Objective: This study aims to build up the RP-HPLC process for Azilsartan and Cilnidipine and authenticate the RP-HPLC process according to ICH validation code Q2R1.

Methods: System suitability testing was performed to discover the qualifying criterion of the method by injecting the identical standard solution of Azilsartan 40μg/ml and Cilnidipine 10μg/ml in mixture/combo in subsequent optimized chromatographic conditions and the chromatogram was recorded. Moreover, the planned method was validated as per ICH guideline Q2R1 for the following parameters: linearity and range, precision, accuracy, robustness, and determined % recovery.

Results: The outcomes of %RSD for retention time and peak area were found to be 0.65 and 1.32 for Azilsartan and 0.85 and 1.90 for Cilnidipine. The correlation coefficient, y-intercept, slope of the regression line were 0.9996, -1127.1, 3313.9, and 0.9993, 1460.2, 2876.4 for Azilsartan and Cilnidipine, respectively. Moreover, the range of this method was observed to be 40-240 μg/ml and 10-60 μg/ml for Azilsartan and Cilnidipine, standard concentrations respectively. The % RSD achieved for precision (repeatability) was observed in the range of 1.57 to 2.43 for Azilsartan and 0.70 to 1.88 for Cilnidipine. The % accuracy was found in the range of 96.96 to 101.92 w/w for Azilsartan and 99.19 to 101.96% w/w for Cilnidipine. The percent recovery values achieved for Azilsartan were in the range of 99.87 to 106.39% w/w and for Cilnidipine in the range of 94.51 to 105.96% w/w.

Conclusion: The author concludes that the simultaneous estimation of Azilsartan and Cilnidipine with predefined objectives was successfully achieved. Moreover, the method was found to be steadfast for the quantification of Azilsartan and Cilnidipine in marketed tablet dosage forms.

Keywords: Azilsartan, Cilnidipine, RP-HPLC, Linearity and range, Precision, %Accuracy, Robustness, Limit of detection, Limit of quantification
separation was performed using Hypersil ODS C18 (250 mm×4.6 mm), 5 μm id column with ambient temperature. The mobile phase seen suitable for the study was Acetonitrile and potassium dihydrogen phosphate buffer of pH 3.0 in the proportion of 80:20% v/v. The flow rate was maintained at 1.2 ml/min. The mobile phase was filtered through a 0.45μ membrane filter and also degassed before use. The injection volume was 10μl and the detector was set at 250 nm. The method run time was 12 min.

Table 1: Optimized chromatographic conditions

| Chromatographic conditions | Column | Mobile phase | Detection wavelength | Flow rate | Temperature | Sample size | Run Time |
|---------------------------|--------|--------------|----------------------|-----------|-------------|-------------|----------|
|                           | C18 (250 mm×4.6 mm), 5μm id | Acetonitrile: phosphate buffer (pH 3.0) 80:20 | 250 nm (Isobestic Point) | 1.2 ml/min | Ambient | 10μl | 12 min |

Linearity
Aliquots of 1.0, 2.0, 3.0, 4.0, 5.0, and 6.0 ml standard stock solution (Azilsartan Medoxomil 400μg/ml and Cilnidipine 100μg/ml) were pulled out and taken into a 10 ml volumetric flask. The volume of the afterward was made up to 10 ml with mobile (acetonitrile 80: KH2PO4 buffer 20, pH 3.0) to find the following solution of 40, 80, 120, 160, 200, 240μg/ml in that order for Azilsartan Medoxomil and 10, 20, 30, 40, 50 and 60 μg/ml for Cilnidipine. All of these standard working solutions of Azilsartan Medoxomil and Cilnidipine (in the mixture) were injected as a mixture in triplicate to the optimized chromatographic parameters and mean peak area was determined [29]. A calibration curve was arranged among the concentration of standard solutions of Azilsartan Medoxomil and Cilnidipine. Mean peak area consequential out of chromatographic measurement with each standard concentration. From the calibration curve equation of the line, correlation coefficient and intercept were calculated. The general equation of a straight line is as mentioned underneath.

\[ y = mx + c \]

Where, \( Y \) = Peak area; \( m \) = slope; \( X \) = measured concentration; \( c \) = intercept.

