WWW.IJRPR.COM

International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

Novel RP-HPLC Method for the Simultaneous Estimation of Candesartan and Hydrochlorothiazide in Bulk and Tablet Formulation

[1]Ravi Shankar Sahu, [2]Shailesh Pradhan, [3]Chandrakant Sahu, [4]Hitesh Bhoi, [5]Nikhil Rathor, [6]Mukesh Patel

[1]M. Pharma, Department of Pharmaceutics, Columbia Institute of Pharmacy, Tekari, Raipur
[2]M. Pharma, Department of Pharmaceutical Quality Assurance, Columbia Institute of Pharmacy, Tekari, Raipur
[3]M. Pharma, Department of Pharmaceutical Quality Assurance, Columbia Institute of Pharmacy, Tekari, Raipur
[4]M. Pharma, Department of Pharmacology, Columbia Institute of Pharmacy, Tekari, Raipur
[5]M. Pharma, School of Pharmacy Chouksey Engineering College, Bilaspur
[6]M. Pharma, Department of Pharmaceutical Quality Assurance, Columbia Institute of Pharmacy, Tekari, Raipur
Email Id pradhanshailesh551@gmail.com

ABSTRACT

No single publication was found while scanning the common international databases for literature on any analytical RP-HPLC approach for the routine simultaneous determination of candesartan (CDS) and hydrochlorothiazide (HCT) combinations in a pharmaceutical tablet formulation. A straightforward, robust, accurate, cost-effective, and easy solution was developed to solve the issue. Establishing a validated RP-HPLC approach for CDS and HCT estimation in bulk and tablet formulation is the aim of this work. The method was created using a 250 mm × 4 mm 5 µm Qualisil-5 BDS C_8 column. ACN and water were used to make up the mobile phase, which was provided at a flow rate of 1.0 mL/min and detected at a wavelength of 272 nm. The experiment was run at room temperature with an injection volume of 10 µL. The retention times of OFL (internal standard), CDS, and HCT were 2.146 min, 2.548 min, and 3.883 min, respectively. Linearity, accuracy, precision, and robustness—the four validation criteria—were all found to be within acceptable bounds. For both CDS and HCT, the calibration plots were produced between 5 and 60 µg/mL, with r^2 values of 0.998 in each case. With an RSD of <2, the recovery of CDS and HCT was determined to be 99.8% and 99.1%, respectively. The regular analysis of CDS and HCT in bulk as well as in tablet dosage form may be carried out using this RP-HPLC technique, which has been proven to be quick, specific, exact, and accurate. With less analytical time and a perfectly defined peak, the separation was complete.

Keywords: Candesartan, Hydrochlorothiazide, RP-HPLC, Bulk, Tablet, Simultaneous estimation

INTRODUCTION

The chemical compound of candesartan (CDS) is 1-[[(cyclohexyloxy)carbonyl]oxy]ethyl 2-ethoxy-1-[[2'-(1*H*-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl]. Angiotensin II receptor antagonist -1H-benzimidazole-7-carboxylate is selective for AT_1 receptors and has a tight binding and gradual receptor dissociation **(Figure 1A)**. It has no agonistic properties. During absorption from the gastrointestinal system, ester hydrolysis quickly transforms it into the drug's active component, candesartan [1].

Chemically, hydrochlorothiazide (HCT), a first-line thiazide class diuretic medication, is 6-chloro-1,1-dioxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7 sulfonamide **(Figure 1B)**. It works by initially boosting salt and water excretion before reducing blood pressure. Extracellular volume is reduced as a result, which lowers cardiac output and renal blood flow. Plasma volume returns to a normal level with continued therapy, although peripheral resistance falls [2].

According to a review of the literature, three HPLC techniques were presented for the quantification of HCT [3-5] alone in biological fluids, while spectrophotometric [6], HPLC [7], and LC-MS [8] methods were devised for the analysis of CDS alone. For the simultaneous assessment of CDS and HCT in pharmaceutical formulations and biological fluids, only a few numbers of analytical techniques, including the spectrophotometric [9], HPLC [10–12], and HPTLC [13] approaches, have been established to date.

