 g/ml of chlorthalidone was used instead of 100 g/ml. Samples were taken when the force was breaking down in different ways, and the drug solution was made to break down over a range of times. To study acid-base breakdown, 9 ml of 0.1N hydrochloric acid and 9 ml of 0.1N sodium hydroxide solutions were added to 10 ml volumetric flasks that each held 1 ml of a chlorthalidone stock solution. At 80 C, the flasks were warmed for 30 minutes. By adding water to 1 ml of a stock solution of chlorthalidone, neutral hydrolysis was done. After that, it was left alone at room temperature for 3 hours. To test oxidative breakdown, 1 ml of stock chlorthalidone solution was mixed with 6% H2O2 and left alone at room temperature for 30 minutes. For a study on how things break down when heated, a layer of chlorthalidone powder about 1 mm thick was put in a petri dish and heated at 105°C for 7 hours. For testing photolytic decline, a sample of the solution was put in a room with 200Whrm-2 of UV light [17-18].

Sample preparation: In the presence of acid and base, samples of chlorthalidone breakdown were neutralised, and the concentration was lowered to 20 g/ml by changing the volume with mobile phase. The samples for thermal, photolytic, neutral, and oxidative degradation were also made at the same quantity. Before the samples were analysed, they were all passed through a 0.45 nylon membrane filter [19-20].

Method Validation: Guidelines from the International Conference on Harmonisation (ICH) were used to test the method. Validations were done on the approach's linearity, precision, accuracy, limit of detection (LOD), limit of measurement (LOQ), and how stable it is [21].

1. **Linearity:** Five replicas of each of five known analyte concentrations in the range of 5–10 g/ml were injected to test the method's linearity. Plotting peak regions versus analyte concentration yielded the calibration curve [22].
2. **Precision:** By comparing three copies of each of the three concentrations of chlorthalidone (5, 10, and 15 g/ml) on different days (intraday and interday), the accuracy of the process was checked. The precision was given as a proportion of the relative standard deviation (%RSD) [23].
3. **Accuracy:** By spiking the tablet formulation solution in chlorthalidone standard solution, three concentration levels of 80% (36 g/ml), 100% (40 g/ml), and 120% (44 g/ml) were used to test the method's accuracy in triplicate. At each step, the drug recovery % was calculated [24].
4. **limit of quantification (LOQ) and Limit of detection (LOD):** The proposed chromatographic condition was used to calculate LOD and LOQ using the signal to noise ratio approach. LOD was regarded as being 3:1 and LOQ as being 10:1 [25].
5. **Robustness:** The method's durability was tested by changing the way the experiment was set up on purpose. Chromatographic settings like flow rate (0.9 ml/min and 1.1 mi/min) and wavelength (316 nm and 314 nm) were used to analyse samples of chlorthalidone standard solution (20 g/ml) [26].

System suitability Test: System appropriateness was evaluated by injecting three replicates of the standard solution and measuring the resulting area under the curve, theoretical plates, resolution, and peak asymmetry [27].

RESULTS AND DISCUSSION:

The analysis of chlorthalidone in both bulk and tablet form can now be performed using a unique, easy, and quick RP-HPLC method. The method was optimised so that the analyte and its degradation product could be separated adequately (enough theoretical plates and resolution between peaks), with sufficient sensitivity and proper peak symmetry (peak tailing factor > 2), in a short amount of time. To achieve this goal, we had to do research on a wide range of factors, such as mobile phase pH, buffer concentration, stationary phase concentration, mobile phase composition, flow velocity, and detector wavelength. Because hydrophilic stationary phases often provide sufficient retention for organic non-polar molecules, C18, a commonly used reversed-phase column, was employed to select the stationary phase. Using a C18 stationary phase column (Agilent C18 250 x 4.6mm, 5 m) and a mobile phase of

methanol and water (70/30 v/v) at a flow rate of 1 ml/min yielded the best results. The absorbance peak occurred at 275 nm, therefore that's what we used to measure with. With a retention time of 2.79 and a cycle duration of 5 minutes, it generated symmetrical peaks and achieved the best separation. System suitability data, including theoretical plate, resolution, and symmetric factor, are displayed in Table 1.

