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Research Article

# Stability-indicating Assay UHPLC Method for Simultaneous Analysis of Cilnidipine and Chlorthalidone in Bulk and Pharmaceutical Matrices

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ABSTRACT

A simple, rapid, precise and accurate stability-indicating ultra-high performance liquid chromatography (UHPLC) method was developed for the simultaneous estimation of Cilnidipine and Chlorthalidone in bulk material and pharmaceutical matrix. Accordingly, the scientific novelty of designed work was to develop a specific and precise stability-indicating UHPLC assay method for the simultaneous quantification of cited drugs in an extended-release fixed dose combinations (FDC). The reversed-phase UHPLC resolution was analyzed with the assistance of UPLC BEH C18 (150 mm × 2.1 mm) with 1.7 µm particle size column at ambient temperature using a solvent system in a proportion of (70:30% v/v) acetonitrile and water with a flow rate of 0.4 mL/minutes of a solvent system. The analytes were supervised at 275 nm by employing photodiode array recognition. The retention times were 1.731 ± 0.02 and 1.061 ± 0.022 minutes for Cilnidipine and Chlorthalidone, respectively. The Cilnidipine and Chlorthalidone have confirmed the linearity ranges of 2.0–12 and 2.5–15 µg/mL, with 0.9998 and 0.9994 determination coefficients. The UHPLC method was effectually validated for accuracy, precision, sensitivity, robustness, ruggedness, and selectivity, and specificity. Moreover, the anticipated UHPLC method's capability to analyze the cilnidipine and chlorthalidone with no obstruction from degradation products.

## Introduction

Cilnidipine (CIL) chemically is 2-Methoxyethyl (2E)-3-phenyl-2-propen-1-yl 2,6-dimethyl-4-(3-nitrophenyl)-1, 4-dihydro-3, 5-pyridinedicarboxylate. It acts as an antihypertensive and is a calcium channel blocker which relaxes blood vessels and makes the heart more efficient at pumping blood throughout the body. The molecular formula is $C_{27}H_{28}N_2O_7$, and molecular weight 492.528 g/moL. It is sparingly soluble in methanol and insoluble in water. It has a pKa value of 11.39. Chlorthalidone (CHL) chemically is 2-chloro-5-(1-hydroxy l-3-oxo-2, 3-dihydro-1H-isoindol-1- yl) benzene-1-sulfonamide. It is a thiazide diuretic which removes extra water and certain electrolytes from the body by increasing the amount of urine produced. Over time it also works by relaxing blood vessels and improving blood flow. Compared with other medications of the thiazide class, chlortalidone has the longest duration of action but a similar diuretic effect at maximal therapeutic doses. The molecular formula is $C_{14}H_{11}ClN_2O_4S$, and molecular weight 338.762 g/moL. CHL is a white powder with a melting point between 236–238°C. It is sparingly soluble in methanol and insoluble in water. It has a pKa value of 8.58. CIL and CHL combination lower blood pressure effectively and also lowers the risk of stroke and heart attack.[1-3] The chemical structures of CIL and CHL are depicted in Figs. 1 and 2, respectively.

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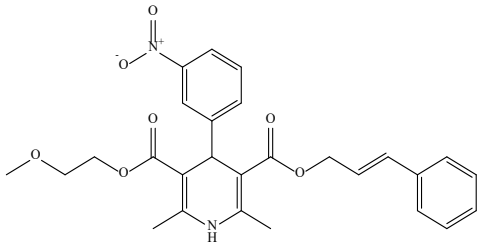

**Fig. 1:** Chemical structure of CIL

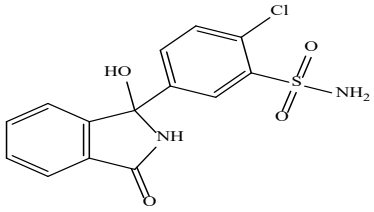

**Fig. 2:** Chemical structure of CHL

The design of a specific and precise analytical assay method is highly advantageous for simultaneous quantification of CIL and CHL in pharmaceutical FDC.

Numerous analytical literature search reports have been addressed for the analysis of cited drugs alone or in combined FDC with other therapeutic agents by exploring LC-MS/MS,[4-11] saxagliptin SAXA HPLC,[12-17] HPTLC,[18-21] and UV-spectrometry[22] in pharmaceutical matrices as well in bioanalytical samples. Consequently, none of these approaches have been deemed highly acceptable due to higher retention times of analytes, excessive consumption of polar organic solvents, more generation of waste, higher rate of flows and unproductive analysis due to an operational cost. However, in order to overcome disadvantages associated with these analytical reports, the ultra-high performance liquid chromatography (UHPLC) technique have been deemed extremely useful for enabling rapid determination of analytes with less process cycle time ensures end-product efficiency by reducing operating costs and shortening run times, faster-resolving power making it more selective and sensitive. Moreover, it uses a novel column material with a minimum particle size to improve sensitivity and reduce polar organic solvent's excessive consumption.