No single publication was found while scanning the common international databases for literature on any analytical RP-HPLC approach for the routine simultaneous determination of CDS and HCT combinations in a pharmaceutical tablet formulation. A straightforward, robust, accurate, cost-effective, and easy solution was developed to solve the issue. Establishing a validated RP-HPLC approach for CDS and HCT estimation in bulk and tablet formulation is the aim of this work.

EXPERIMENTAL

Chemical

Micro Labs Ltd., Sikkim, India, received CDS and HCT as a kind gift sample. The local pharmacy store was the source for the Candelong H® pill (10 mg CDS and 10 mg HCT), which is a product of Micro Labs Ltd., Sikkim, India. We bought HPLC grade water and acetonitrile (ACN) from Merck Life Science Ltd. in Bengaluru, India.

Instrumentation

The solvent delivery module LC-20AD Shimadzu liquid chromatography pump with 10 µL loop and SPD-M20A PDA detector made up the HPLC system, which was run using LCMS solution software. The separation procedure made use of a Qualisil-5 BDS C8 column (250 mm × 4.6 mm; 5 µm). On a Shimadzu® (Kyoto, Japan) AUW220D balance, the weighing was done. We measured the pH with a digital VSI® VSI-1B pH meter from Mohali, India. Sonication took place using a Transonic Digital S sonicator from Mumbai, India.

Mobile Phase

Selection of the mobile phase

The mobile phase for the solutes' elution must be properly chosen. The theoretical plates, peak purity index, and peak symmetry were used to choose the mobile phase. Buffer systems and an eluant such acetonitrile, methanol, or another solvent were used to start the investigation. Elution with a similar ratio of buffer KH_2PO4 and methanol led to low-intensity peaks with a lot of tailing. The use of acetonitrile and KH_2PO4 buffer (pH 4.8) led to the production of a wide peak with tailing, which was an improvement over the prior experiment. When water was used as the buffer, the peak symmetry much improved and tailing was decreased, but the solutes were still not sufficiently eluted. ACN was mixed with water to produce a sharp peak with a nice Gaussian peak. The most theoretical plates and highest peak purity index were created at the 84:16 v/v ratio. The mobile phase was filtered through a 0.45 µm membrane filter after being degassed under vacuum. The mobile phase was allowed to equilibrate until it reached a stable baseline.

Preparation of Mobile Phase

The ratio of water: ACN was 84:16 v/v. Before being filtered under vacuum using a 0.45 µm membrane filter, the aforementioned solution was sonicated for 5 minutes to degas it.

Preparation of Diluent

ACN: methanol of HPLC grade (50:50 v/v) was created and used as a diluent all across the investigation.

Chromatographic Condition

The method was created using a 250 mm × 4 mm 5 µm Qualisil-5 BDS C_8 column. ACN and water were used to make up the mobile phase, which was provided at a flow rate of 1.0 mL/min and detected at a wavelength of 272 nm. The experiment was run at room temperature with an injection volume of 10 µL.

Standard stock solution

100 mg each of CDS and HCT were precisely weighed before being added to two different 100 mL volumetric flasks with 50 mL of diluent. The contents were dissolved using sonication for five minutes. After being brought up to the mark using diluent, the volume was diluted to the necessary concentrations.

Preparation of Internal standard

A 100 mL volumetric flask containing 50 mL of diluent was filled with OFL after it had been precisely weighed (100 mg). Using sonication, the substance was dissolved for 10 minutes. The amount was raised to 100 mL using diluent, then the aliquot was further diluted to a concentration of 10 µg/mL.