Precision
The precision of the method was studied by assessment and repeatability and intermediate precision. Across the range, the three standards (viz. 60, 140, and 220μg/ml for Azilsartan Medoxomil and 15, 35, and 55μg/ml for Cilnidipine likewise) were selected, and three replicates of the same were injected into the optimized chromatographic conditions to determine peak area. Appropriate statistical analysis was performed to calculate statistical parameters [30]. Repeatability was studied by measurements of three standards and its three replicates in a day. However, the intermediate precision was studied on different days.

% recovery (% accuracy) by standard addition method
Preparation of standard concentrations of azilsartan medoxomil and cilnidipine
The 40μg/ml and 10 μg/ml standard solutions of Azilsartan Medoxomil and Cilnidipine were prepared as procedure mentioned above in triplicate and kept in three different volumetric flasks.

Preparation of sample concentrations of azilsartan medoxomil and cilnidipine
Twenty tablets of the combined dosage form of Azilsartan Medoxomil and Cilnidipine (Myotan CN 40/10, labeled claim Azilsartan Medoxomil 40 mg, Cilnidipine 10 mg) were weighed; average weight (0.1844 gm) was determined and powdered. Powder equivalent to 40 mg of Azilsartan Medoxomil, 0.1844g (10 mg of Cilnidipine) was weighed and pulled out to 100 ml of mobile phase to achieve the sample stock solution of Azilsartan Medoxomil 400μg/ml (100 μg/ml for Cilnidipine). The resulting sample solution was filtered through a 0.45μ membrane filter and degassed using ultrasonication for 3 cycles each of 10 min. From the sample stock solution, an aliquot of 1.0 ml was taken with a micropipette, transferred to a 10 ml volumetric flask, and diluted up to the mark with mobile phase to acquire a consequential solution of 40μg/ml for Azilsartan Medoxomil (10μg/ml for Cilnidipine). Likewise,

**Fig. 1: Structure of drugs**

Formulation used
Myotan 40 tablets (Synochem Pharmaceutical Ltd.), with the strength of Azilsartan Medoxomil 40 mg and Cilnidipine 10 mg were purchased from the local medical store at Aurangabad. This formulation was utilized to study the relevance of the present method for estimation of Azilsartan Medoxomil and Cilnidipine in the marketed tablet dosage form.

Preparation of standard stock solution of azilsartan medoxomil and cilnidipine
Weighed accurately 40 mg of Azilsartan Medoxomil and 10 mg Cilnidipine and transferred to an identical 10 ml volumetric flask containing a mixture of acetonitrile: phosphate buffer (pH 3.0) (80:20), the mobile phase. The volume was made up to the mark with the help of the mobile phase. The consequential standard stock solutions of Azilsartan Medoxomil (400μg/ml) and Cilnidipine (100μg/ml) were filtered through a 0.45μ membrane filter and ultrasonicated for 3 cycles each of 10 min. This standard stock was employed for preparing various concentration solutions required in the different validation parameters.

Preparation of working solution
Aliquot 1.0 ml stock solution was taken from the above standard stock solution of Azilsartan Medoxomil and Cilnidipine. The aliquot was transferred to an identical 10 ml volumetric flask. It was then diluted up to 10 ml using mobile phase to attain resultant solution consisting of 40μg/ml of Azilsartan Medoxomil and 10μg/ml Cilnidipine. This outfitted solution was degassed by an ultrasonicator for 10 min.