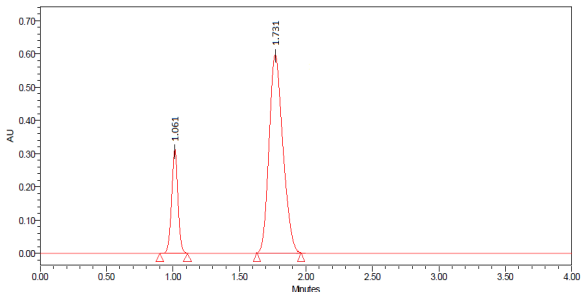


**Fig. 3:** Chromatogram of CIL and CHL

Therefore, the present work was accordingly undertaken by employing the merits mentioned earlier to design a cost-effective, rapid and precise UHPLC assay method for quantifying CIL and CHL in the pharmaceutical FDC. Moreover, the application of proposed work to assess intrinsic stability behavior of the CIL and CHL under distinct conditions of stressors.

## Materials And Methods

### Chemicals and Reagents

CIL and CHL pure drug samples were generously gifted by Amegh Pharma Pvt. Ltd., India. Nexovas CH (label claim CIL -10 mg and CHL - 6.25 mg) and Nexovas CH (label claim CIL 10 mg and CHL 12.5 mg) tablets used were manufactured by Macleods Pharmaceuticals PVT. LTD., India. Methanol and acetonitrile HPLC grade was purchased from Merck, LTD., India.

### UHPLC Method Developement

#### *Instrumentation*

The reversed-phase UHPLC was selected to achieve the objectives of the development of a stability-indicating assay method. Agilent UPLC system equipped with ACQ-PDA detector was used. Saperation was carried out using UPLC BEH $C_{18}$ column (150 2.1 mm, i.d., 1.7 µm particle size). Data acquisition and integration was performed using Empower 3 software.

#### *Stock standard solution preparation*

A combined stock standard solution was prepared by dissolving the precise quantity of 10 mg of CIL and 12.5 mg of CHL into 100 mL of calibrated flask consisting of 50 mL of methanol, manually shaken for 10 minutes. Finally, volume was diluted to obtain 100 and 125 µg/mL concentrations of CIL and CHL, respectively.

#### *Working solution of analysis*

A working solution of CIL and CHL were prepared by moving accurate volume of 1.0 mL into 10 mL of the calibrated flask from stock standard solution. Lastly, the volume was diluted to the mark to get the concentrations of 10 and 12.5 µg/mL of CIL and CHL, respectively.

#### *Chromatographic Conditions for Resolution of CIL and CHL*

The identification and quantification of a compound of interest (CIL and CHL) from non-interest components with acceptable selectivity, specificity, and sensitivity with rapid analysis were verified on UPLC BEH $C_{18}$ (150 2.1 mm) with 1.7 µm particle size column at ambient temperature using a solvent system in a proportion of (70:30 % *v/v*) acetonitrile: water. Before the execution of chromatographic analysis, the solvent system was filtered through a 0.2 µm membrane (Ultipor $N_{66}$ Nylon 6, 6) and sonication of it for 20 minutes. A 10 µL of fixed volume

(working solution) was injected. The chromatogram was studied at a detection wavelength of 275 nm. Ahead of injection of working solution, UPLC BEH $C_{18}$ column was saturated for at least 20 minutes with the solvent system flowing through the column. The total analysis time for quantification of CIL and CHL was below 4 minutes. The R*t* of CIL and CHL were 1.731 ± 0.02 minutes and 1.061 ± 0.022 minutes, respectively, the chromatogram is depicted in Fig. 3.

### *Assay of Marketed FDC of CIL and CHL*

The assay of CIL and CHL in the marketed pharmaceutical formulation was performed for two different pharmaceutical matrices. Twenty tablets of Nexovas CH (label claim CIL- 10 mg and CHL- 6.25 mg and CIL- 10 mg and CHL- 12.5 mg) were evaluated to estimate the average weight of the tablets. After making the final concentrations of 6 and 7.5 µg/mL of CIL and CHL, respectively; the peak area was estimated for selected peak.

### *Stress Degradation Studies for CIL and CHL*

The present UHPLC method was investigated according to Q1A (R2) guidelines of ICH references for hydrolysis, oxidation, thermal (dry heat and wet heat stress) and photolysis as per the references of Q1B. The stressors, the preference of their concentration and sample's processing were predicted on a pre-developed research experiment. Subsequently, it was found that the CIL and CHL were practically insoluble in water; thus, the stress studies were initiated by dissolving the stressor in methanol. The slight changes in mobile phase composition and flow rate were made to resolve all the potential degradants.[23-25]

### *Acidic Hydrolysis*

Acidic hydrolytic stress studies for CIL and CHL were carried out by precisely solubilizing 10 mg of CIL and CHL separately into calibrated flask consisting of 10 mL of 1 M methanolic HCl. The resulting solution was moved into a 50 mL RBF and refluxed at 80°C for 1 hours on a thermostatic water bath. Adequate aliquots of stress-induced CIL and CHL samples (1.0 mL) were withdrawn and subjected to neutralization with equal concentration 1 M methanolic NaOH solution. Afterwards, 0.1 mL of resulting solutions were diluted with a solvent system to obtain the concentrations of 10 µg/mL of CIL and CHL each and then samples were addressed as per the designed UHPLC method.