Analysis of drugs in marketed formulation

Twenty pills in total were weighed and properly pulverized. With 50 mL of diluent added, 0.2758 mg of tablet powder equivalent to 10 mg of CDS and 10 mg of HCT was precisely weighed and added to a 100 mL volumetric flask. The sample was sonicated for 15 minutes to dissolve it, and then diluent was used to raise the volume to the necessary amount. A membrane filter with a 0.22 µm pore size was used to filter the resulting solution. After that, the filtrate was diluted to create solutions with 20 µg/mL each of CDS and HCT. The diluted sample was examined using HPLC.

Method validation

The USFDA's guidance together with the Q2A and Q2B standards from the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) was used to verify the approach.

Linearity and Range

The linearity of the technique was examined for the CDS and HCT tests using seven different solute concentrations, ranging from 5 to 60 µg/mL. The diluent was used to make the solutions, and an equal amount was then injected into the HPLC device to calculate the peak area. The average area and concentration of each solute were plotted on a linearity graph. Additionally, the regression coefficient's r^2 value was calculated [13].

Accuracy

By adding reference drug solutions at concentrations of 50%, 100%, and 150%, the suggested method's accuracy was evaluated (recovery). Three times the experiment was run, and the findings were reported as a percentage of recovery. Based on the concentrations, the % relative error was calculated and appropriately drawn [14].

Precision

Spiking concentrations of 40%, 60%, and 80% six times in a single day (intra-day) and on three distinct days allowed researchers to assess the recommended method's accuracy in terms of intra-day and inter-day variability (inter-day). The toughness research also involved two analysts. The data was expressed as a percentage relative error precision [15].

Robustness

By changing the flow rate from 0.8 mL/min to 1.2 mL/min while leaving all other chromatographic parameters alone, the robustness of the procedure was examined [16].

Systems suitability parameters

Injecting five times the standard solution and tracking data like retention length, peak area, theoretical plates, and tailing factor allowed for the determination of the analytical method's repeatability profile [17].

Limit of detection and quantification

The limit of detection (LOD) is the lowest concentration that any analytical method can detect, albeit it is not required to specify the precise quantity [18].

The limit of detection (LOD) was determined by the formula:

$$\mathbf{LOD = 3.3\ (\sigma / S)}$$

Where, **σ =** standard deviation of response; **S** = slope of the calibration curve. The slope S may be estimated from the calibration curve of the analyte.

The smallest amount that can be quantified using any analytical procedure with a specific level of accuracy and precision is known as the limit of quantification (LOQ) [19].

The limit of quantification (LOQ) is determined by the formula:

$$\mathbf{LOQ = 10\ (\sigma / S)}$$

Where, **σ =** standard deviation of response; **S** = slope of the calibration curve. The slope S may be estimated from the calibration curve of the analyte.

RESULTS AND DISCUSSION

Method development and optimization of chromatographic conditions

Since there were no existing equivalent processes, the new methodology was entirely developed by trial and error. However, the choice of the stationary phase was significantly influenced by earlier investigations. Peak tailing was minimized and the robustness of the analytical technique was significantly increased by keeping the mobile phase at a low pH. The use of acidic pH was more strongly justified since silica-based reversed-phase columns with high basic pH caused dissolution. It was also found that the pKa of the solute and the pH of the mobile phase were relatively comparable, allowing them to remain in the unionized form. As a result, two units were used to calculate the pH value.

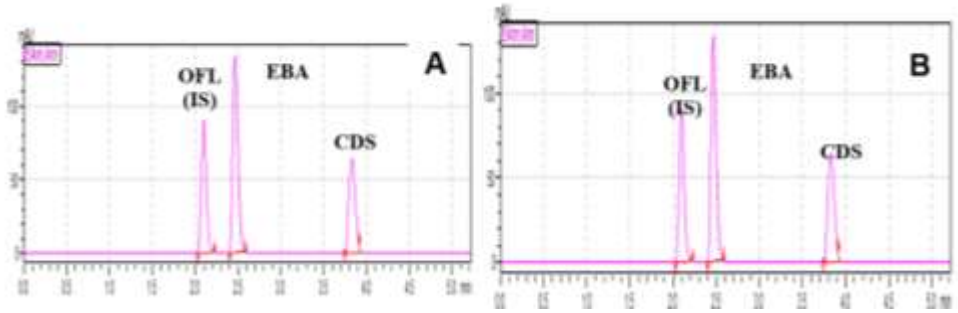

Figure 1. Chromatograms: (A) Standard solution (B) Sample solution.