Procedures
System suitability testing
This test was performed using 40μg/ml and 10μg/ml Azilsartan Medoxomil and Cilnidipine, respectively. The study was conducted using six repeated measurements in the optimized chromatographic conditions, as illustrated in table 1.
aliquots of 0.8 and 1.2 ml were pulled out from the sample stock solution (400μg/ml and 100μg/ml) to acquire the operational sample solutions of 32 and 48μg/ml (8 and 12μg/ml for Cilnidipine, respectively). The three sample solutions of combined dosage form viz. 32, 40, and 48μg/ml and 8, 10, and 12μg/ml (Azilsartan Medoxomil and Cilnidipine, respectively) were labeled as three levels of percent recovery testing viz. 80, 100, and 120% in that order.

**Preparation of test solution for % recovery**

40μg/ml and 10 μg/ml standard solution of a mixture of Azilsartan Medoxomil and Cilnidipine was spiked into every sample solution of combined dosage form viz. 32, 40 , and 48μg/ml and 8, 10 , and 12μg/ml to attain test solutions at 80%, 100% and 120% levels correspondingly. Each of these 3 percent recovery levels was injected in triplicate in optimized chromatographic conditions of the projected method. The mean peak area for each percent recovery level was determined. The recovery was calculated from the following formula [31].

\[
\% \text{ Recovery} = \frac{\text{sample peak area}}{\text{standard peak area}} \times \frac{\text{standard concentration}}{\text{sample concentration}} \times 100
\]

**Robustness**

The robustness of the proposed simultaneous method of Azilsartan Medoxomil and Cilnidipine was studied by deliberate redecoration in method parameters [24, 25]. In the present experimentation, the method parameters viz. detector wavelength in ‘nm’, the flow rate of the mobile phase in ‘mL/min’, and organic concentration of the mobile phase were altered as per table 2. The standard solution with concentrations of Azilsartan Medoxomil (40μg/ml) and Cilnidipine (10μg/ml) was selected for this examination. It was maintained stable throughout the robustness study till all planned variations were effected. The measurements were made in triplicate.

| Method parameter | Standard | Variation 1 | Variation 2 |
|------------------|----------|-------------|-------------|
| Wavelength in ’nm’ | 250      | 251         | 249         |
| Flow rate of mobile phase in ml/min (±0.1 ml/min) | 1.2   | 1.3         | 1.1         |
| Organic conc. of Mobile phase (±2%) | 80     | 82          | 78          |

**LOD and LOQ determination**

LOD and LOQ were calculated based on the standard deviation of the response and the slope using the following formulae.

\[
\text{LOD} = \frac{3.3 \times \sigma}{\text{Slope}}
\]

\[
\text{LOQ} = \frac{10 \times \sigma}{\text{Slope}}
\]

Where σ = the standard deviation of the responses.

The slope was estimated from the calibration curve. The standard deviation of the responses was calculated by determining the standard deviation of the y-intercept of the regression line. The latter was used as a standard deviation [26, 27].

**RESULTS**

**System suitability testing (SST)**

This was performed by six repeated measurements of the standard solutions of Azilsartan Medoxomil and Cilnidipine (40μg/ml and 10 μg/ml). The results acquired were as tabulated in table 3.

| S. No. | Peak area Azilsartan medoxomil | RT in ‘min.’ Azilsartan medoxomil | Peak area Cilnidipine | RT in ‘min.’ Cilnidipine |
|--------|---------------------------------|-----------------------------------|-----------------------|--------------------------|
| 1      | 50992                           | 4.03                              | 31847                 | 7.21                     |
| 2      | 52431                           | 4.01                              | 33281                 | 7.16                     |
| 3      | 51426                           | 3.99                              | 32231                 | 7.12                     |
| 4      | 50987                           | 3.98                              | 32011                 | 7.11                     |
| 5      | 52297                           | 3.97                              | 33069                 | 7.06                     |
| 6      | 52310                           | 3.96                              | 33021                 | 7.05                     |
| Avg. area (n = 6) | 51740.50                       | 3.99                              | 32576.67              | 7.12                     |
| SD    | 683.77                          | 0.03                              | 617.70                | 0.06                     |
| %RSD  | 1.32                            | 0.65                              | 1.90                  | 0.85                     |

n = 6; SD: Standard deviation of the responses; %RSD: % relative standard deviation, the representative chromatogram observed in system suitability testing was as depicted in fig. 2.

