International peer reviewed open access journal

Journal of Medical Pharmaceutical and Allied Sciences

Journal homepage: www.jmpas.com CODEN: JMPACO

Research article

Development and validation of stability indicating RP-HPLC method for estimation of cyclandelate in bulk drug and capsule dosage form

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Received - 25-11-2023**, Revised -** 10-12-2023, **Accepted -** 26-12-2023 **(DD-MM-YYYY)**

Refer This Article

Binit Patel, Archita Patel, Dilip Ghava, Rikeeta Padiya, Pravinkumar Darji, 2023. Development and validation of stability indicating rp-hplc method for estimation of cyclandelate in bulk drug and capsule dosage form. Journal of medical pharmaceutical and allied sciences, V 12 - I 6, Pages - 6247 – 6253. Doi: https://doi.org/10.55522/jmpas.V12I6.5943.

ABSTRACT

For the quantification of Cyclandelate (CYL) in bulk medication and capsule dosage form in the presence of degradation products, a quick, accurate, and simple RP-HPLC method was devised. On the Phenomenex Luna C18 (250 mm x 4.6mm, 5 m) column, effective chromatographic separation was accomplished. The separation was made simple by employing the Isocratic elution program with Acetonitrile and buffer pH 3.0 (0.1% Triethylamine in water followed by pH correction with Trifluoroacetic acid) in the ratio of 70:30 (%v/v) at a flow rate of 1.2 mL/min, and an eluent was monitored at 220 nm. The degradation products were found to be isolated from the principal peak after being subjected to force degradation with acid, alkali, peroxide, thermal, and photolytic agents. The technique was linear in the range of 30-200 µg/mL, with a correlation coefficient of 0.9975. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 8.3 µg/mL and 25.1 µg/mL respectively. The presented method was validated for parameters such as system appropriateness, specificity, linearity, accuracy, precision, and robustness. The robustness investigation was part of the validation research as well. The outcomes for every metric are acceptable by ICH standards. Therefore, the method can be considered to be rapid and accurate for high-throughput analysis of this drug even when degradation products are present.

Keywords: Validation, Stability Indicating, Degradation Product, Cyclandelate (CYL)

INTRODUCTION

The IUPAC name of Cyclandelate is (3, 3, 5-trimethylcyclohexyl) 2-hydroxy-2-phenylacetate. It is a vasodilator used to treat Raynaud's disease, arteriosclerosis, and claudication. It has been researched for its effectiveness against migraines and is also used to relieve overnight leg cramps [1-2]. Cyclandelate Capsule is not cited in any pharmacopeia. Excipients may contribute to incompatibility by changing the moisture content, the pH of the microenvironment, serving as a catalyst for degradation, or contributing to an impurity that promotes drug product deterioration. Hence, the degradation study was performed using bulk drugs. Various methods have been reported, Zang et al. develop dissolution method[8], Bhoir et al. develop SFC and HPLC-based assay method for the estimation of Cyclandelate using the Reverse phase HPLC[10]. Forconi et al. discover plasma concentration estimation using the Reverse Phase HPLC technique, while G Andermann optimizes the Gas Chromatography method for CYL combination product [11, 16]. After a

thorough literature search, it was observed that no RP-HPLC-DAD method was found for the estimation of degradation study in Cyclandelate Capsule. As a result, it was determined that conducting a study to investigate how Cyclandelate Capsules degrade would ensure the final pharmaceutical formulation's quality, efficacy, and safety. The current research's goal was to validate the method for identifying the pertinent compounds in order to assess the physical and chemical stability of Cyclandelate when mixed with excipients and placed in a capsule under varied storage conditions in accordance with the ICH guidance document. Regulations for the identification, qualification, and control of impurities in pharmacological substances and their formed products are advised by the International Conference on Harmonization (ICH). All routine contaminants at or above the 0.1% level should be recognized using appropriate analytical procedures, as advised by ICH [3-16].

MATERIALS AND METHOD

Reagents and chemicals

The CYL sample was provided as a gift from Step-Up Pharmaceuticals for the purpose of this experiment. The HPLC grade Trifluoroacetic acid (TFA), Methanol, Acetonitrile, and Triethylamine (TEA) were obtained from Merck. The Sodium hydroxide pellets and Hydrochloric acid were of analytical quality obtained from Finar Chemicals Ltd. The experiment utilized Milli-Q water exclusively.