### *Alkaline Hydrolysis*

Alkaline hydrolytic stress studies for CIL and CHL were carried out by precisely solubilizing 10 mg of CIL and CHL separately into calibrated flask consisting of 10 mL of 0.5 M methanolic NaOH. The resulting solution of CIL was preserved in the dark at room temperature for 24 hours to avoid a certain level of substantial degradation due to light; while CHL resulting solution was moved into a 50 mL of RBF; attached with a reflux condenser and refluxed at 80°C for 2 hours on a thermostatic water bath. Adequate aliquots of stress-induced CIL and CHL samples were withdrawn and subjected to neutralization with equal concentration (1.0 mL) 0.5 M methanolic HCl solution. Afterwards, 0.1 mL of resulting solutions were diluted with a solvent system to obtain the solutions of 10 µg/mL of CIL and CHL and were addressed as per the designed UHPLC method.

### *Neutral Hydrolysis*

To analyse hydrolysis influence in a neutral condition, investigation was done by precisely solubilizing 10 mg of CIL and CHL discretely into a 10 mL calibrated flask with methanol as a stressor. The resulting solution was preserved in the dark at room temperature for 10 days to avoid a certain level of substantial degradation by light. An adequate aliquot of stress-induced CIL and CHL samples (0.1 mL) were withdrawn and diluted with a solvent system to obtain the concentrations of 10 µg/mL of CIL and CHL and were investigated. It was noticed that, both the analytes were practically stable with neutral hydrolysis as no degradation was noticed when subjected to neutral hydrolysis at room temperature for ten days.

### *Oxidative Degradation*

Oxidative stress studies were carried out by precisely solubilizing 10 mg of CIL and CHL separately into a calibrated flask (6% $H_2O_2$ *v/v* for CIL and 3% $H_2O_2$ *v/v* for CHL). Finally, the volume was diluted to the mark of a calibrated flask with methanol. The resulting solutions were preserved in the dark at room temperature for 3 days. An adequate aliquot of stress-induced samples (0.1 mL) were withdrawn and diluted with a solvent system to obtain the concentrations of 10 µg/mL of CIL and CHL each and were investigated.

### *Photodegradation*

The photolysis was performed using the solid samples (spreading as a thin layer on a petri dish) exposed to the illumination of ≥210cWh/$m^2$ at 30°C with UV radiation, i.e., for short UV-254 nm and long UV-360 nm for 10 consecutive days. An adequate aliquot of stress-induced CIL and CHL samples (0.1 mL) were withdrawn and diluted with a solvent system to obtain the concentrations of 10 µg/mL CIL and CHL each and were analysed. It was observed that both drugs were stable at the proposed condition as no degradation was noticed.

### *Thermal Degradation (Dry and Wet Heat)*

The thermal (dry heat) degradation was performed by introducing approximately 100 mg quantity of CIL and CHL separately into a sealed ampoule and placing it into the digital controlled thermostatic hot air oven at 60°C for 10 hours. From the same, precise quantity of 10 mg of CIL and CHL separately dissolved in methanol and an adequate

**Table 1:** System suitability parameters

| *Parameters* | *Retention times (Rt)* | | *Theoretical plates (USP plate count)* | | *Tailing factor* | | *Resolution* | |
|---|---|---|---|---|---|---|---|---|
| Analytes | CIL | CHL | CIL | CHL | CIL | CHL | CIL | CHL |
| Results [n=6 ± SD] | 1.731 ± 0.02 | 1.061 ± 0.022 | 8035.56 ± 0.12 | 10345.14 ± 0.21 | 1.04 ± 0.06 | 1.16 ± 0.07 | 16.56 | |

n= number of determinations.

aliquot of stress-induced samples (0.1 mL) of the resulting solution was diluted with a solvent system to obtain the concentrations of 10 µg/mL of CIL and CHL each and were analysed. In the proposed experiment, CIL and CHL were nearly stable under dry heat.

The thermal (wet heat) degradation was performed by keeping stock solutions (1 mg/mL) in the digital controlled thermostatic hot air oven at 60°C for 10 hours. An adequate aliquot of stress-induced samples (0.1 mL) of the resulting solution was diluted with a solvent system to obtain the concentrations of 10 µg/mL of CIL and CHL and were analyzed.

### *Validation of Designed UHPLC Method*

The Q2 (R1) (confirmation), Q1A(R2) (stress studies) and Q1B (photolysis) procedures of the International Council for Harmonization (ICH) of technical requirements for pharmaceuticals for human use were employed for confirmation of the designed stability-indicating UHPLC assay method for simultaneous quantification for CIL and CHL in FDC.

## Results And Discussion

### Validation of UHPLC Method

The optimized conditions for the estimation of CIL and CHL were used to validate the designed UHPLC method.

### *System Suitability Parameters*

The system suitability parameters were investigated using 10 and 12.5 µg/mL concentrations of CIL and CHL, respectively (six determinations). Standard deviation (SD) and relative standard deviation (RSD%) were estimated for responses (peak area and R*t*). The RSD% values of responses were within a 2% range, suggesting that the system development was suitable. The tailing factor and the number of USP plates were both found to be within reasonable limits. The results of analysis are depicted in Table 1.