![Fig. 2: The chromatogram observed in SST of azilsartan medoxomil and cilnidipine](image-url)
Linearity
The linearity of the method was seen in the range of 40-240 μg/ml and 10-60 μg/ml for Azilsartan Medoxomil and Cilnidipine (AZL and CIL), respectively. The study was performed with three replicates measurements of each standard solution of AZL as well as CIL. The average area is explored in Table 4. The calibration curve was constructed against the average peak area and the standard concentrations of AZL and CIL. The calibration curves observed were as exposed in fig. 3a and 3b. The equation of regression line, slope, and y-intercept were estimated and shown in fig. 3a and 3b.

Precision
The precision of the presented method was studied by measuring three standards and three replicates of each covering total of nine determinations. Repeatability was assessed by measuring three standards and three replicates on the same day. However, intermediate precision (ruggedness) was studied on three different days. The results observed are shown in Table 5 for repeatability and Table 6, 7, etc. for an intermediate precision. The statistical parameters like standard deviation and relative standard deviation were also calculated and shown in Tables 5, 6, and 7.

Table 4: Outcomes of the linearity experiment of azilsartan medoxomil and cilnidipine

| Conc. (μg/ml) | AZL Avg. peak area (n = 3) | CIL Avg. peak area (n = 3) | SD | %RSD |
|--------------|---------------------------|---------------------------|----|------|
| 40           | 47217                     | 29975                     | 1122.02 | 366.85 | 2.38 | 1.22 |
| 80           | 39712                     | 57877                     | 735.02  | 316.39 | 0.78 | 0.55 |
| 120          | 138730                    | 88724                     | 31682.68 | 708.53 | 1.61 | 0.80 |
| 160          | 184721                    | 116918                    | 1108.06 | 554.25 | 0.60 | 0.47 |
| 200          | 231071                    | 147240                    | 5781.09 | 718.36 | 0.25 | 0.49 |
| 240          | 271189                    | 172064                    | 724.85  | 1705.31 | 0.27 | 0.99 |

n = 3: results three repeated injections; SD: standard deviation; %RSD: % relative standard deviation; AZL: Azilsartan Medoxomil; CIL: Cilnidipine

Fig. 3a: Calibration curve of azilsartan medoxomil

Fig. 3b: Calibration curve of cilnidipine

Table 5: Outcomes of the precision (repeatability) experiment of AZL and CIL (Day 1)

| Conc. (μg/ml) | AZL Avg. peak area (n = 3) | CIL Avg. peak area (n = 3) | SD | %RSD |
|--------------|---------------------------|---------------------------|----|------|
| 60           | 72948.33                  | 45636.00                  | 1145.27 | 858.31 | 1.57 | 1.88 |
| 140          | 172065.00                 | 109402.00                 | 2691.16 | 1248.77 | 1.56 | 1.14 |
| 220          | 265062.67                 | 168074.67                 | 6498.57 | 1173.26 | 2.43 | 0.70 |

n = 3: three repeated injections; Avg. Peak area: Average peak area of three repeated measurements; SD: standard deviation; %RSD: %relative standard deviation
The method as per ICH guideline Q2R1 viz. accuracy and specificity. The % recovery assessment is a trial to discover two parameters of corresponding peak area respectively. The % amount found of AZL and CIL in this experiment in these three cases were explored in Tables 8, 9, and 10, concentration, and the mobile phase’s flow rate. The results attained were varied viz. wavelength, the mobile phase’s organic the method parameters. In this research work, three parameters were varied viz. wavelength, the mobile phase’s organic concentration, and the mobile phase’s flow rate. The results attained in these three cases were explored in Tables 8, 9, and 10, respectively. The % amount found of AZL and CIL in this experiment was calculated from the regression equation using the corresponding peak area.