Preparation of standard solution

A final concentration of 1000 µg per mL was created by weighing and dissolving about 50 mg of the CYL working standard in 50 ml of the volumetric flask using 10 mL of acetonitrile. The final volume was then created with water. In order to create the final working standard solution, which has 100 µg per mL, the same solution was further diluted with diluent. (Note: Mobile phase was used as a diluent in this experiment).

Preparation of test solution from pharmaceutical formulation

Weighed and transferred powder equivalent to 50 mg of CYL into a 50 mL volumetric flask. 30mL of diluent was added, and it was sonicated for 15 minutes with periodic shaking. Using diluent, the final volume was made to be 50 mL with a concentration of 1000 µg per mL. The sample solution was filtered via a 0.45µ Nylon filter and diluted with diluent to make the final concentration 100 µg per mL.

Optimization of chromatographic conditions

A trial-and-error process was used to optimize the chromatographic conditions. Numerous trials were taken with different ranges of buffers in composition with suitable solvents to achieve proper elution of CYL. On a conclusion basis, the mobile phase in combination with Acetonitrile: 0.1% TEA buffer pH3.0 (adjusted with Trifluoroacetic acid) with a ratio of 70:30, 1.2 mL/minute flow rate with Phenomenex Luna C18 column (250 X 4.6mm, 5µm) was chosen for better elution pattern. The injection volume was 20µL and 220 nm wavelength was selected for the symmetry of the analyte peak.

Forced degradation study

The drug was exposed to acidic, alkaline, oxidizing, and heating conditions as part of the forced degradation investigation. CYL was put under a variety of forced degradation conditions to partially degrade the medication, ideally between 20 and 40%. For the purpose of validating specificity, the study offers some information on stability under stress conditions and degraded samples.

Effect of acid hydrolysis

Weighed and transferred powder equivalent to 50 mg of CYL into a 50 mL volumetric flask. 30mL of diluent was added, and it was sonicated for 15 minutes with periodic shaking. Added 1.0 mL of 0.1N HCl solution to it and mix well. The acidic solution was refluxed for 2 hours at room temperature and neutralized with 0.1N NaOH as appropriate and diluted to the mark with a diluent to obtain a solution of 1000 µg/mL. The sample solution was filtered via a 0.45µ Nylon filter and diluted with diluent to make the final concentration 100 µg per mL.

Effect of base hydrolysis

Weighed and transferred powder equivalent to 50 mg of CYL into a 50 mL volumetric flask. 30mL of diluent was added, and it was sonicated for 15 minutes with periodic shaking. Add 5.0 mL of 0.1N NaOH solution to it and mix well. The basic solution was refluxed for 3 hours at 80°C in the water bath and neutralized with 0.1N HCl as appropriate and diluted to the mark with a diluent to obtain a solution of 1000 µg/mL. The sample solution was filtered via a 0.45µ Nylon filter and diluted with diluent to make the final concentration 100 µg per mL.

Effect of oxidative study

Weighed and transferred powder equivalent to 50 mg of CYL into a 50 mL volumetric flask. 30mL of diluent was added, and it was sonicated for 15 minutes with periodic shaking. Add 1.0 mL of 30% Hydrogen Peroxide solution to it and mix well. The solution was refluxed for 2 hours at room temperature and diluted to the mark with a diluent to obtain a solution of 1000 µg/mL. The sample solution was filtered via a 0.45µ Nylon filter and diluted with diluent to make the final concentration 100 µg per mL.

Effect of thermal study

Weighed and transferred powder equivalent to 50 mg of CYL into a 50 mL volumetric flask. 30mL of diluent was added, and it was sonicated for 15 minutes with periodic shaking. The solution was kept at 80°C for 30 minutes in the water bath and diluted to the mark with a diluent to obtain a solution of 1000 µg/mL. The sample solution was filtered via a 0.45µ Nylon filter and diluted with diluent to make the final concentration 100 µg per mL.

Validation of the analytical method

The analytical method was validated in accordance with the ICH guidelines Q2 (R1) for Specificity, Linearity, Accuracy, Precision, Limit of detection, Limit of quantitation, and Robustness.

Specificity

The analytical method's specificity was used to weed out any potential interference from blank samples, stressed samples, and impurities at the retention time of the CYL analyte peak.

Linearity

The linearity of CYL was plotted by a series of dilutions in the concentration ranges of 30 to 200 µg per mL. Utilizing a plot of peak area vs. concentration to build a linear connection, the regression correlation was attained.