### *Linearity*

The linearity for CIL and CHL were assessed using the six working solutions in the range of 2–12 and 2.5–15 µg/mL concentrations of CIL and CHL respectively. The calibration plots of peak area against the µg/mL concentrations for both analytes were plotted and analyzed using the linear regression equation to develop a relationship as a calibration curve. Calibration plots are depicted in Fig. 4a for CIL and 4b for CHL.

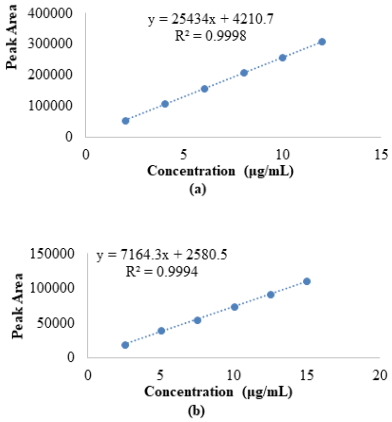


**Fig. 4:** (a) Calibration curve for CIL and (b) Calibration curve for CHL.

### *Accuracy*

The accuracy of the designed UHPLC method for CIL and CHL was addressed in the context of %recovery and accomplished at three distinct levels, i.e., 80, 100, and 120%. The %recovery was exercised by adding a fixed amount of CIL and CHL standards to pre-analyzed tablet solution (CIL- 4 and CHL- 5 µg/mL) and resulting solution was ultimately addressed using the established method. The results of the %recovery of the planned method are given in Table 2.

### *Precision*

The precision analysis of the UHPLC method for CIL and CHL was investigated for intra and inter-day and repeatability variability. The three distinct concentrations 4, 6 and 8 µg/mL of CIL and 5, 7.5 and 10 µg/mL of CHL

**Table 3:** Summary of validation parameters.

| *Parameters* | *UHPLC method* | |
|---|---|---|
| | *CIL* | *CHL* |
| Linearity | | |
| Range (µg/mL) | 2.0-12 | 2.5-15 |
| Determination coefficient ($r^2$) | 0.9998 | 0.9994 |
| Sensitivity | | |
| LoD (µg) | 0.18 | 0.23 |
| LoQ (µg) | 0.56 | 0.71 |
| Robustness | Robust | |
| Specificity | Specific | |

[1]SD= standard deviation, [2]RSD%= %relative standard deviation.

**Table 2:** %Recovery and precision investigation for CIL and CHL

| *Initial amount [µg/mL, n=3]* | | *Level of recovery study CIL* | *Total amount found [µg/mL]* | | *%Recovery* | | *%RSD* | |
|---|---|---|---|---|---|---|---|---|
| | | | *CHL* | *CIL* | *CHL* | *CIL* | *CHL* | |
| CIL- 4, CHL-5 | | 80 % | 7.21 | 8.98 | 100.37 | 99.63 | 0.33 | 0.57 |
| CIL- 4, CHL-5 | | 100 % | 7.99 | 9.98 | 99.80 | 99.76 | 0.13 | 0.35 |
| CIL- 4, CHL-5 | | 120 % | 8.81 | 11.01 | 100.40 | 100.29 | 0.3 | 1.56 |
| Concentration [µg/mL, n=3] | | Precision analysis | Total amount found [µg/mL] | | %Amount found | | %RSD | |
| 4 | 5.0 | | 4.08 | 4.97 | 101.99 | 99.53 | 0.94 | 1.16 |
| 6 | 7.5 | Intra-day Precision | 5.96 | 7.48 | 99.74 | 99.87 | 0.39 | 0.33 |
| 8 | 10 | | 8.0 | 9.91 | 100.08 | 99.19 | 0.18 | 0.34 |
| 4 | 5.0 | | 4.03 | 4.97 | 100.09 | 99.55 | 0.58 | 0.78 |
| 6 | 7.5 | Inter-day Precision | 5.95 | 7.42 | 99.32 | 99.10 | 0.38 | 0.99 |
| 8 | 10 | | 8.0 | 9.93 | 100.06 | 99.40 | 0.23 | 0.26 |
| 6 | 7.5 | Repeatability [n=6] | 5.96 | 7.46 | 99.93 | 99.47 | 0.49 | 0.46 |

n= number of determinations.

**Table 4:** Results of force degradation studies for CIL

| *Stressor conditions* | *Number of degradants* | *Rt of degradants (min)* | *%degradation* |
|---|---|---|---|
| Acidic hydrolysis | | | |
| 1 M HCL reflux for 80 for 1-hour | 2.0 | 1.042<br>3.729 | 12.810<br>2.298 |
| Alkaline hydrolysis<br>0.5 M NaOH at room temperature for 24 hours | 4.0 | 1.090<br>2.721<br>2.982<br>3.743 | 2.298<br>5.655<br>1.512<br>0.684 |
| Neutral hydrolysis | | | |
| At room temperature for 10 days | 0 | Stable | |
| Oxidation | | | |
| 6% $H_2O_2$ at room temperature for 3.0 days | 3.0 | 0.423<br>0.875<br>1.345 | 5.922<br>2.535<br>27.390 |
| Photolysis | | | |
| Illumination of ≥210Wh/$m^2$ at 30°C with UV radiation, i.e., for short UV-254 nm and long UV-360 nm for 10 days | 0 | Stable | |
| Thermal degradation | | | |
| Dry heat<br>Sealed ampoule consisting of 100 mg of CIL at 60°C for 10 hours | 0 | Stable | |
| Wet heat digital controlled thermostatic hot air oven at 60°C for 10 hours | 1.0 | 2.561 | 2.959 |