Method validation has been done in accordance with the ICH requirements to ensure that the technique is suitable for its intended use. Linearity, accuracy, precision, LOD, LOQ, and resilience were required for method validation. According to the calibration curve made from the peak area vs. analyte concentration (Table 2), the method was linear in the range of 5-10 g/ml with an excellent correlation value (r2 = 0.990). To examine the procedure's accuracy, a sample solution containing 20 g/ml of chlorthalidone was employed. The method's tolerable accuracy was shown by values for the percent relative standard deviation that were less than 2% (Table 3). The method's accuracy was estimated by recovery experiments to be between 99.0% and 101.0% (on average 100%) (Table 4). The sensitivity of the procedure was shown by the determination of the LOD and LOQ to be 0.40 and 1.20 g/ml, respectively (Table 2). The methodology's flexibility in terms of flow rate (0.1 ml/min) and maximum (1 nm) showed how reliable the established HPLC procedure was. Results showed that retention time and percent RSD values under 2 were unaffected by the study's chosen analyte concentration of 20 g/ml (Table 5).

Table 6 lists the outcomes of investigations on chlorthalidone's forced deterioration. The results showed that chlorthalidone degrades more readily under acidic and oxidative conditions than under alkaline, thermal, or photolytic conditions. The drug peak and its degradation product peak were sufficiently separated from one another (fig. 1, 2 and 3)

Table 1: System the suitability study of the indicated method

Compound	**t_R in min**	**N (**Theoretical plate**)**	**Rs (**Resolution**)**	**Symm. (**Symmetric factor**)**
Chlorthalidone	2.79	4010	4.54	1.6

N = theoretical plate; t_R= retention time; Symm.= symmetric factor; Rs = resolution

Table 2: Parameters for the quantification of chlorthalidone using a method

Parameters	**Results**
Regression equation	Y = 1390.49 X + 3689.90
Correlation coefficient (r^2)	0.990
Linear range (µg/ml)	5-25
LOD (µg/ml)	0.40
LOQ (µg/ml)	1.20

X is the concentration of Chlorthalidone in µg/ml; Y is the peak area at 275 nm

Table 3: Chlorthalidone's intra- and inter-day precision

Chlorthalidone (µg/ml)	**Intraday Precision**			**Interday Precision**		
	Mean	**SD**	**% RSD**	**Mean**	**SD**	**% RSD**
5	131129.8	112.10	0.090	130050	1081.0	0.79
10	211669.4	70.99	0.039	21450	120689	0.60
15	27596	51.90	0.020	308826	19999	0.59

RSD is relative standard deviation

Table 4: Recovery study of Chlorthalidone

Compound	Level in %	API Added in µg/ml	Spiked quantity in µg/ml	conc. Found in µg/ml	%Recovery	%RSD
Chlorthalidone	80	20	16	34.90	99.90	0.35
	100	20	20	39.90	99.40	0.96
	120	20	24	44.10	99.45	1.89

Table 5: Robustness study of Chlorthalidone

Parameter	Changes made	Chlorthalidone		%RSD
		Retention time	SD	
Flow rate	0.90 ml/min	3.015	1700.10	0.60
	1.0 ml/min	3.266	890.60	0.30
	1.10 ml/min	2.665	3310.3	1.19
Detection wavelength	274 nm	3.015	2310	0.79
	275 nm	2.936	690.60	0.30
	276 nm	2.934	1421.4	0.49

Table 6: Chlorthalidone investigation of force depreciation

Stress Condition	Condition Degradation	Number of degradants	Conc. Found in µg/ml	% Degradation
Acidic	80ºC for 30 min (0.1N HCl)	1	8.00	70.50
Alkaline	80ºC for 30 min (0.1N NaOH)	1	15.99	61.50
Neutral	Room temp. for 3hr water	-	14.99	56.99
Oxidative	Room temp. for 30 min 6% H_2O_2	-	12.00	79.60
Thermal	105ºC for 7 hr.	-	13.90	18.5
Photolytic	18 hr UV light	-	15.50	8.90