The HPLC procedure, which was designed utilizing a Qualisil-5 BDS C8 column (250 mm 4 mm, 5 m), was used to perform the elution. With an injection volume of 10 L, ACN and water were mixed in a ratio of 84:16 v/v, delivered at a flow rate of 1.0 mL/min, and detected at a wavelength of 272 nm at the column's ambient temperature. The mobile phase-containing blank solution had no such peak and a clear baseline, indicating a steady elution mechanism. **Figure 1B** shows that the retention times of the sample solutions OFL (internal standard), CDS, and HCT were 2.146 min, 2.548 min, and 3.883 min, respectively. These times nearly matched those of the standard solution **(Figure 1A)**. It was discovered that the percentage label claim for the sold product Candelong H® was 99.80% for MKN and 99.10% for HCT, respectively **(Table 1)**. This demonstrated unequivocally that the recommended analytical approach was precise, accurate, and reliable for regular medicine combination analysis in tablets.

Table 1: Assay of the marketed formulation.

Marketed formulation	Drug	Label claim (mg/tablet)	Estimated amount (mg/tablet)	% label claim
Candelong H	CDS	10	9.98	99.80% SD (± 0.241)
	HCT	10	9.91	99.10% SD (± 0.279)

Method validation

Linearity and range

With linear regression equations of y = 10.988x - 0.4287 for CDS and y = 1.264x - 1.154 for HCT, respectively, both substances demonstrated extremely good linearity between concentration and peak area in the range of 5-60 µg/mL. The entire regression coefficient values were 0.998, indicating that all of the results were very linear **(Figure 2)**

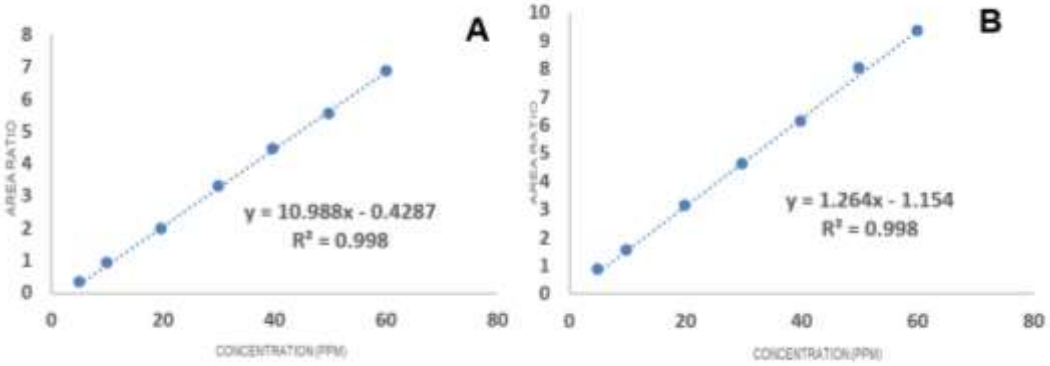

Figure 2. Linearity plot of (A) CDS and (B) HCT.

Accuracy

The Y-intercept and slope of the graph were used to establish, in part, the percent recovery characteristic of the suggested approach for simultaneous analysis by utilizing the calibration curve. The observed % RSD values for CDS in all three concentrations were 0.2234, 0.0761, and 0.1948. In the case of HCT, the % RSD values were 0.2772, 0.0728, and 0.1667 for each of the three concentrations. The US Pharmacopeia's acceptance standard of less than 2% was satisfied by all of them **(Table 2)**. In the end, the procedure showed that the data obtained was accurate.