Accuracy

By using the conventional addition approach to calculate CYL recoveries, the accuracy of the method was evaluated. Recovery was carried out in the range of 50%, 100%, and 150% of drug concentration and each level was injected in triplicate.

Precision

The proposed strategy's intra-day and inter-day precision was evaluated by comparing equivalent responses on the same day and a separate day for 3 different levels of CYL at 50%, 100%, and 150% of drug concentration.

Limit of Detection and Limit of Quantification

The limit of detection (LOD) and the limit of quantification (LOQ) were calculated using the standard deviation of response (N) and slope (S) of the calibration curve.

$$LOD = 3.3 \times N/S$$

$$LOQ = 10 \times N/S$$

Robustness

The robustness investigation was performed by varying the flow rate, mobile phase ratio, and wavelength detecting parameters of the approach. Robustness was performed by altering wavelength (± 2 nm), flow rate (± 0.1mL/min), and mobile phase ratio (± 2% absolute). The changes were noted and compared against normal conditions.

RESULT AND DISCUSSION

Selection of Chromatographic Conditions

Several trials were carried out combining different mobile phase ratios at varying flow rates with various USP column types, such as C8 and C18, to achieve appropriate symmetry of the analyte peak, CYL. By considering the pKa value and solubility of the compound numerous trials were taken for mobile phase selection. Alongside solvent composition in the mobile phase, alteration in the pH of the buffer plays a vital role to achieve better separation of analyte peak and degradation peak. Finally, 0.1% TEA buffer pH3.0 (adjusted with Trifluoroacetic acid) with Acetonitrile in ratio of 30:70 (v/v) with Phenomenex Luna C18 column (250 X 4.6mm, 5µm) at 1.2 mL per minute flow shows better separation of analyte peak. The injection volume was adjusted to 20µL and the analyte peak was detected at 220 nm. The analyte peak elutes within the time frame of 9 minutes. The chromatograms of the standard and the same were shown in Figure 1 and 2.