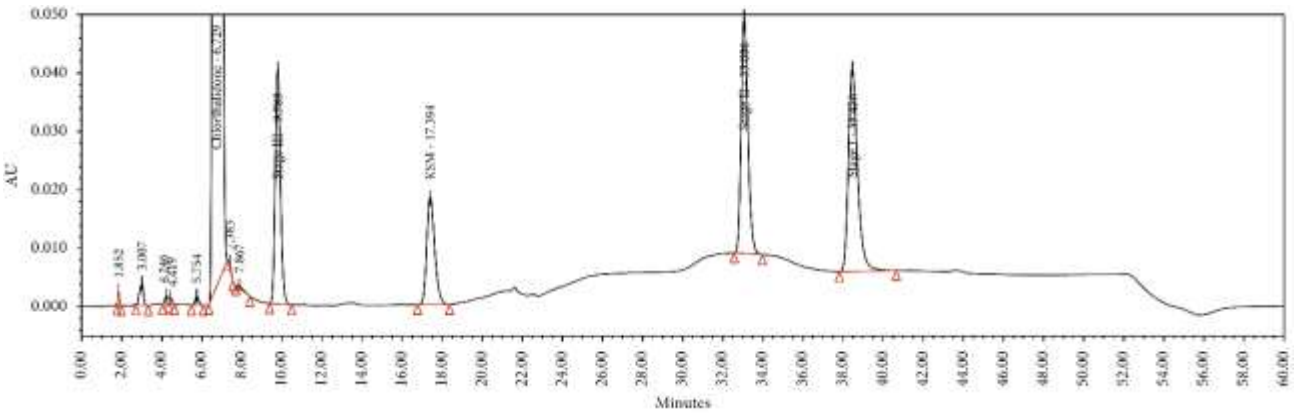

Figure 1: Chlorthalidone API and contaminants from the manufacturing process were added to a spiked solution.