were addressed using assay at different time frame for intra and inter-day precision and for three successive days of analysis by the guidelines of ICH. In addition to this, repeatability assessed using six determinations of 6 µg/mL of CIL and 7.5 µg/mL of CHL concentrations. The outcomes of intra and inter-day precisions and repeatability are disclosed in Table 2.

### *Sensitivity*

To calculate the sensitivity of the designed UHPLC method, LoD and LoQ were estimated using standard deviation (N) of outcomes of the CIL and CHL (n=3) and calibration curve slope (B). Serial working dilutions of 2– 4 µg/mL of CIL and 2.5–4.5 µg/mL of CHL ranges have been prepared and examined. The results of sensitivity experiments are presented in Table 3.

### *Robustness*

Robustness analysis of the designed UHPLC method was carried out by attempting to make significant changes in %proportion of acetonitrile in a solvent system, the temperature of the column oven compartment and flow

**Table 5:** Results of force degradation studies for CHL.

| *Stressor conditions* | *Number of degradants* | *Rt of degradants (min)* | *%degradation* |
|---|---|---|---|
| Acidic hydrolysis | | | |
| 1 M HCL reflux for 80 for 1 hour | 3.0 | 1.621<br>1.846<br>2.145 | 6.761<br>26.902<br>9.463 |
| Alkaline hydrolysis<br>0.5 M NaOH at room temperature for 24 hours | 4.0 | 1.130<br>2.105<br>2.205<br>2.513 | 28.839<br>3.202<br>2.074<br>12.257 |
| Neutral hydrolysis | | | |
| At room temperature for 10 days | 0 | Stable | |
| Oxidation | | | |
| 3% $H_2O_2$ at room temperature for 3.0 days | 2.0 | | 2.932<br>1.542 |
| Photolysis | | | |
| Illumination of ≥210Wh/$m^2$ at 30°C with UV radiation, i.e., for short UV-254 nm and long UV-360 nm for 10 days | 0 | Stable | |
| Thermal degradation | | | |
| Dry heat<br>Sealed ampoule consisting of 100 mg of CHL at 60°C for 10 hours | 0 | Stable | |
| Wet heat<br>Digital controlled thermostatic hot air oven at 60 °C for 10 hours | 0 | Stable | |

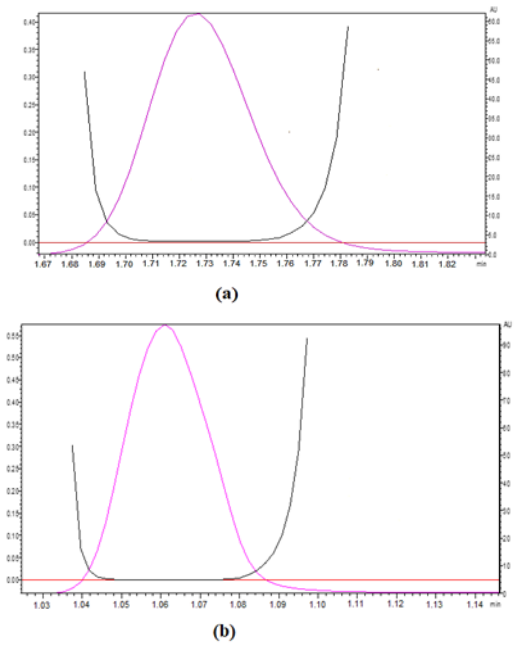


**Fig. 5:** (a) Peak purity spectrum of CIL and (b) Peak purity spectrum of CHL.

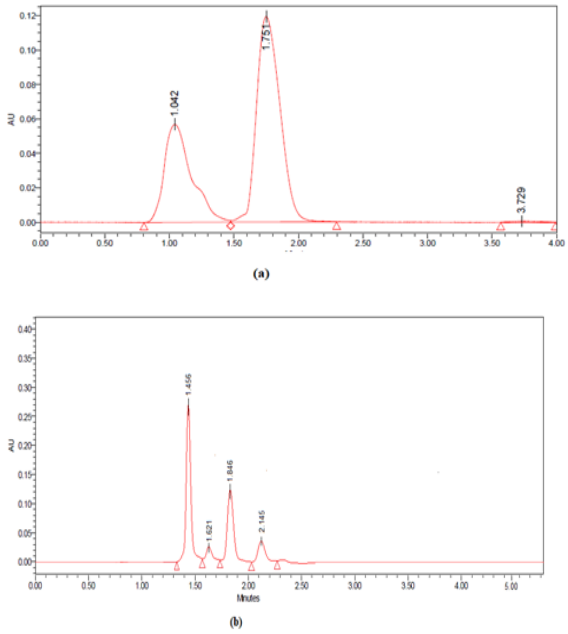


**Fig. 6:** Chromatogram of acidic hydrolysis of CIL (a) and CHL (b).

rate. The influence of each of the independent variable was determined for the peak areas of CIL and CHL. The results of robustness experiments are presented in Table 3.

