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# Analytical method development and validation of cilnidipine and ramipril by using reverse phase high performance liquid chromatography

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**Abstract**

**Objective:** the current investigation was pointed at developing and progressively validating novel, simple, responsive and stable rp-hplc method for the measurement of active pharmaceutical ingredients of cilnidipine and ramipril.

**Methods:** a simple, selective, validated and well-defined stability that shows isocratic rp-hplc methodology for the quantitative determination of cilnidipine and ramipril. The chromatographic strategy utilized x-bridge phenyl column of dimensions 250x4.6 mm, 5 micron, using isocratic elution with a mobile phase of acetonitrile and 0.1 percent triethyl amine (60:40). A flow rate of 1 ml/min and a detector wavelength of 242 nm utilizing the pda detector were given in the instrumental settings. Validation of the proposed method was carried out according to an international conference on harmonization (ich) guidelines.

**Results:** lod and loq for the two active ingredients were established with respect to test concentration. The calibration charts plotted were linear with a regression coefficient of $r^2 > 0.999$, means the linearity was within the limit. Recovery, specificity, linearity, accuracy, robustness, ruggedness were determined as a part of method validation and the results were found to be within the acceptable range.

**Conclusion:** the proposed method to be fast, simple, feasible and affordable in assay condition. During stability tests, it can be used for routine analysis of the selected drugs.

**Key words:** cilnidipine, ramipril, rp-hplc, development, validation.

## Introduction

Cilnidipine is a calcium channel blocker [1,2]. Cilnidipine is approved to treat hypertension [3,4]. It is a calcium antagonist accompanied with l-type [5,6] and n-type calcium channel [7] blocking functions. Unlike other calcium antagonists, cilnidipine can act on the n-type calcium channel in addition to acting on the l-type calcium channel. Cilnidipine decreases blood pressure [8,9] and is used to treat hypertension [10], and its comorbidities [11,12]. Due to its blocking action at the l-type and n-type calcium channel, cilnidipine dilates both arteriols and venules, reducing the pressure in the capillary bed. Cilnidipine is vasoselective and has a weak direct dromotropic [13] effect, a strong vasodepressor [14,15] effect, and an arrhythmia-inhibiting [16,17] effect.

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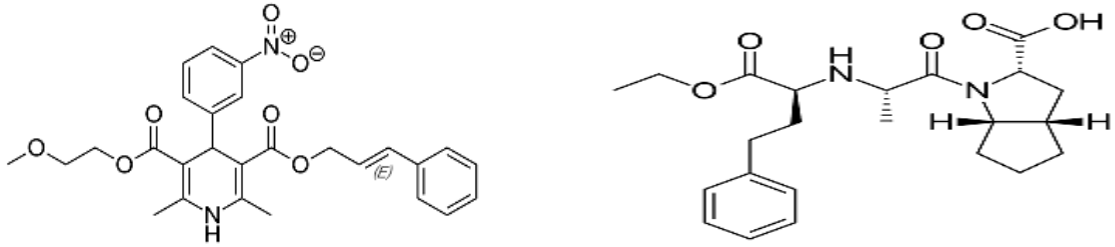
Ramipril is a medication used to treat high blood pressure, heart failure [18,19] and diabetic kidney disease [20,21]. Also used to prevent cardiovascular disease [22,23] in those at high risk. It is a reasonable initial treatment for high blood pressure. It is taken by mouth. Common side effects include headaches, dizziness [24,25], feeling tired and cough. Serious side effects may include liver problems [26], angioedema [27], kidney problems and high blood potassium [28,29]. Use in pregnancy and breast feeding is not recommended. It is an ace inhibitor [30] and works by decreasing rennin-angiotensin-aldosterone [31] system activity.



**A** **b**

**Fig. 1: structure of (a) cilnidipine and (b) ramipril**

**Materials and method**

**Chemicals:** acetonitrile, hplc-grade formic acid, water, were purchased from merck india ltd, mumbai, india. Apis of cilnidipine, ramipril standards were procured from glenmark, mumbai.