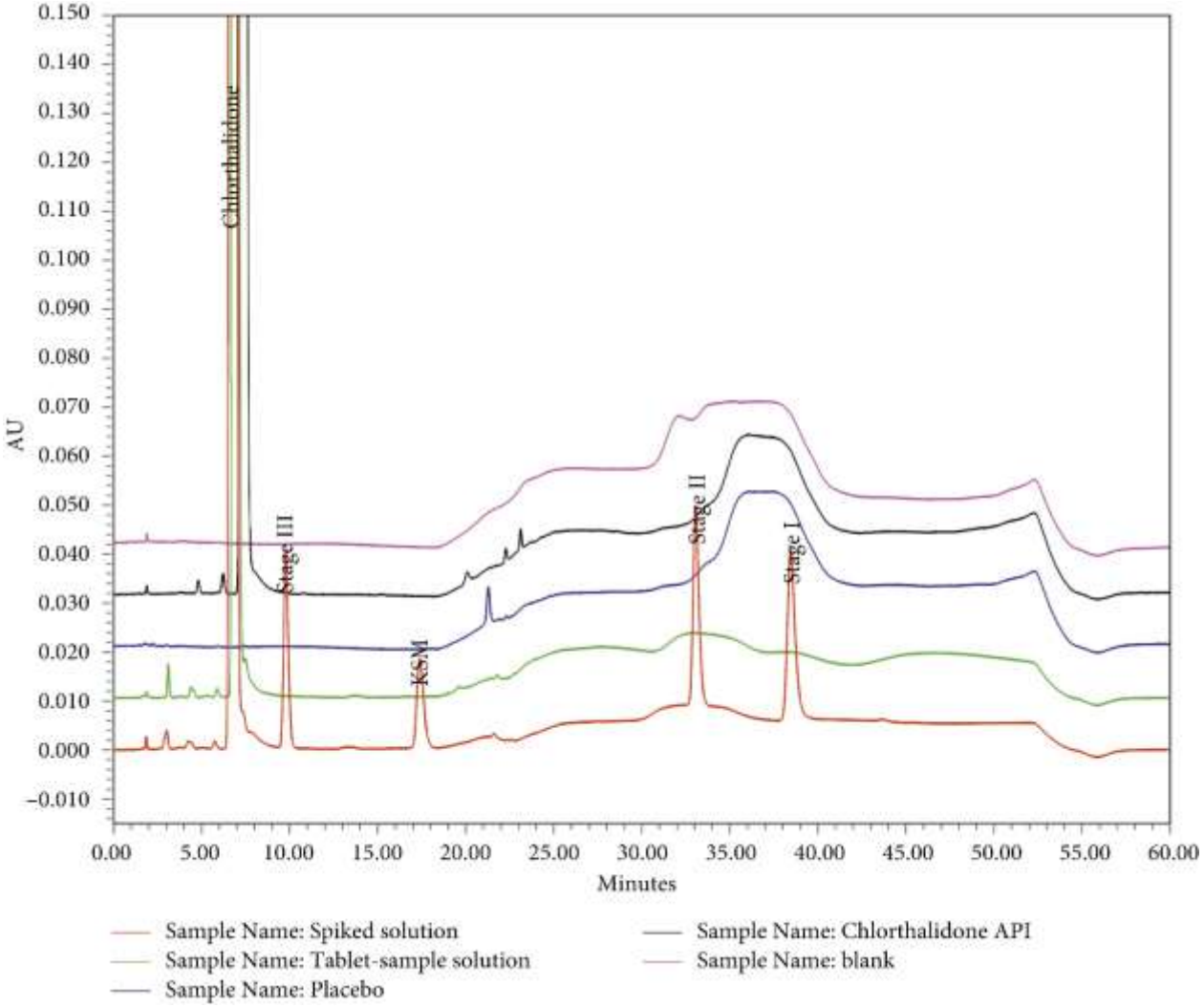

Figure 2: Chromatographic images of the blank solution, placebo solution for chlorthalidone tablets, formulation solution for chlorthalidone tablets, and chlorthalidone API should all be superimposed.

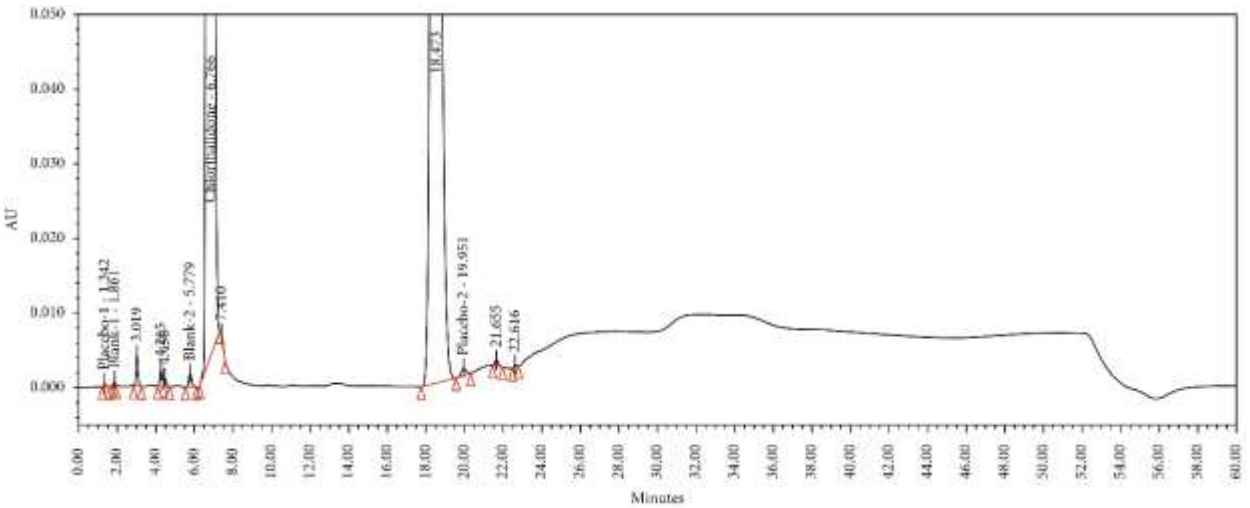

Figure 3: Chlorthalidone API sample with a stressed acid chromatogram.