Figure 1: Chromatogram of CYL Standard Solution

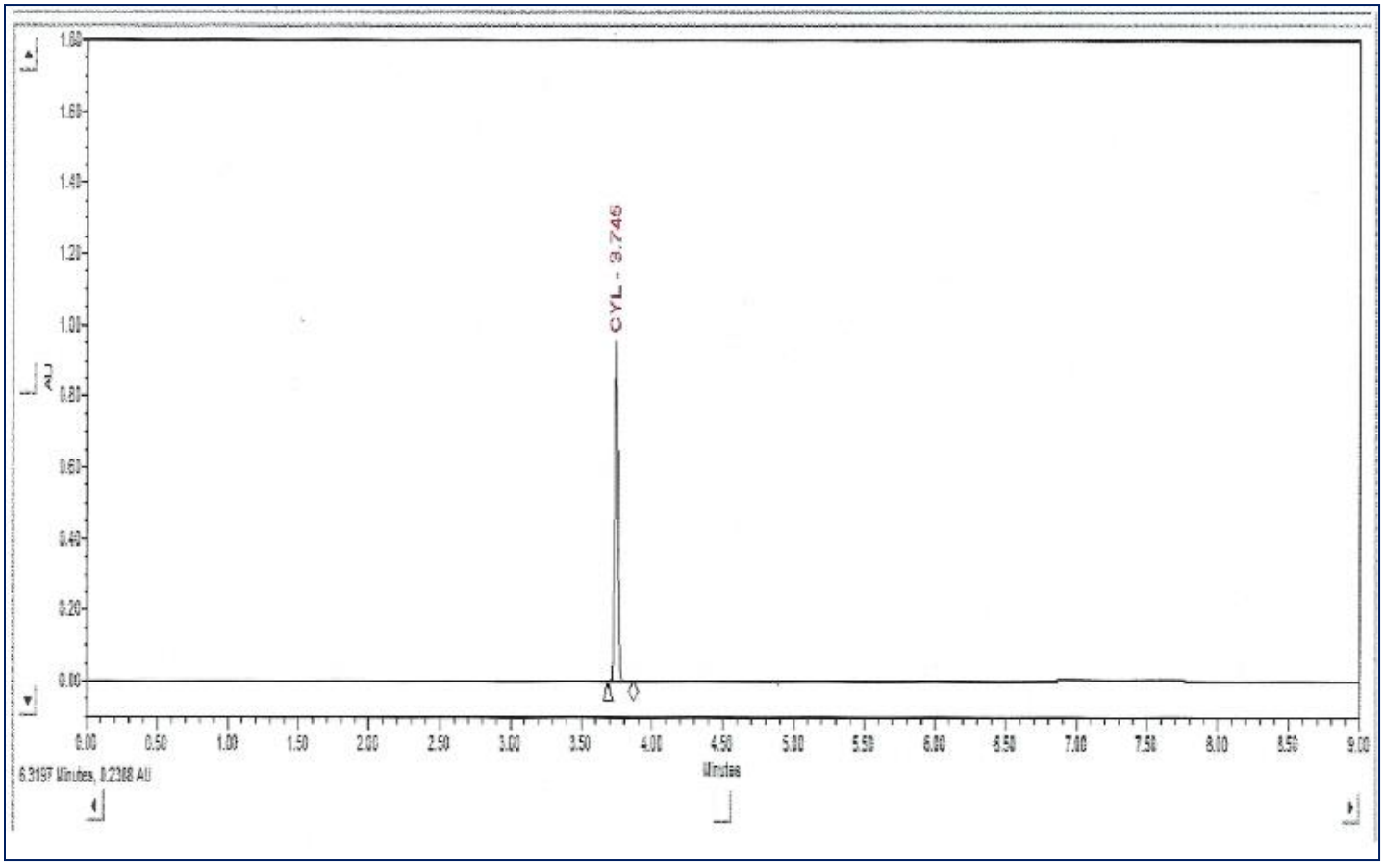
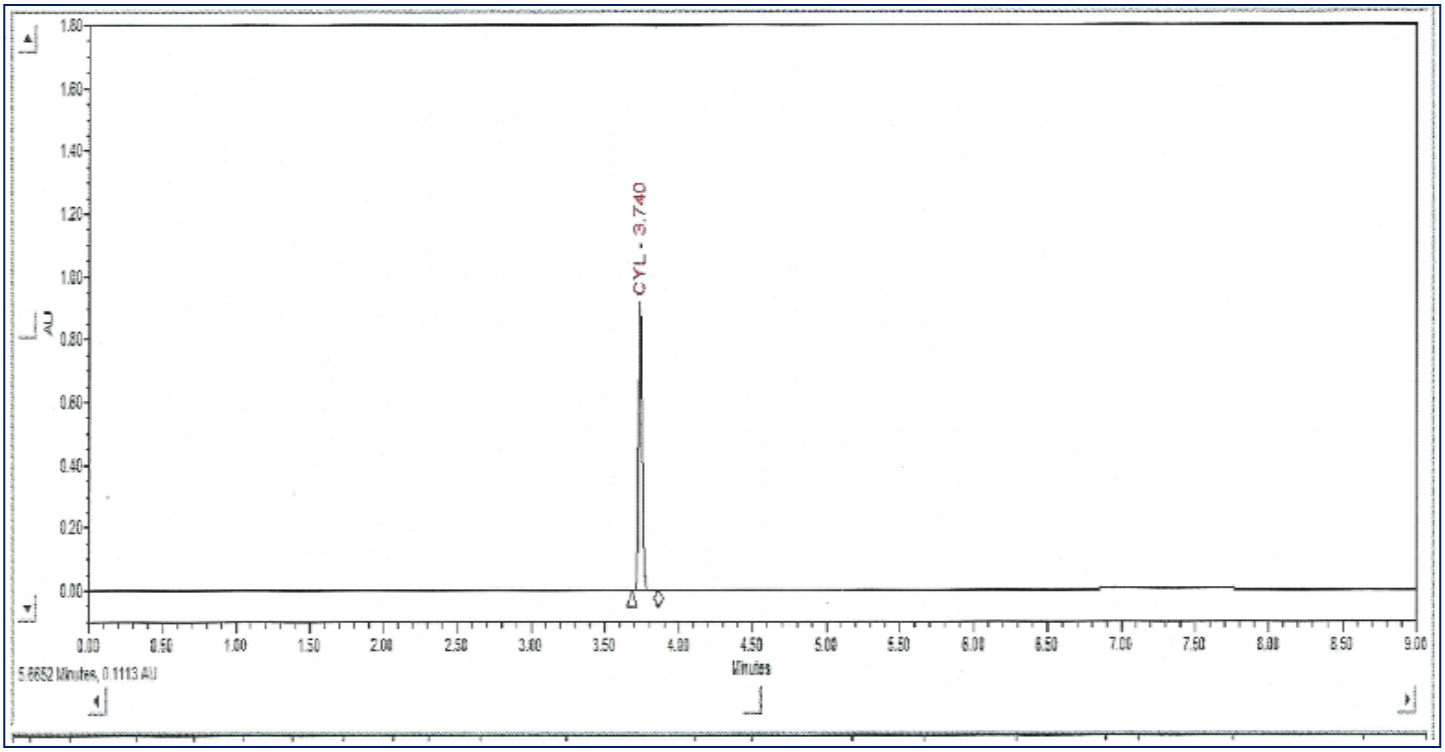