**The instrumentation:** waters alliance liquid chromatography (model 2695) monitored with empower 2.0 data handling system and a detector of photo diode array (model 2998) was used for this study.

**Method optimization:** to optimize the chromatographic conditions, different ratios of phosphate buffer and the acetonitrile in the mobile phase with isocratic and gradient mode was tested. However the mobile phase composition was modified at each trial to enhance the resolution and also to achieve acceptable retention times. Finally 0.1% triethyl amine buffer and acetonitrile with isocractic elution was selected because it results in a greater response of active pharmacy ingredient. During the optimization of the method various stationary phases such as $c_8$, $c_{18}$ and amino, phenyl columns were tested. From these trials the peak shapes were relatively good with x-bridge phenyl column of 250 x 4.6mm, 5 µ with a pda detector. The mobile phase flow rate has been done at 242nm in order to obtain enough sensitivity. By using above conditions we get retention times of cilnidipine and ramipril were about 3.0 min and 7.2 min with a tailing factor of 1.11 & 0.78. The number of theoretical plates for cilnidipine and ramipril were 8634, 9261 which indicate the column's successful output the % rsd for six replicate injections was around 0.51% and 0.63%, the proposed approach suggests that it is extremely precise. According to ich guidelines, the method established was validated.

Till today there are no hplc methods were reported in the literature, but only few methods are developed in individual analysis of cilnidipine and ramipril. Hence we developed method for the simultaneous quantification of cilnidipine and ramipril. The developed hplc method was utilized for the estimation of the combined drugs by *in vitro* method.

**Validation procedure**

The analytical parameters such as system suitability, precision, specificity, accuracy, linearity, robustness, lod, loq, forced degradation and stability were validated according to ich q2 (r1) guidelines.

**Preparation of buffer:** 1 ml of triethyl amine is dissolved in 1 lt of hplc grade water and filter through 0.45 µ filter paper.

**Chromatographic conditions:** the hplc analysis was performed on reverse phase hplc system with isocratic elution mode using a mobile phase of acetonitrile and 0.1% triethyl amine and x-bridge phenyl (250x4.6 mm, 5 µ) column with a flow rate of 1 ml /min.

**Diluent:** mobile phase was used as diluent.

**Preparation of the standard stock solution:** for standard stock solution preparation, add 70ml of diluents to 10mg of cilnidipine and 10 mg of ramipril taken in a 100 ml volumetric flask and sonicate for 10 minutes to fully dissolve the contents and then make up to the mark with diluent.

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**Preparation of standard solution:** 5 ml of solution is drawn from the above normal stock solution into a 50ml volumetric flask and diluted up to the level.

**Results and discussion**

The main analytical challenge during development of a new method was to separate active pharmacy ingredients. In order to provide a good performance the chromatographic conditions were optimized.

**System suitability:** in system suitability injecting standard solution and reported usp tailing and plate count values are tabulated in table 1 and the standard chromatogram was shown in figure 2.

**Table 1:** results of system suitability

| System suitability parameter | Acceptance criteria | Drug name | |
|---|---|---|---|
| | | Cilnidipine | Ramipril |
| Usp plate count | Nlt 2000 | 8634 | 9261 |
| Usp tailing | Nmt 2.0 | 1.11 | 0.78 |
| Usp resolution | Nlt 2.0 | - | 13.67 |
| % rsd (n=6) | Nmt 2.0 | 0.51 | 0.63 |