Conclusion

In order to perform an analysis of chlorthalidone, a cutting-edge HPLC method was devised and then verified. The validation criteria demonstrated that the procedure was quick, precise, accurate, sensitive, and resilient. The method's specificity was successfully tested under stress, which showed that the testing was successful. It's possible that the regular analysis of chlorthalidone might benefit from and be applicable to this proposed procedure.

Funding support:

Nil

Conflict of interest:

Nil

REFERENCES:

1. Mhaske, R.A., Sahasrabudhe, S. and Mhaske, A.A., 2012. RP-HPLC method for simultataneous determination of irbesartan, losartan, hydrohlorothiazide and chlorthalidone-application to commercially available drug products. International journal of pharmaceutical sciences and research, 3(4), p.1116.
2. Mhaske, R.A., Garole, D.J., Mhaske, A.A. and Sahasrabudhe, S., 2012. RP-HPLC method for simultataneous determination of amlodipine besylate, valsartan, telmisartan, hydrochlorothiazide and chlorthalidone: application to commercially available drug products. International Journal of Pharmaceutical Sciences and Research, 3(1), p.141.
3. Kayesh, R., Jahan, M.S. and Sultan, M.Z., 2017. Development using response surface methodology and validation of a stability-indicating RP-HPLC method for simultaneous estimation of azilsartan medoxomil and chlorthalidone in solid dosage form. Chromatographia, 80, pp.593-603.
4. Sharma, V.K., Koka, A., Yadav, J., Sharma, A.K. and Keservani, R.K., 2016. Self-micro emulsifying drug delivery systems: A strategy to improve oral bioavailability.
5. Kharat, C., Shirsat, V.A., Kodgule, Y.M. and Kodgule, M., 2020. A validated RP-HPLC stability method for the estimation of chlorthalidone and its process-related impurities in an API and tablet formulation. International Journal of Analytical Chemistry, 2020.
6. Mhaske, R.A., Sahasrabudhe, S., Mhaske, A.A. and Garole, D.J., 2012. RP-HPLC method for simultataneous determination of atorvastatin calcium, olmesartan medoxomil, candesartan, hydrochlorothiazide and chlorthalidone-application to commercially available drug products. International Journal of Pharmaceutical Sciences and Research, 3(3), p.793.
7. Keservani, R.K., Bandopadhyay, S., Bandyopadhyay, N. and Sharma, A.K., 2020. Design and fabrication of transdermal/skin drug-delivery system. In Drug Delivery Systems (pp. 131-178). Academic Press.
8. Keservani, R.K. and Sharma, A.K., 2014. Flavonoids: emerging trends and potential health benefits. Journal of Chinese Pharmaceutical Sciences, 23(12), pp.815-822.
9. Keservani, R.K., Sharma, A.K. and Kesharwani, R.K., 2017. An overview and therapeutic applications of nutraceutical and functional foods. Recent advances in drug delivery technology, pp.160-201.