Figure 2: Chromatogram of CYL Sample Solution



Specificity

The analytical method was found specific, as there was no interference of diluent at the retention of CYL.

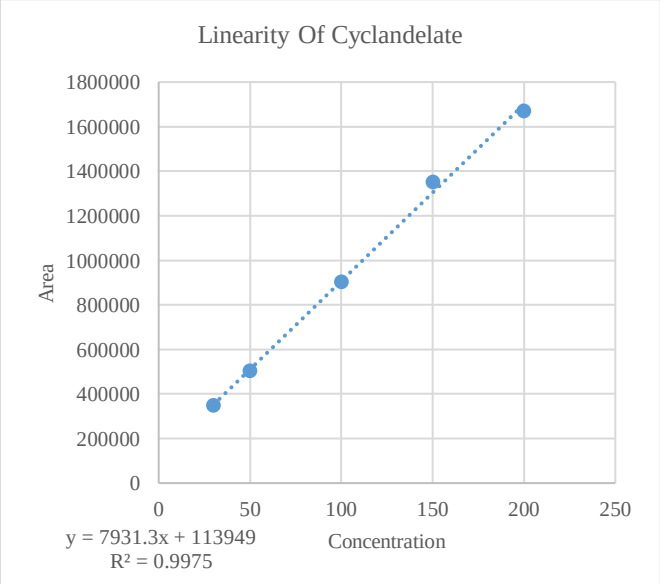
Linearity

Peak area against concentration was used to plot the linearity with the range from 30 to 200 µg/mL and the correlation coefficient was found 0.9975, which is in line with the guideline shown in Figure 3, and the result of the same is shown in Table 1.

Table 1: Result of Cyclandelate Linearity

Parameter	Result
Linearity Range	30-200µL
Linearity	Y = 7931.3x + 113949
Correlation coefficient	0.9975
LOQ µg/mL	12.22(µg/mL)
LOD µg/mL	4.031(µg/mL)

Figure 3: Linearity plot of Cyclandelate



Accuracy

Recovery was carried out in the range of 50%, 100%, and 150% of drug concentration for Cyclandelate. The recovery values were found within the expected range of 95% - 105%. The results were mentioned in Table 2.

Table 2: Result of Cyclandelate Recovery

Recovery Level	% Recovery			%Recovery Avg.	%RSD
	Set- 1	Set- 2	Set- 3		
50	98.5	99.5	98.2	98.7	0.69
100	102.5	99.1	101.2	100.9	1.70
150	101.5	102.1	98.9	100.8	1.69

Precision

The % RSD for Inter-day precision was found to be 1.25%, while the % RSD for Intraday precision was found to be 0.63%. The results indicate the method is precise.

Robustness

The method was found to be trustworthy because just slight changes in the mobile phase's composition, flow rate, and detector wavelength had a substantial impact on the results. The results for the same have been depicted in Table-3.

Limit of detection (LOD) and Limit of quantification (LOQ)

The limit of detection and limit of quantification for CYL was found 4.031 µg/mL and 12.22 µg/mL respectively.