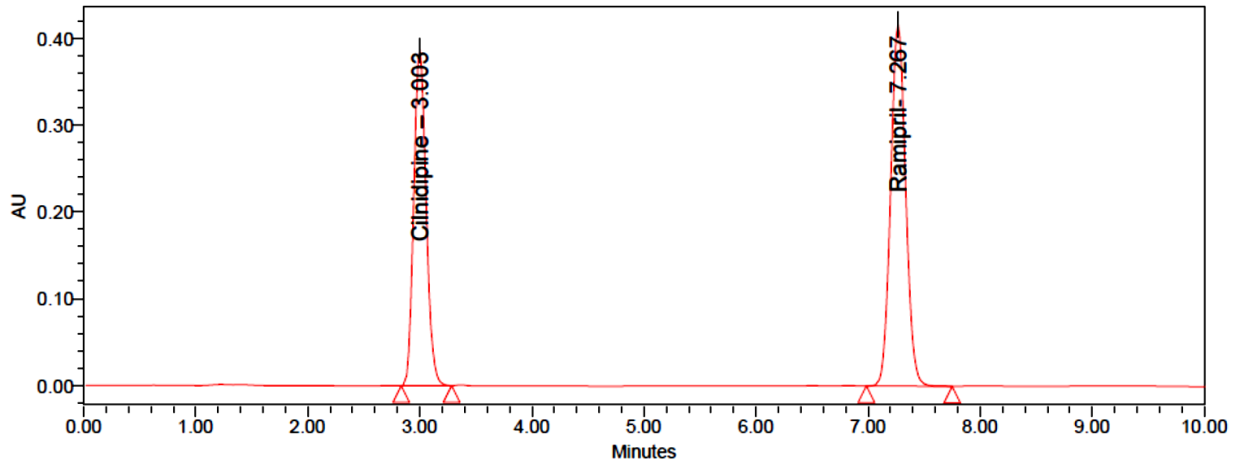


**Fig. 2: chromatogram of standard**

**Specificity:** in this test method placebo, standard and standard solutions were analyzed individually to examine the interference. The below figure shows that the active ingredients were well separated from blank and their excipients and there was no interference of placebo with the principal peak. Hence the method is specific.