**Table 6: Outcomes of intermediate precision of AZL and CIL (Day 2)**

| Conc. (μg/ml) | Azilsartan Medoxomil (AZL) | Cilnidipine (CIL) | SD | %RSD |
|--------------|-----------------------------|-------------------|----|------|
| AZL          |                             |                   |    |      |
| CIL          |                             |                   |    |      |
| 60           | 15                          | 7128.23           | 45008 | 981.92 | 846.39 | 1.10 | 1.88 |
| 140          | 35                          | 15650.033         | 102620 | 2883.95 | 801.89 | 1.84 | 0.78 |
| 220          | 55                          | 251937.33         | 160109 | 3995.75 | 1428.81 | 1.59 | 0.89 |

n = 3: three repeated injections; Avg. Peak area: Average peak area of three repeated measurements; SD: standard deviation; %RSD: %relative standard deviation

**Table 7: Outcomes of intermediate precision of AZL and CIL (Day 3)**

| Conc. (μg/ml) | Azilsartan Medoxomil (AZL) | Cilnidipine (CIL) | SD | %RSD |
|--------------|-----------------------------|-------------------|----|------|
| AZL          |                             |                   |    |      |
| CIL          |                             |                   |    |      |
| 60           | 15                          | 71095.33          | 44809.00 | 738.76 | 985.22 | 1.04 | 2.20 |
| 140          | 35                          | 154319.00         | 99522.33 | 2659.98 | 597.47 | 1.72 | 0.60 |
| 220          | 55                          | 249555.00         | 158592.00 | 3078.19 | 842.82 | 1.23 | 0.53 |

n = 3: three repeated injections; Avg. Peak area: Average peak area of three repeated measurements; SD: standard deviation; %RSD: %relative standard deviation

**Table 8: Results acquired for robustness experiment with variation in detector wavelength for a mixture of AZL and CIL at 40 and 10 ppm, respectively**

| λ in ‘nm’ | Azilsartan Medoxomil (AZL) | Cilnidipine (CIL) | Avg. area* | Avg. measured conc. (μg/ml)* | % amount found (w/w) | Inference |
|----------|-----------------------------|-------------------|------------|-----------------------------|----------------------|-----------|
| 250      | 47217                       | 29975             | 41.21      | 9.91                        | 103.03               | 99.13     | Complied | Complied |
| 251      | 48258                       | 30119             | 38.95      | 9.96                        | 97.38                | 99.63     | Complied | Complied |

*n = 3 Average area of three repeated measurements; *Estimated from regression equation; AZL: Azilsartan Medoxomil; CIL: Cilnidipine

**Table 9: Results acquired for robustness experiment with variation organic concentration of the mobile phase for a mixture of AZL and CIL at 40 and 10 ppm, respectively**

| Organic conc. % | Azilsartan Medoxomil (AZL) | Cilnidipine (CIL) | Avg. area* | Avg. measured conc. (μg/ml)* | % amount found (w/w) | Inference |
|-----------------|-----------------------------|-------------------|------------|-----------------------------|----------------------|-----------|
| 80              | 47217                       | 29975             | 41.21      | 9.91                        | 103.03               | 99.13     | Complied | Complied |
| 78              | 48482                       | 30673             | 40.07      | 10.16                       | 100.19               | 101.56    | Complied | Complied |
| 82              | 47452                       | 30395             | 39.16      | 10.06                       | 97.90                | 100.59    | Complied | Complied |

*n = 3 Average peak area of three repeated measurements; *Estimated from regression equation; AZL: Azilsartan Medoxomil; CIL: Cilnidipine