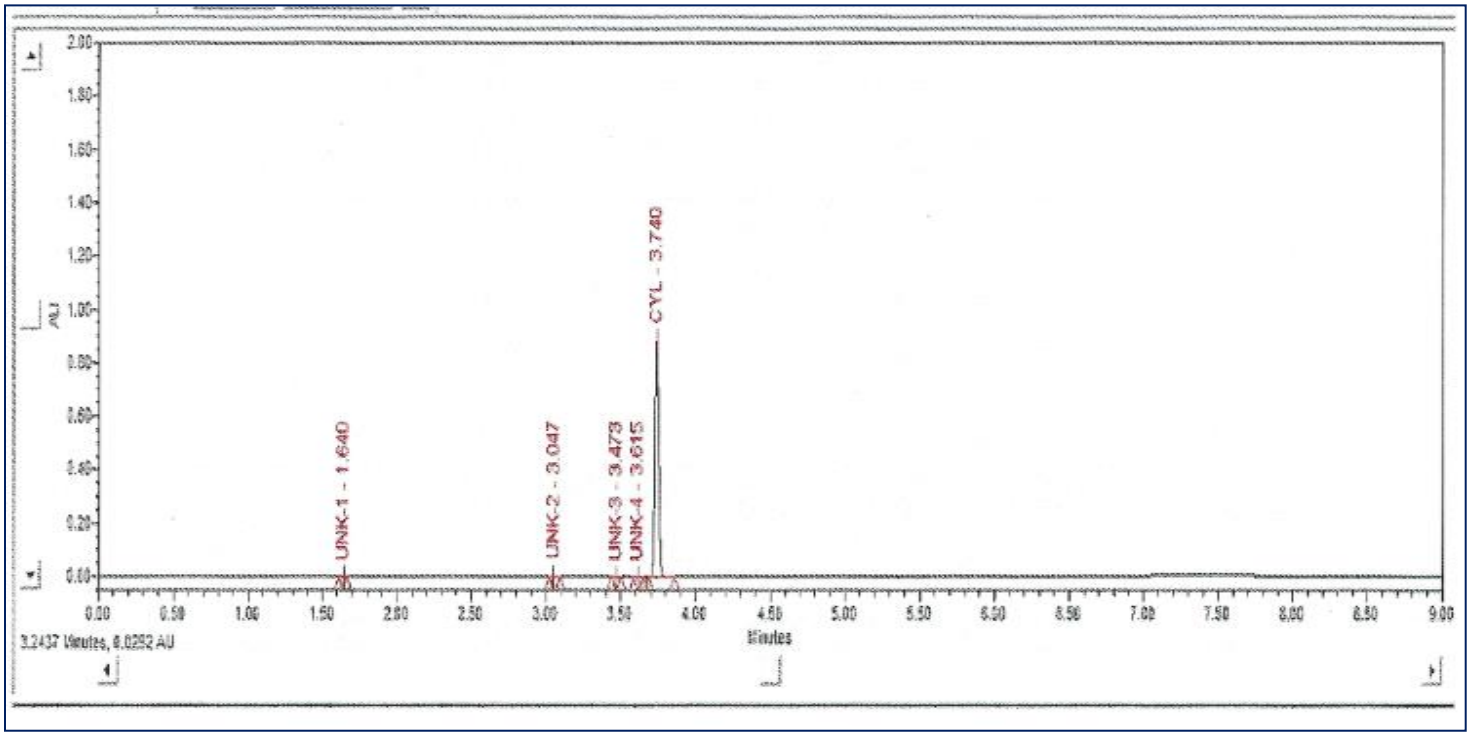
System Suitability

The relative standard deviation for the standard preparation's five replicate injections was under the limit of 2.0%, indicating that the results were deemed adequate. The USP Theoretical plates, USP Tailing factor, and retention time were also taken into account while determining the suitability of the system.

Table 3: Result of Cyclandelate Robustness

Condition	As Such (Normal)	Flow Plus 1.3 mL/min	Flow Minus 1.1 mL/min	Plus Wavelength (222nm)	Minus Wavelength (218nm)	Plus Organic ACN: Water (72:28 %v/v)	Minus Organic ACN: Water (68:32 %v/v)
Mean (Area)	905089	8887315	928914	886520	927300	916090	895733
%RSD	0.53	0.43	0.42	0.35	0.37	0.33	0.22
USP Plates	4847	6304	6279	4900	4752	6325	9\6107
USP Tailing	1.2	1.1	1.2	1.2	1.1	1.1	1.2
Retention Time (min)	5.4	4.1	6.5	5.4	5.4	3.1	7.9

Figure 4: Chromatogram of CYL Acid Degradation – Sample



Estimation of Cyclandelate in Marketed Formulation

The proposed RP-HPLC method for CYL determination was effectively used for the capsule dosage form. It was determined that the percentage of CYL was adequate and comparable to the label claim. The result for the analysis was depicted in Table 4.