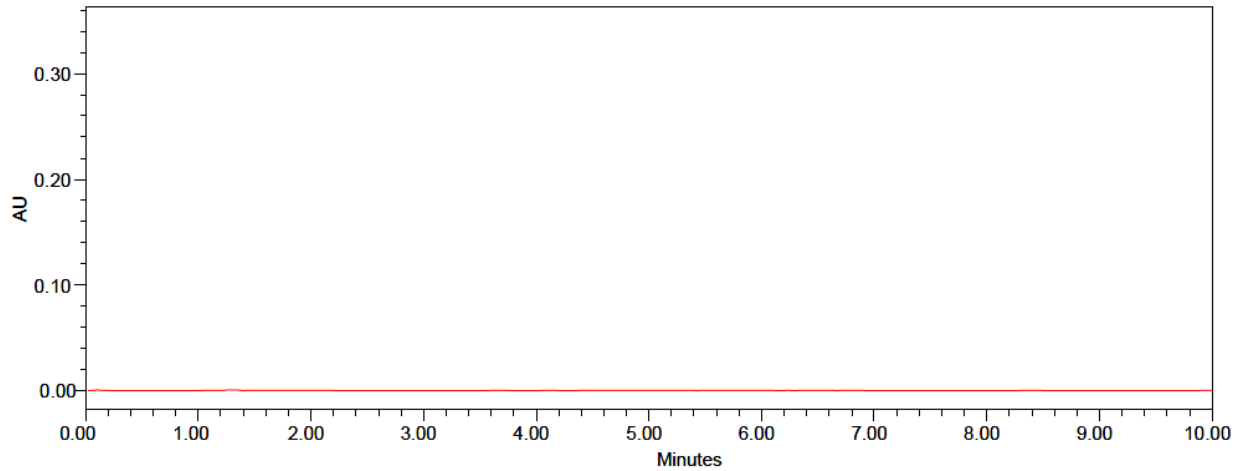


**Fig. 3: chromatogram of blank**

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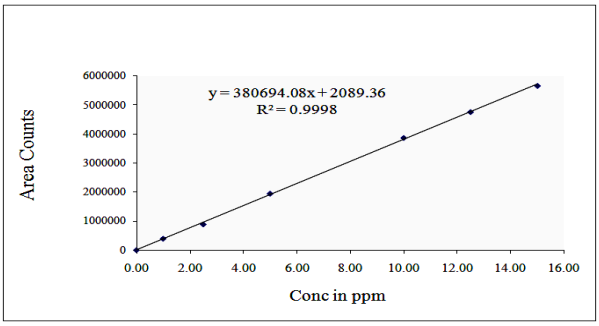
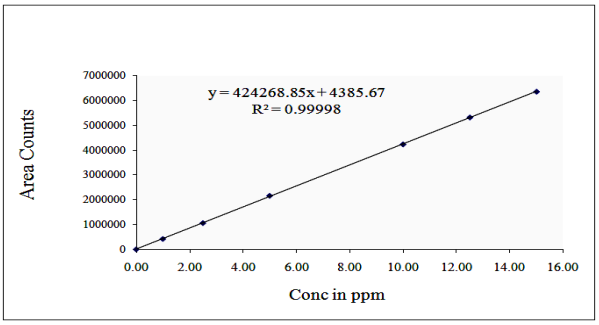
**Linearity:** the area of the linearity peak versus different concentrations has been evaluated for cilnidipine, ramipril, as 10, 25, 50, 100, 125, 150 percent respectively. Linearity was performed in the range of 17.5-262.5µg/ml of cilnidipine and 1-15µg/ml of ramipril. The correlation coefficients achieved greater than 0.999 for all.

**Table 2: linearity of cilnidipine and ramipril**

| S.no | Conc. µg/ml | Cilnidipine area count | Conc. µg/ml | Ramipril area count |
|---|---|---|---|---|
| 1 | 1.00 | 391971 | 1.00 | 419865 |
| 2 | 2.50 | 887459 | 2.50 | 1056954 |
| 3 | 5.00 | 1947935 | 5.00 | 2158749 |
| 4 | 10.00 | 3869390 | 10.00 | 4233016 |
| 5 | 12.50 | 4763458 | 12.50 | 5319841 |
| 6 | 15.00 | 5666340 | 15.00 | 6358642 |
| Correl coef | | 0.99980 | | 0.99998 |
| Slope | | 380694.08 | | 424268.85 |
| Intercept | | 2089.36 | | 4385.67 |




Cilnidipine




ramipril

**Fig. 4: calibration plots of (a) cilnidipine (b) ramipril**

**Accuracy:** in this method, accuracy was conducted in triplicate by analyzing active pharma ingredient standard solution at three kinds of concentration levels of 50, 100 and 150% of each at a specified limit. For all impurities, percentage recoveries were measured and found to be within the limit. The accuracy and reliability of the developed method were established. The percentage recovery values were found to be in acceptable range. The results are given in table 3.

**Table 3: results of accuracy**

| S. No | % level | Cilnidipine % recovery | Ramipril % recovery |
|---|---|---|---|
| 1 | 50 | 99.6 | 99.5 |
| 2 | 100 | 99.3 | 99.5 |
| 3 | 150 | 99.0 | 99.6 |

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**Intraday precision:** six replicates of a standard solution containing cilnidipine (10µg/ml) and ramipril (10µg/ml) were analysed on the same day. Peak areas were calculated, which were used to calculate mean, sd and %rsd values.

**Table 4: intraday precision results of cilnidipine and ramipril**

| **Cilnidipine** | | | | **Ramipril** | | |
|---|---|---|---|---|---|---|
| **S.no** | **Conc.(µg/ml)** | **Area counts** | **% assay as is** | **Conc.(µg/ml)** | **Area counts** | **% assay as is** |
| 1 | 10 | 3932418 | 99.4 | 10 | 4250638 | 100 |
| 2 | | 3901582 | 98.7 | | 4247745 | 99.9 |
| 3 | | 3914570 | 99 | | 4231502 | 99.5 |
| 4 | | 3928269 | 99.3 | | 4221078 | 99.3 |
| 5 | | 3917321 | 99.1 | | 4285941 | 100.8 |
| 6 | | 3951651 | 99.9 | | 4239865 | 99.7 |
| % rsd | 0.41 | | | 0.52 | | |
| Mean | 99.2 | | | 99.9 | | |
| Sd | 0.408 | | | 0.524 | | |