**Table 10: Results acquired for robustness experiment with variation in mobile phase flow rate in ‘ml/min’ for a mixture of AZL and CIL at 40 and 10 ppm, respectively**

| Flow rate ‘ml/min’ | Azilsartan Medoxomil (AZL) | Cilnidipine (CIL) | Avg. area* | Avg. measured conc. (μg/ml)* | % amount found (w/w) | Inference |
|-------------------|-----------------------------|-------------------|------------|-----------------------------|----------------------|-----------|
| 1.2               | 47217                       | 29975             | 41.21      | 9.91                        | 103.03               | 99.13     | Complied | Complied |
| 1.1               | 51562                       | 32482             | 42.81      | 10.78                       | 107.02               | 107.85    | Complied | Complied |
| 1.3               | 45093                       | 28455             | 37.07      | 9.38                        | 92.67                | 93.85     | Complied | Complied |

*n = 3 Average area of three repeated measurements; # Estimated from regression equation; AZL: Azilsartan Medoxomil; CIL: Cilnidipine

**Robustness**

The robustness of the method was studied to establish that the technique remains unaffected by minor but purposeful variations in the method parameters. In this research work, three parameters were varied viz. wavelength, the mobile phase’s organic concentration, and the mobile phase’s flow rate. The results attained in these three cases were explored in Tables 8, 9, and 10, respectively. The % amount found of AZL and CIL in this experiment was calculated from the regression equation using the corresponding peak area.

**% Accuracy by % recovery method**

The % recovery assessment is a trial to discover two parameters of the method as per ICH guideline Q2R1 viz. accuracy and specificity. The accuracy of the method was studied with the planned process by determining the recovered amount of Azilsartan Medoxomil and Cilnidipine by the spike method. A known quantity of standard solutions of drugs (40 and 10μg/ml of Azilsartan Medoxomil and Cilnidipine as API) were spiked to a sample solution of Azilsartan Medoxomil and Cilnidipine (32, 40, 48μg/ml for Azilsartan Medoxomil and 8, 10, 12 μg/ml for Cilnidipine) representing 80, 100 and 120 % levels.

The results observed for % accuracy are shown in Tables 11 and 12 for AZL and CIL, respectively. To establish the specificity of the method, a blank followed by a sample was injected. It was observed that no interference due to commonly used excipients was seen. The representative chromatogram observed in the recovery study at the 120% level is shown in fig. 4.
HPLC method for Azilsartan Medoxomil and Cilnidipine in bulk as

**DISCUSSION**

Extensive literature was studied before the development of the RP-HPLC method for Azilsartan Medoxomil and Cilnidipine in bulk as API. Riddhi J. Jani et al. developed a spectrophotometric method to simultaneously estimate Azilsartan Medoxomil Kamedoxomil and Cilnidipine in a synthetic mixture. Beer’s law was obeyed in the concentration range of 2-14 μg/ml. The process was validated as per ICH guidelines [34]. No other method was observed in the literature for simultaneous estimation of Azilsartan Medoxomil and Cilnidipine. The analytical methods (like HPLC, HPTLC, and UV) for combinations of Cilnidipine or Azilsartan Medoxomil were found to be reported with other drugs. Lakshamana Rao et al. developed a simultaneous method for Chlorthalidone and Cilnidipine. The separation was achieved on C18 (150 x 4.6 mm, 5 μ) column using acetonitrile and buffer in the ratio of 35:65 v/v [29]. Leena sawaikar et al. reported stability-indicating the RP-HPLC method for simultaneous determination of Chlorthalidone and Cilnidipine in human plasma by RP-HPLC. The separation was carried out in isocratic mode on the C18 stationary phase using acetonitrile and buffer as the mobile phase. The Rt observed for Olmesartan medoxomil and Cilnidipine were 2.47 and 6.32 min, respectively [32]. Similarly, few methods with a combination of Azilsartan Medoxomil and other drugs were also found in the literature. Naazneen S et al. reported the RP-HPLC method for simultaneous estimation of Azilsartan Medoxomil medoxomil and Chlorthalidone in the solid dosage form. Hypersil BDS C18 column (100 x 4.6 mm) was used as the mobile phase. The Rt observed for Olmesartan medoxomil and Cilnidipine were 2.47 and 6.32 min, respectively [32]. Similarly, few methods with a combination of Azilsartan Medoxomil and other drugs were also found in the literature. Naazneen S et al. reported the RP-HPLC method for simultaneous estimation of Azilsartan Medoxomil medoxomil and Chlorthalidone in the solid dosage form. Hypersil BDS C18 column (100 x 4.6 mm) was used as the mobile phase. The Rt observed for Olmesartan medoxomil and Cilnidipine were 2.47 and 6.32 min, respectively [32].