10. Chaudhary, B.R. and Dave, J.B., 2017. Development and validation of stability indicating gradient RP-HPLC method for simultaneous estimation of telmisartan and chlorthalidone in bulk API and fixed dose combination. World J Pharm Res, 6(10), pp.1015-1029.
11. Sharma, A., Mishra, A. and Sharma, S., 2016. Stability indicating simultaneous validation of telmisartan, cilnidipine and chlorthalidone with forced degradation behavior study by RP-HPLC in tablet dosage form. International Journal of Chemistry and Pharmaceutical Sciences, 7, pp.6-12.
12. Sravani, P., Rubesh Kumar, S., Duganath, N. and Devanna, N., 2014. Method development and validation for the simultaneous estimation of azilsartan and chlorthalidone by RP-HPLC in pharmaceutical dosage form. Int J Pharm Sci. 2014; 4 (5): 725, 729.
13. Sharma, A.K., Keservani, R.K., Dadarwal, S.C., Choudhary, Y.L. and Ramteke, S., 2011. Formulation and in vitro characterization of cefpodoxime proxetil gastroretentive microballoons. Daru: Journal of Faculty of Pharmacy, Tehran University of Medical Sciences, 19(1), p.33.
14. Kumar, T.H., Begum, A. and Sankar, D.G., 2019. Determination of azilsartan medoximil and chlorthalidone in tablets exposed to forced degradation by using RP-HPLC. Biomedical Research, 30(5), pp.10-35841.
15. Patel, B., Chaudhary, A. and Gami, S., 2019. RP-HPLC method development and validation for simultaneous estimation of benidipine hydrochloride, telmisartan and chlorthalidone in Tablet.
16. Surana, K., Chaudhary, B., Diwaker, M. and Sharma, S., 2018. Benzophenone: A ubiquitous scaffold in medicinal chemistry. MedChemComm, 9(11), pp.1803-1817.
17. Kesharwani, R.K., Vishwakarma, V.K., Keservani, R.K., Singh, P., Katiyar, N. and Tripathi, S., 2020. Role of ADMET tools in current scenario: application and limitations. Computer-Aided Drug Design, pp.71-87.
18. Keservani, R.K. and Karthikeyan, C., 2010. Chewing gum as a drug delivery system. Archives of Applied Science Research, 2(2), pp.79-99.
19. Keservani, R.K., Sharma, A.K. and Kesharwani, R.K. eds., 2017. Drug Delivery Approaches and Nanosystems, Volume 1: Novel Drug Carriers. CRC Press.
20. MP, P., Smitha, M. And Das, G., 2020. Estimation of Chlorthalidone API Tablet for Stability Indication and Validation using RP-HPLC Techniques. International Journal of Pharmaceutical Research (09752366).
21. Masthanamma, S.K., 2014. Stability Indicating RP-HPLC method for determination of Azilsartan medoxomil in Pharmaceutical dosage form. Research Journal of Pharmacy and Technology, 7(2), pp.168-172.
22. Pawar, V.T., Pawar, S.V., More, H.N., Kulkarni, A.S. and Gaikwad, D.T., 2017. RP-HPLC method for simultaneous estimation of cilnidipine and chlorthalidone. Research Journal of Pharmacy and Technology, 10(11), pp.3990-3996.
23. Vanaja, N., Preethi, C., Manjunath, S.Y. and Pal, K., 2015. Method development and validation for simultaneous estimation of telmisartan and chlorthalidone by RP-HPLC in pharmaceutical dosage form. Asian Journal of Pharmaceutical Analysis, 5(4), pp.171-177.
24. Rai, K. and Rao, N., 2020. Development And Validation Of Novel Rp-Hplc Method For Related Substances In Chlorthalidone And Fimasartan Formulations. hypertension, 11, p.12.
25. Naazneen, S. and Sridevi, A., 2014. Stability-indicating RP-HPLC method for the simultaneous estimation of azilsartan medoxomil and chlorthalidone in solid dosage forms. International Journal of Pharmacy and Pharmaceutical Sciences, 6(6), pp.236-43.
26. Sonawane, S., Jadhav, S., Rahade, P., Chhajed, S. and Kshirsagar, S., 2016. Development and validation of stability-indicating method for estimation of chlorthalidone in bulk and tablets with the use of experimental design in forced degradation experiments. Scientifica, 2016.
27. Kanthale, S.B., Thonte, S.S. and Mahapatra, D.K., 2019. Stability indicating RP-HPLC method for the simultaneous estimation of ivabradine and metoprolol in bulk and tablet formulation. Journal of Applied Pharmaceutical Science, 9(4), pp.137-144.