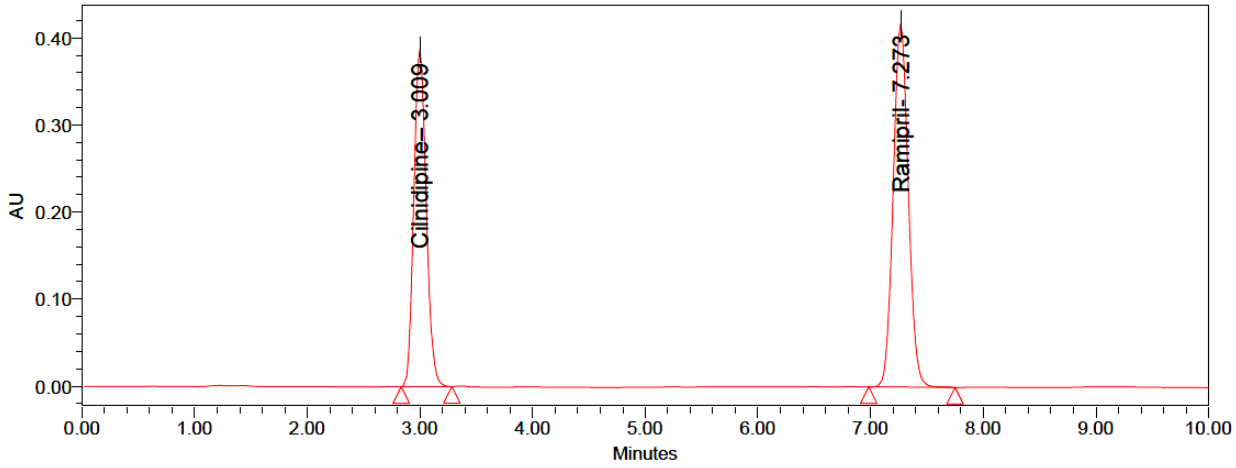


**Fig. 5: chromatogram of method precision**

**Inter-day precision:** six replicates of a standard solution containing cilnidipine (10µg/ml) and ramipril (10µg/ml) were analysed on a different day. Peak areas were calculated which were used to calculate mean, sd and %rsd values. The present method was found to be precise as the rsd values were less than 2% and also the percentage assay values were close to be 100%. The results are given in table 5.

**TABLE 5: INTER-DAY OUTCOMES OF ACCURACY OF CILNIDIPINE AND RAMIPRIL**

| **Cilnidipine** | | | | **Ramipril** | | |
|---|---|---|---|---|---|---|
| **S.no.** | **Conc.(µg/ml)** | **Area counts** | **% assay as is** | **Conc.(µg/ml)** | **Area count** | **% assay as is** |
| 1 | | 3958214 | 100.1 | 10 | 4201587 | 98.8 |