### *Specificity and Selectivity*

It was noticed that there was no other specific intervention was recorded around the R*t* of CIL and CHL; neither the baseline exhibits a substantial unavoidable noise. Thus, the proposed method is quite well selective and specific. The peak-purity spectrum for CIL and CHL are depicted in Fig. 5. The summary of validation parameters is present in Table 3.

### *Stress Degradation Studies of CIL and CHL*

Analysis of stress conditions and retention time of CIL and CHL degradation products are depicted in Table 4 and

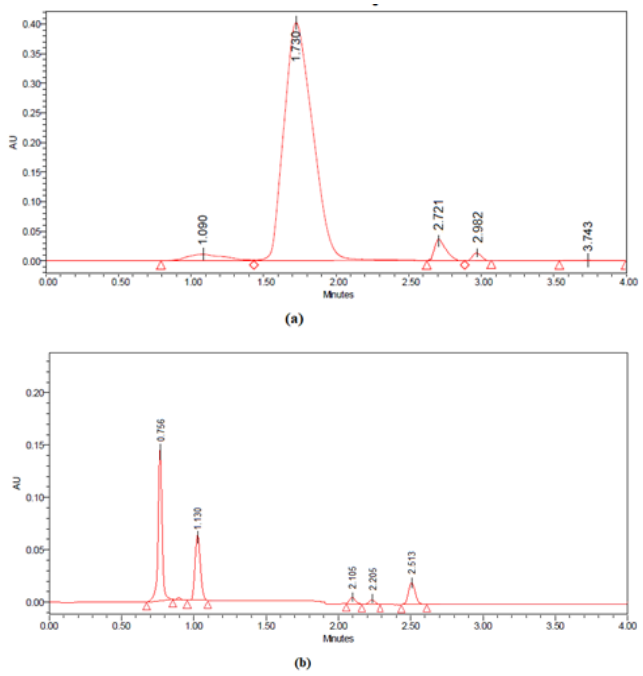


**Fig. 7:** Chromatogram of alkaline hydrolysis of CIL (a) and CHL (b).

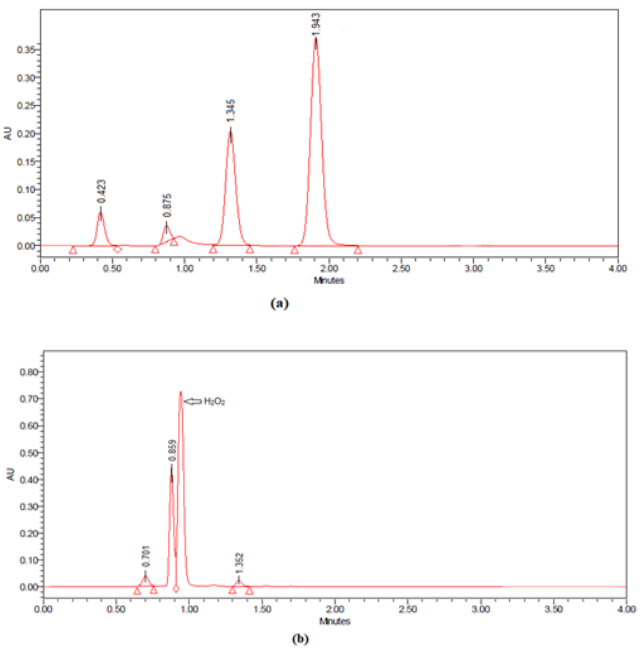


**Fig. 8:** Chromatogram of oxidative hydrolysis of CIL (a) and CHL (b).

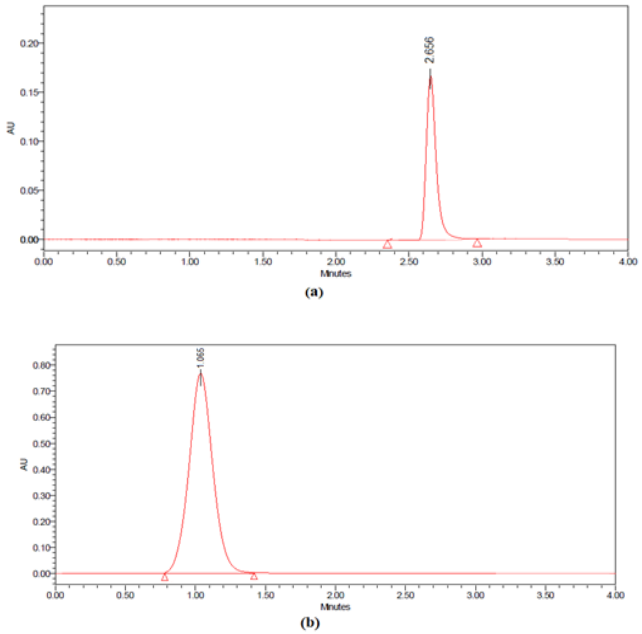


**Fig. 9:** Chromatogram of photolysis of CIL (a) and CHL (b).

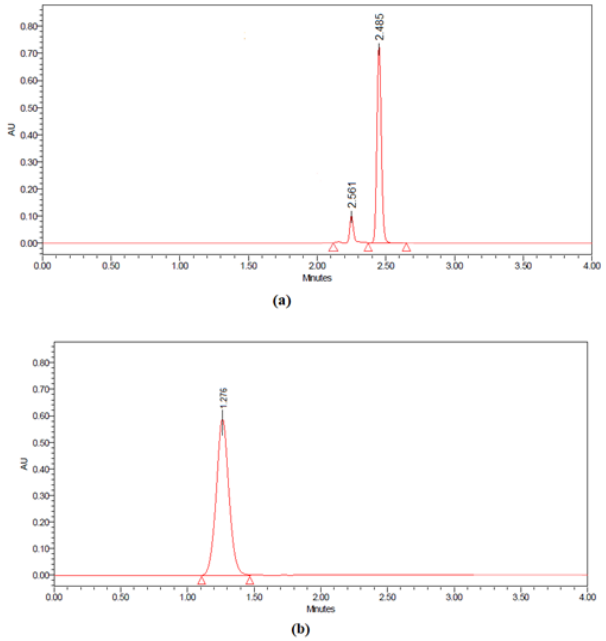


**Fig. 10:** Chromatogram of wet heat analysis of CIL (a) and CHL (b).

Table 5. Two degradation products by acidic hydrolysis for CIL and three degradation products for CHL as shown in Fig. 6, four degradation products for CIL, and CHL each were formed through alkaline hydrolysis as shown in Fig. 7. While at neutral conditions, CIL and CHL were found to be stable. The oxidative stress testings in 6% $H_2O_2$ formed three degradation product for CIL; while two degradation was noticed for CHL in 3% $H_2O_2$ as shown in Fig. 8. In photolysis conditions, CIL and CHL were found to be stable as shown in Fig. 9. The thermal stress (dry and wet heat) produced one degradation product for CIL in wet heat while in dry heat found to be stable and CHL found to be stable in both dry and wet heat as shown in Fig. 10. In all cases, the designed UHPLC method's capability to analyse the intact CIL and CHL with no obstruction from impurities (degradation products) signifies the stability-indicating potential of the proposed investigation and therefore address the specificity of the method.

## Conflicts Of Interests

The authors declare no conflict of interests.

## Conclusion

For the simultaneous estimation of CIL and CHL in pharmaceutical FDC, a novel and rapid stability-indicating UHPLC assay method was developed and successfully validated. In all cases, the anticipated UHPLC method's capability to analyze the intact CIL and CHL with no obstruction from impurities (degradation products) signifies the stability-indicating potential of the anticipated investigation and consequently, addresses the specificity of the method. The developed UHPLC method was cost-effective, efficient, specific and it can be used for the quality control laboratories for quantification of CIL and CHL in FDC.