Table 2: % Recovery for CDS and HCT.

% Recovery	50 %		100%		150%	
Particulars	**CDS**	**HCT**	**CDS**	**HCT**	**CDS**	**HCT**
Amount present (mg)	10	10	10	10	10	10
	10	10	10	10	10	10
	10	10	10	10	10	10
Amount of Std. added (mg)	4.9	4.7	9.7	9.6	14.8	14.4
	4.6	4.4	9.7	9.3	14.3	14.2
	4.7	4.6	9.7	9.6	14.6	14.4
Amount of Recovered (mg)	14.70	14.55	19.60	19.40	24.50	24.25
	14.51	14.24	19.47	19.13	24.11	23.88
	14.55	14.51	19.54	19.33	24.41	24.11
% Recovery	99.08	99.35	99.80	99.29	99.20	99.38
	99.52	99.05	99.26	99.16	99.21	99.11
	99.35	99.60	99.83	99.28	99.54	99.08
Mean	99.31	99.33	99.63	99.35	99.31	99.16
SD	0.2218	0.2753	0.0786	0.0723	0.1934	0.1652
% RSD	0.2234	0.2772	0.0761	0.0728	0.1948	0.1667

Precision

Both intra-day and inter-day variability testing for precision data showed that the method was extremely accurate across the measured ranges for CDS and HCT. With an RSD of less than 2%, the peak area of the sample solution consistently matched that of the standard solution. For CDS in the concentration range of 10–30 µg/mL, the% RSDs in terms of intraday precision lay in the range of 0.83%–0.91%, whereas for HCT in the concentration range of 10–30 µg/mL, the% RSDs in terms of intraday accuracy fall in the range of 0.83%–0.92% **(Table 3)**. The percentage RSDs were found to be between 0.7668 and 0.8494% and 0.4130 and 0.8303%, respectively, for the interday precision for the medicines CDS and HCT in the concentration range of 10 to 30 µg/mL **(Table 4)**. When the ruggedness test was carried out between two analysts for both medications in the tested range, the percentage RSDs were within the allowed limit of 2%, i.e. 0.0077-0.0091 for CDS and 0.0038-0.0047 for HCT **(Table 5)**.

Table 3: Intraday Precision.

S. No.	CDS			HCT		
	Concentration (µg/mL)	**± SD**	**% RSD**	**Concentration (µg/mL)**	**± SD**	**% RSD**
1	10	0.894427	0.91	10	0.894427	0.92
2	20	0.752773	0.76	20	0.816497	0.83
3	30	0.816497	0.83	30	0.816497	0.83

Table 4: Interday Precision.

S. No.	Day	CDS			HCT		
		Concentration (µg/mL)	± SD	% RSD	Concentration (µg/mL)	± SD	% RSD
1	Day 1	10	0.8366	0.8494	10	0.4082	0.4130
	Day 2						
	Day 3						
2	Day 1	20	0.7527	0.7668	20	0.8164	0.8303
	Day 2						
	Day 3						
3	Day 1	30	0.8366	0.8494	30	0.8164	0.8275
	Day 2						
	Day 3						

Table 5. Ruggedness study.

Drug	Parameter	Concentration (µg/mL)	±SD	%RSD
CDS	Analyst 1	10	0.8366	0.0091
	Analyst 2	10	0.8164	0.0077
HCT	Analyst 1	10	0.8944	0.0047
	Analyst 2	10	0.7527	0.0038

Robustness

The chromatogram for both drugs was significantly altered as a result of the deliberate alteration of flow rate, one of the most important chromatographic parameters. When the flow rate was increased or decreased by 0.2 mL/min, there was a little (5%) change in the peak area, theoretical plates, retention time, and tailing factor for both CDS and HCT **(Table 6)**. The purposeful modification of the flow rate for all of the drugs demonstrated the capability of the proposed approach to survive such modifications.