Table 4: Market formulation analysis (n=5)

Dosage Formulation	Label Value (mg)	% Drug found Mean ± SD	% RSD
Capsule	200	99.05 ± 1.79	1.8

Figure 5: Chromatogram of CYL Base Degradation – Sample

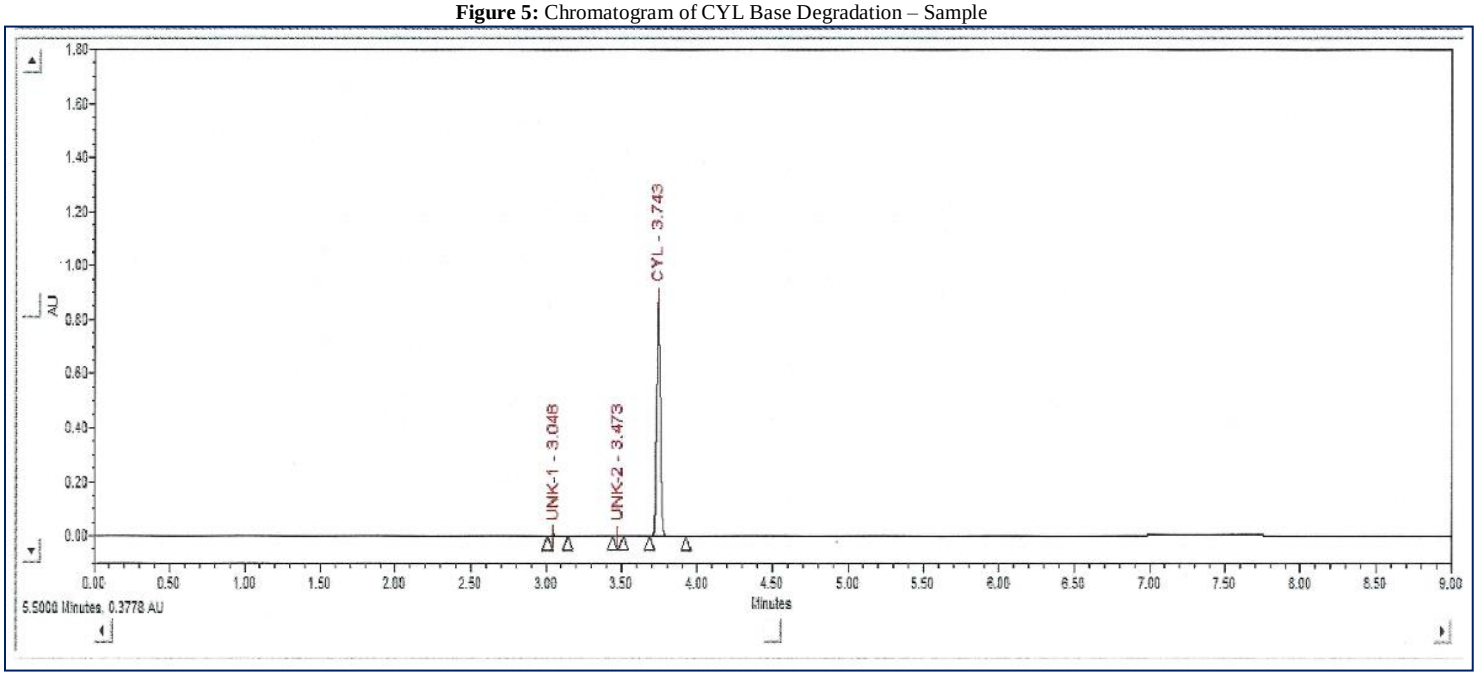
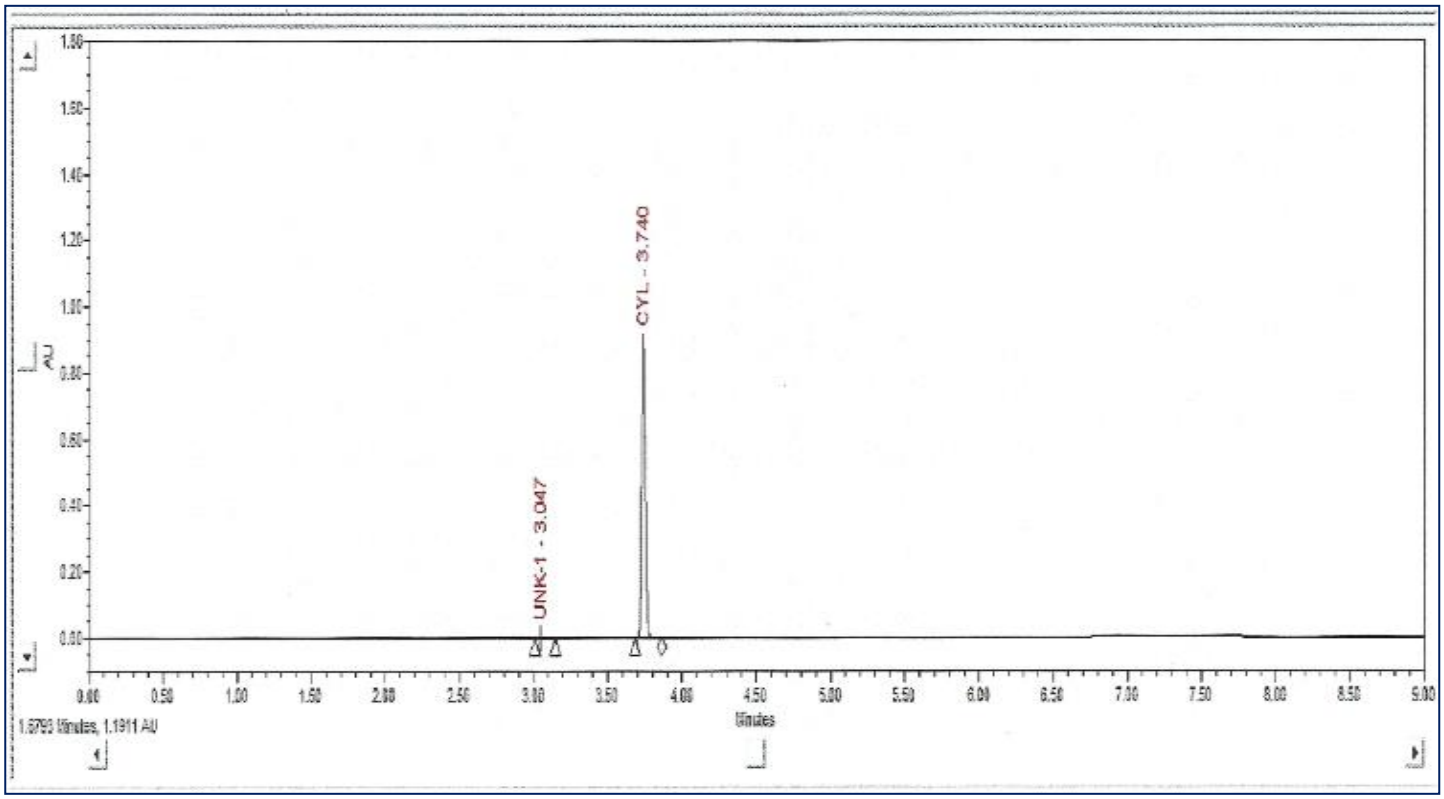