**LOD and LOQ**

In the proposed method, the LOD and LOQ were estimated by the standard deviation of the responses and the slope. The standard deviation of the responses in turn was calculated from the standard deviation of the y-intercept of the regression line. The standard deviation and the slope were then put in consequent formulae and the standard deviation of the responses in turn was calculated from the standard deviation of the response and the slope. The approximate limit of detection (LOD) and limit of quantitation (LOQ) were as LOD and LOQ for Azilsartan Medoxomil and Cilnidipine were 2.47 and 6.32 min, respectively [32].

**Table 11: Observations noted for % recovery study of azilsartan medoxomil at three levels**

| % Recovery level | Conc. of standard spiked (μg/ml) | Conc. of the sample (μg/ml) | Total Avg. peak area* (test conc.) (μg/ml) | Avg. peak Area of sample conc. (μg/ml) | Amount recovered (μg/ml) | % Recovery (w/w) | Inference |
|------------------|----------------------------------|-----------------------------|-------------------------------------------|---------------------------------------|--------------------------|-----------------|-----------|
| 80               | 40                               | 32                          | 91912                                     | 44695                                 | 36.71                    | 106.39          | Complied   |
| 100              | 40                               | 40                          | 100695                                    | 53478                                 | 44.51                    | 101.84          | Complied   |
| 120              | 40                               | 48                          | 110168                                    | 62951                                 | 52.91                    | 99.87           | Complied   |

*n = 3 Average peak area of three repeated measurements.

**Table 12: Observations noted for % recovery study of cilnidipine at three levels**

| % Recovery Level | Conc. of standard spiked (μg/ml) | Conc. of the sample (μg/ml) | Total Avg. peak area* (test conc.) (μg/ml) | Avg. peak Area of sample conc. (μg/ml) | Amount recovered (μg/ml) | % Recovery (w/w) | Inference |
|------------------|----------------------------------|-----------------------------|-------------------------------------------|---------------------------------------|--------------------------|-----------------|-----------|
| 80               | 10                               | 8                           | 54734                                     | 24759                                 | 8.10                     | 94.51           | Complied   |
| 100              | 10                               | 10                          | 64675                                     | 34700                                 | 11.56                    | 105.96          | Complied   |
| 120              | 10                               | 12                          | 69149                                    | 39174                                 | 13.11                    | 99.66           | Complied   |

*n = 3 Average peak area of three repeated measurements.

**Table 13: LOD and LOQ observed for azilsartan medoxomil and cilnidipine**

| Standard drug solution | LOD (μg/ml) | LOQ (μg/ml) |
|-----------------------|-------------|-------------|
| Azilsartan Medoxomil  | 2.73        | 8.29        |
| Cilnidipine           | 1.30        | 3.95        |

*LOD: Detection Limit; LOQ: Quantitation Limit

**DISCUSSION**

Extensive literature was studied before the development of the RP-HPLC method for Azilsartan Medoxomil and Cilnidipine in bulk as...
stationary and the mobile phase, respectively. The Rt noted for Azilsartan Medoxomil and Chlorthalidone were 5.54±0.5 and 2.36±0.1. The author claimed that the method was suitable and economical [16]. The quality by design (QbD) approach was employed to simultaneously estimate Azilsartan Medoxomil Medoxomil and Chlorthalidone using RP-HPLC by Chawla et al. The author used RP-HPLC MINITAB software for optimization of the method parameters. The method was validated as per ICH guidelines and proved to be accurate, precise, and robust [3]. Vekariya Paras et al. have also developed and validated the RP-HPLC method for simultaneous determination of Azilsartan Medoxomil Medoxomil and Chlorthalidone using solid-phase extraction technique. The PDA detector at wavelength 254 nm was employed for the detection of the eluents. The author stated that the method could be employed to study the bioavailability and bioequivalence of Azilsartan Medoxomil Medoxomil Potassium [33].