Table 6. Robustness study.

		CDS			HCT		
Robustness parameter		Retention Time (Rt)	USP Tailing	%RSD of peak areas	Retention Time (Rt)	USP Tailing	%RSD of peak areas
Flow rate	0.8	3.79	0.892	0.75	2.49	1.345	0.82
	1	3.8	0.895	0.76	2.5	1.347	0.83
	1.2	3.86	0.896	0.77	2.52	1.349	0.84
Avg.		3.8166	0.8943	0.76	2.5033	1.347	0.83
SD		0.0378	0.0020	0.01	0.0152	0.002	0.01
%RSD		0.9919	0.2327	1.3157	0.6101	0.1484	1.2048

Systems suitability parameters

The system suitability features of the suggested technique demonstrated a good level of repeatability and may be applied to routine study of drug combinations. The average retention time (Rt) and mean theoretical plate (TP) for the suggested method for MKN were 3.886 minutes and 7643 minutes, respectively. The Rt and TP for HCT were 2.551 minutes and 6892, respectively **(Table 7)**. A tailing value of less than 2% in every case showed no

specific tailing. The symmetric and asymmetric components of an ideal Gaussian peak with complete peak symmetry (asymmetric factor = 1) are of equal magnitude. The assertion that the suggested method met the US Pharmacopoeia monograph's minimal requirements (minimum theoretical plates of 2000 and tailing factor of less than 2%), demonstrating outstanding resolution, isolation, column efficacy, and repeatability. The separation factor (α) and resolution factor (Rs) were much higher than the ICH limits and essential guidelines of 1 and 1.5, respectively, showing that the recommended analytical procedure generates stronger isolation of both peaks with significantly less tailing and greater resolution. The approach may be applied to routine analysis because of its high precision, reproducibility, and accuracy.

Table 7. System suitability and validation parameter.

Parameters	CDS	HCT
Theoretical plates	7643	6892
Resolution	8.2	3.4
Asymmetric factor	0.953	1.136
Retention time (min)	3.886	2.551

Limit of detection and quantification

While the LOD and LOQ for CDS and HCT were 0.152 µg/mL and 0.2382 µg/mL, respectively, and 0.1322 µg/mL and 0.1752 µg/mL, respectively, these results show the method's excellent ability to simultaneously detect the solute at its lowest possible concentration from a mixture or formulation.

CONCLUSION

The new RP-HPLC method was developed for the simultaneous measurement of candesartan and hydrochlorothiazide from the combination dosage form at 272 nm using a C_8 column with water and acetonitrile. The run time of the drug components was little under 5 minutes, making short daily quality assurance application in industries feasible. The approach was validated in accordance with ICH requirements for linearity, accuracy, specificity, intraday and interday, precision, and robustness. The theoretical plates, % RSD, and tailing values all complied with the US Pharmacopoeia's minimal standards. This will be very beneficial for quality assurance and control for chemists. The method's great precision, repeatability, and accuracy make it suitable for regular analysis.

REFERENCES

1. Mahapatra DK, Bharti SK. Handbook of Research on Medicinal Chemistry: Innovations and Methodologies. Taylor & Francis; 2017.

2. Mahapatra DK, Bharti SK. Medicinal Chemistry with Pharmaceutical Product Development. CRC Press; 2019.

3. Naseem AC, Mohammad B, Enas A and Khalid Ibrahim HA, “Determination of Candesartan cilexetil in tablet dosage forms and dissolution testing samples by first derivative UV spectrophotometric method” Analytical Letters, 2009, 42(14), 2232 – 2243.

4. Subba Rao DV, Radhakrishnanand P, Suryanarayana MV, Himabindu V,”A stability-indicating LC method for Candesartan cilexetil” Chromatographia, 2007, 66 (7/8), 499- 507.