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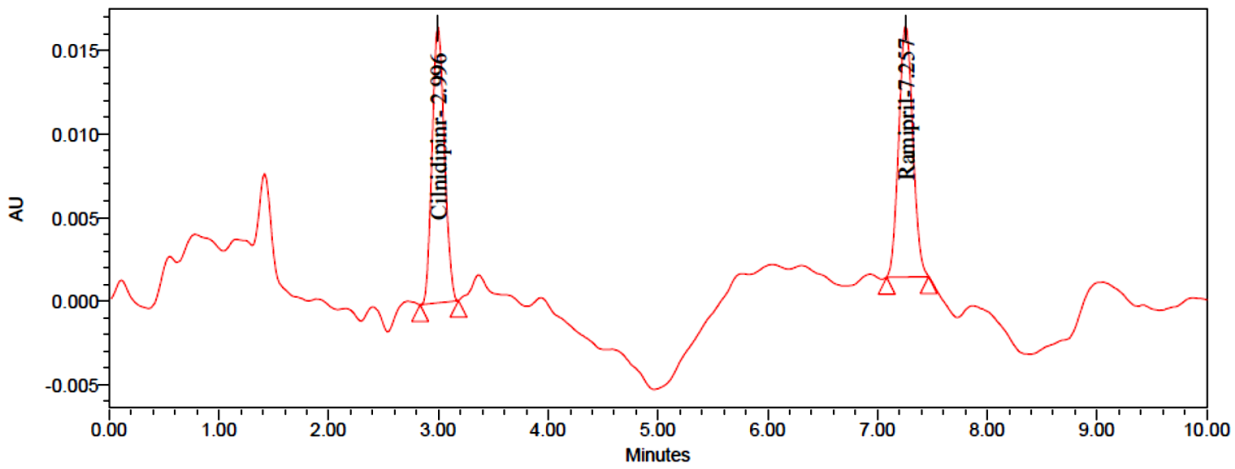
| | | | | | | |
|---|---|---|---|---|---|---|
| 2 | 10 | 3913206 | 99.0 | | 4196583 | 98.7 |
| 3 | | 3915427 | 99.0 | | 4236512 | 99.7 |
| 4 | | 3926539 | 99.3 | | 4215784 | 99.2 |
| 5 | | 3904362 | 98.7 | | 4265341 | 100.3 |
| 6 | | 3941488 | 99.7 | | 4265312 | 100.3 |
| %rsd | 0.52 | | | 0.72 | | |
| Mean | 99.3 | | | 99.5 | | |
| Sd | 0.518 | | | 0.713 | | |

**Lod and loq:** the lod concentrations for cilnidipine are 0.015 µg/ml and s/n values is 7 and ramipril 0.013 µg/ml and s/n value 6. The loq concentration for cilnidipine 0.1 µg/ml and their s/n values are 25 and ramipril 0.1 µg/ml and s/n value is 25. The method is validated as per the ich guidelines.

**TABLE 6: LOD AND LOQ FOR CILNIDIPINE AND RAMIPRIL**

| **Cilnidipine** | | | | **Ramipril** | | | |
|---|---|---|---|---|---|---|---|
| **Lod** | | **Loq** | | **Lod** | | **Loq** | |
| Concentration | S/n | Concentration | S/n | Concentration | S/n | Concentration | S/n |
| 0.015µg/ml | 7 | 0.1 µg/ml | 25 | 0.013µg/ml | 6 | 0.1 µg/ml | 25 |




**A**

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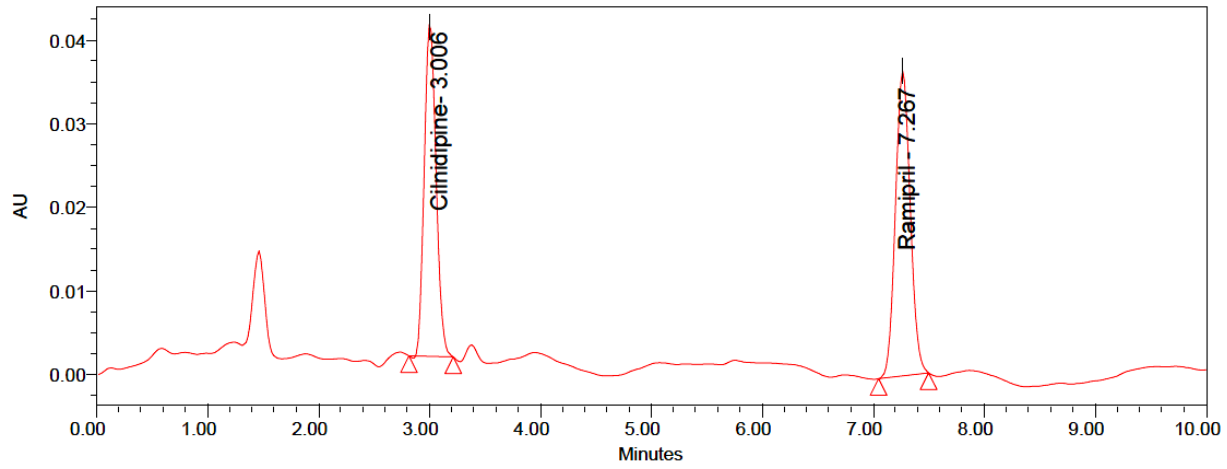