## Acknowledgment

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## References

1. Sweetman SC, Blake PS. Martindale, The complete drug reference. Edn 36, Pharmaceutical press, London, 2010, pp. 453, 460, 1243, 1245.
2. Chaudhari SR, Shirkhedkar AA. An Investigative Review for Pharmaceutical Analysis of 1, 4-Dihydropyridine-3, 5-Dicarboxylic Acid Derivative: Cilnidipine. Critical Reviews in Analytical Chemistry. 2020;1-10. https://doi.org/10.1080/10408347.2020.1718483.
3. https://www.1mg.com/generics/cilnidipine-chlorthalidone-401058.
4. Lee HW, Seo JH, Lee HS, Jeong SY, Cho YW, Lee KT. Development of a liquid chromatography/negative-ion electrospray tandem mass spectrometry assay for the determination of cilnidipine in human plasma and its application to a bioequivalence study. Journal of Chromatography B. 2008; 862(1-2):246-251. https://doi.10.1016/j.jchromb.2007.11.016.
5. Zeng H, Wang F, Zhu B, Zhong W, Shan W, Wang J. Study of the structures of photodegradation impurities and pathways of photodegradation of cilnidipine by liquid chromatography/Q-Orbitrap mass spectrometry. Rapid Communications in Mass Spectrometry. 2016; 30(15):1771-1778. https://doi.10.1002/rcm.7657.
6. Krishnam R, Shanmukh JV, Palusa SKG. Isolation and characterization of novel degradation products in cilnidipine by LC-QTOF-MS/MS, LCMS n, 2D-NMR and FTIR. New Journal of Chemistry. 2018; 42(1):634-646. https://doi.org/10.1039/C7NJ02781H.
7. Lee KR, Chae YJ, Lee JH, Kim DD, Chong S, Shim CK, Chung SJ. Quantification of cilnidipine in human plasma by liquid chromatography-mass spectrometry. Journal of Liquid Chromatography & Related Technologies. 2012; 35(2):308-320. https://doi.org/10.1080/10826076.2011.597078.
8. Khuroo A, Mishra S, Singh O, Saxena S, Monif T. Simultaneous determination of atenolol and chlorthalidone by LC–MS–MS in human plasma. Chromatographia. 2008; 68(9-10):721. https://doi.10.1365/s10337-008-0755-7.
9. Gonzalez O, Iriarte G, Rico E, Ferreirós N, Maguregui MI, Alonso RM, Jiménez RM. LC–MS/MS method for the determination of several drugs used in combined cardiovascular therapy in human plasma. Journal of Chromatography B. 2010; 878(28):2685-2692. https://doi.10.1016/j.jchromb.2010.07.026.
10. Zhang X, Zhai S, Zhao R, Ouyang J, Li X, Baeyens WR. Determination of cilnidipine, a new calcium antagonist, in human plasma using high performance liquid chromatography with tandem mass spectrometric detection. Analytica Chimica Acta. 2007; 600(1-2):142-146. https://doi.10.1016/j.aca.2006.11.072.
11. Zeng H, Wang J, Zhu B, Shao P, Zhong W. Rationality Evaluation of Packaging Materials and Study on Impurity Profiling of Cilnidipine Preparations by HPLC-Q-TOF/MS. Current Pharmaceutical Analysis. 2020; 16(2):220-230. https://doi.10.2174/1573412914666180517100626.
12. Kachave RN, Yelmame SS, Mundhe AG. Quantitative estimation of cilnidipine and valsartan in rat plasma by RP-HPLC: its pharmacokinetic application. Future Journal of Pharmaceutical Sciences. 2021; 7(1):1-7. https://doi.org/10.1186/s43094-020-00153-8.
13. Rupareliya RH, Joshi HS. Stability indicating simultaneous validation of telmisartan and cilnidipine with forced degradation behavior study by RP-HPLC in tablet dosage form. ISRN Chromatography. 2013; 1:1-6. https://doi.org/10.1155/2013/461461.
14. Raju VB, Gandhi BM, Sumanth KS, Srinivas K, Neeraja TNVL. RP-HPLC Method Development and validation for simultaneous estimation of telmisartan and ramipril in pure and pharmaceutical dosage forms. Asian Journal of Research in Chemistry. 2017; 10(2):179. https://doi.10.5958/0974-4150.2017.00030.X.
15. Kachave RN, Kale M, Wagh RD. Simultaneous estimation of cilnidipine and valsartan by RP-HPLC in tablet formulation. Eurasian Journal of Analytical Chemistry. 2016; 11(5):245-253. https://doi.10.12973/ejac.2016.137a.
16. El-Gindy A, Emara S, Mostafa A. HPLC and chemometric-assisted spectrophotometric methods for simultaneous determination of atenolol, amiloride hydrochloride and chlorthalidone. Il Farmaco. 2005; 60(3):269-278. https://doi.10.1016/j.farmac.2004.11.013.
17. Elgawish MS, Mostafa SM, Elshanawane AA. Simple and rapid HPLC method for simultaneous determination of atenolol and chlorthalidone in spiked human plasma. Saudi Pharmaceutical Journal. 2011; 19(1):43-49. https://doi.org/10.1016/j.jsps.2010.10.003.
18. Desai D, Vashi N, Dalvadi H, Desai S, Hinge M. HPTLC method development and validation of cilnidipine and metoprolol succinate in combined dosage form. Pharmaceutical Methods. 2016; 7(1):28-34. https://doi.10.5530/phm.2016.7.5.
19. Rathod RH, Patil AS, Shirkhedkar AA. Novel NP and RP-HPTLC in praxis for simultaneous estimation of chlorthalidone and cilnidipine in bulk and pharmaceutical formulation. Analytical Chemistry Letters. 2018; 8(6):862-871. https://doi.org/10.1080/22297928.2018.1527252.
20. Youssef RM, Maher HM, El-Kimary EI, Hassan EM, Barary MH. Validated stability-indicating methods for the simultaneous determination of amiloride hydrochloride, atenolol, and chlorthalidone using HPTLC and HPLC with photodiode array detector. Journal of AOAC international. 2013; 96(2):313-323. https://doi.org/10.5740/jaoacint.11-347.
21. Joglekar AP. Method Development and Validation for the Estimation of Telmisartan and Chlorthalidone in Bulk and Pharmaceutical Dosage form by HPTLC Method. Research Journal of Pharmacy and Technology. 2015; 8(4):376-381. https://doi.10.5958/0974-360X.2015.00063.3.
22. Soni IJ, Panchal HJ. Development and validation of dual wavelength UV spectrophotometric method for simultaneous estimation of cilnidipine and olmesartan medoxomil in tablet dosage form. Indian Journal of Pharmaceutical and Biological Research. 2014; 2(01):76-81. https://doi.10.30750/ijpbr.2.1.12.
23. Stability Testing of New Drug Substances and Products. International Conference on Harmonization. IFPMA, Geneva, 1993.
24. Stability Testing of New Drug Substances and Products. International Conference on Harmonisation. IFPMA, Geneva, 2005.
25. Guideline IH. Validation of analytical procedures: text and methodology. Q2 (R1). 2005 Nov;1(20):05.

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