Figure 6: Chromatogram of CYL Thermal Degradation – Sample



Results of the degradation Study

A Stress study was conducted under various conditions and results with chromatogram were shown in Figures 4 - 6. CYL undergoes 6.8% degradation in acid stress conditions and two distinct unknown peaks elutes at 3.048 and 3.473 min respectively. Whereas, CYL undergoes almost 15.5 % degradation in alkaline stress conditions showing unknown peak elute at 3.047, 3.473, and others at minor % levels different retention time. CYL undergoes 2.9 % degradation in peroxide conditions and no major unknown peak is observed as a degradation product in this stress condition. On the other side, CYL shows 1.8 % degradation in thermal conditions with no significant degradant product elution observed. The peak purity for CYL in drug product degradation was found above 0.990 at each stress condition, demonstrating that no additional impurities merge throughout the retention of time CYL.

CONCLUSION

A precise, selective, and particular RP-HPLC-DAD method was successfully created and validated for the detection of CYL in the presence of degradation products. The procedure's reliability is shown by the outcomes of precision and accuracy. There is No significant interference was observed at the retention time of CYL and the linear response observed for a calibration range of 30-200 µg/mL. The stress study results show No significant degradation products observed in peroxide, thermal, and photolytic condition. It shows slight degradation in acid hydrolysis condition. On the other hand, considerable deterioration was seen when under alkaline stress. The CYL was well resolved with degradant products with an acceptable peak purity index proves the stability indicating the nature of the compound. The aforementioned technique is reliable and can be used to estimate CYL at the laboratory level in future applications.

ACKNOWLEDGEMENT

Step-Up Pharmaceuticals provided Cyclandelate as a gift sample for this study, which the authors are grateful for.

Conflicts of interest

The authors affirm that they have no known financial or interpersonal conflicts that would have appeared to have an impact on the research presented in this study.

Funding source: None

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