**B**

**FIG. 6: CHROMATOGRAM OF (A) LOD AND (B) LOQ**

**Robustness:** the conditions of the experiment were designed to test the robustness of established system intentionally altered, such as flow rate, mobile phase in organic percentage in all these varied conditions. Robustness results for cilnidipine and ramipril found to be within the limit and results are tabulated in table 7.

**TABLE 7: ROBUSTNESS DATA OF CILNIDIPINE AND RAMIPRIL**

| Parameter name | % rsd | |
|---|---|---|
| | Cilnidipine | Ramipril |
| Flow minus (0.8 ml/min | 1.32 | 0.96 |
| Flow plus (1.2 ml/min) | 0.15 | 0.51 |
| Organic minus (-10%) | 0.11 | 0.37 |
| Organic plus (+10%) | 0.12 | 0.21 |

**Degradation studies:** the ramipril and cilnidipine standard was subjected into various forced degradation conditions to effect partial degradation of the drug. Studies of forced degradation have carried out to find out that the method is suitable for products of degradation. In addition, the studies provide details about the conditions during which the drug is unstable, in order that the measures are often taken during formulation to avoid potential instabilities.

**Acid degradation:** acid degradation was done by using 1n hcl and 15.4% of cilnidipine and 14.7% of ramipril degradation was observed.

**Alkali degradation**: alkali degradation was done at 1n naoh and 14.9% of cilnidipine and 14.2% of ramipril degradation was observed.

**Peroxide degradation:** peroxide degradation was performed with 30% hydrogen peroxide and 13.2% cilnidipine, 13.9% of ramipril degradation was observed.

**Reduction degradation:** reduction degradation was performed with 30% sodium bi sulphate solution, 12.4% cilnidipine and 12.5% ramipril degradation was observed.

**Thermal degradation:** in thermal degradation the standard was degraded to 12.1% of cilnidipine and 11.3% of ramipril.

**Degradation of hydrolysis:** in hydrolysis degradation the standard was degraded to 1.6% of cilnidipine and 1.1% of ramipril.

All degradation results are tabulated in table 9.

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**Table 9: forced degradation results of cilnidipine and ramipril**

| Degradation condition | Cilnidipine | | Ramipril | |
|---|---|---|---|---|
| | % assay | %deg | % assay | % deg |
| Acid degradation | 84.6 | 15.4 | 85.3 | 14.7 |
| Alkali degradation | 85.1 | 14.9 | 85.8 | 14.2 |
| Peroxide degradation | 86.8 | 13.2 | 86.1 | 13.9 |
| Reduction degradation | 87.6 | 12.4 | 87.5 | 12.5 |
| Thermal degradation | 87.9 | 12.1 | 88.7 | 11.3 |
| Hydrolysis degradation | 98.4 | 1.6 | 98.9 | 1.1 |

**Conclusion**

We present in this article simple, selective, validated and well-defined stability that shows gradient rp-hplc methodology for the quantitative determination of cilnidipine and ramipril. All the products of degradation formed during the stress conditions and the related active pharma ingredients are well separated and peaks were well resolved from each other and separate with an appropriate retention time indicating that the proposed method to be fast, simple, feasible and affordable in assay condition. Therefore the developed method during stability tests, it can be used for routine analysis of production standards and to verify the quality of drug standards during stability studies.

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**Conflicts of interest:** none

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