5. Surbhi Mehta, Ravi PS, Rajkamal P and Saranjit S, “LC and LC-MS/TOF studies on stress degradation behaviour of candesartan cilexetil” J. Pharm. Biomed. Anal., 2010, 52 (3), 345-354. 6. Be-Sheng K, Arun M, David RO and Kin-Kai H, “Columnswitching High Performance Liquid Chromatographic (HPLC) determination of Hydrochlorothiazide in rat, dog and human plasma” Pharmaceutical Research, 1990, 7 (12), 1257- 1261.

7. Richter K, Oertel R and Kir W, “New sensitive method for the determination of hydrochlorothiazide in human serum by high-performance liquid chromatography with electrochemical detection” J. Chromatogra. A, 1996, 729 (1- 2), 293-296.

8. Dragica Z, Traj e S, Petar M, “Development of solid-phase extraction method and its application for determination of hydrochlorothiazide in human plasma using HPLC” Biomedical Chromatography, 2004, 18 (2), 71-76.

9. Erk N, “Application of first derivative UV-spectrophotometry and ratio derivative spectrophotometry for the simultaneous determination of candesartan cilexetil and hydrochlorothiazide” Pharmazie, 2003, 58 (11), 796-800.

10. Qutab SS, Razzaq SN, Ashfaq M, Shuja ZA and Khan IU “Simple and sensitive LC–UV method for simultaneous analysis of hydrochlorothiazide and candesartan cilexetil in pharmaceutical formulations” Acta Chromatographica, 2007, 19, 119-129.

11. Erk N, “Simultaneous Analysis of Candesartan Cilexetil and Hydrochlorothiazide in Human Plasma and Dosage Forms Using HPLC with a Photodiode Array Detector” J. Liq. Chromatogra. & Rel. Tech.” 2003, 26 (15), 2581 – 2591.

12. Alaa Khedr, “Simultaneous Determination of Candesartan Cilexetil and Hydrochlorothiazide by High-Performance Liquid Chromatography” The J. King Abdulaziz UniversityMedical Sciences, 2008, 15 (2), 3-13.

13. Kanthale SB, Thonte SS, Mahapatra DK, 2019. Stability Indicating RP-HPLC Method for the Simultaneous Estimation of Ivabradin and Metoprolol in Bulk and Tablet Formulation. J Appl Pharm Sci. 9(4), 137-144.

14. Prakash O, Mahapatra DK, Singh R, et al, 2018. Development of a New Isolation Technique and Validated RP-HPLC method for Quercetin and Kaempferol from Azadirachta indica leaves. Asian J Pharm Anal. 8(3), 164-168.

15. Kanthale SB, Thonte SS, Mahapatra DK, 2019. Development of Validated Stability Indicating RP-HPLC Method for the Estimation of Glecaprevir and Pibrentasvir in Bulk and Pharmaceutical Dosage Form. J Appl Pharm Sci. 9(6), 52-60.

16. Deodhe S, Dhabarde DM, Kamble MA, et al, 2017. Development and validation of a novel stability indicating RP-HPLC method for the estimation of entecavir in tablet formulation. Eur J Anal Chem. 12(3), 223-235.

17. Deodhe S, Dhabarde DM, Kamble MA, et al, 2017. Novel stability indicating RP-HPLC method for the estimation of pinaverium bromide in tablet formulation: Assay development and validation. Eur J Anal Chem. 12(2), 3-16.

18. Puranik M, Shambharkar S, Nimbalkar S, et al, 2020. Comparison of UV Spectrophotometric and RP-HPLC Method for the Estimation of Deflazacort in Solid Dosage Form. J Appl Pharm Sci. 10(7), 82-88.

19. Sawale V, Dhabarde DM, Mahapatra DK, 2017. Development and validation of UV spectrophotometric method for simultaneous estimation of olmesartan medoxomil and chlorthalidone in bulk and pharmaceutical dosage form. Eur J Anal Chem. 12(1), 55-66.