The presented method’s author did not find any RP-HPLC method in literature showcasing the simultaneous estimation of Azilsartan Medoxomil Medoxomil and Cilnidipine in the mixture as bulk. Therefore, this research was planned to develop an RP-HPLC method for simultaneous estimation of Azilsartan Medoxomil Medoxomil and Cilnidipine in marketed tablet dosage form. The method was developed employing C18 (250 mm×4.6 mm), 5μm id, and Acetonitrile 80: Potassium dihydrogen phosphate buffer (Phosphate elution mode with a 1.2 ml/min mobile phase flow rate. The sample mobile phase and flow rate at various pH of the aqueous phase was observed for AZL and CIL correspondingly. The linearity was seen in the range of 40-240 μg/ml for AZL and 10-60 μg/ml for CIL. The robustness of the method was studied by deliberate variations in the method parameters viz. wavelength in ‘nm’, organic conc. of the mobile phase in % v/v, and flow rate. The respective average peak area of both the drugs was kept in the regression equation to estimate the average measured conc. of AZL and CIL. The %assay was also calculated from the average measured concentration and standard concentration. As shown in table 8 the %assay was found to be 97.38-103.03% w/w and 99.13-107.59% w/w for AZL and CIL, respectively. Similarly for organic concentration variation it was 97.90-103.03% w/w and 99.13-101.56% w/w (table 9). Finally, variation in flow rate the %assay was seen as 92.67 to 103.03% w/w and 93.85-107.85% w/w (table 10) for AZL and CIL, respectively.

The results were seen well within the boundaries prescribed for AZL and CIL. Therefore, it was observed that the presented method was robust.

The accuracy of the method was studied by estimation of %recovery using marketed tablet dosage form. The results observed were as tabulated in table 11 for AZL and table 12 for CIL. From the outcomes of this experiment, the %accuracy for AZL was noticed in the range of 99.87-106.39% w/w (table 11). Similarly, the %accuracy for CIL was noted in the range of 94.51-105.96% w/w. The outcomes were within acceptance criteria for AZL and CIL as per their respective compendial standards. LOD and LOQ of the AZL were 2.73 and 8.29μg/ml respectively. Furthermore, LOD and LOQ for Cilnidipine were 1.30 and 3.95μg/ml respectively (table 12). The specificity of the method was studied by injecting blank solution followed by injection of the sample solution from the tablet dosage form. The blank conc. showed no peak and the sample showed two peaks at the position of AZL and CIL, respectively. Therefore, this has suggested no interference in detecting AZL and CIL by commonly used excipients used in manufacturing the marketed tablet dosage form. This proved specificity (selectivity) of the method for estimation of AZL and CIL.

CONCLUSION

RP-HPLC method was successfully developed to simultaneously estimate Azilsartan Medoxomil and Cilnidipine in the mixture as API. Also, the method was productively tested for its applicability for convention analysis of Azilsartan Medoxomil and Cilnidipine in combined marketed tablet dosage form with results in compliance with the standards. The method was also proved unambiguous for estimating Azilsartan Medoxomil and Cilnidipine in the sample matrix of the tablet dosage form (marketed formulation). Hence, the presented method can be successfully employed for a custom analysis of Azilsartan Medoxomil and Cilnidipine in marketed tablet dosage forms.

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AUTHORS CONTRIBUTIONS

Ms. Swati Andhale has generated the research plan, prepared and revised the manuscript and Prof. Dr. A. G. Nikalje has provided guidance and supervision to carry out this study. Also, Prof. Dr. A. G. Nikalje has supported data analysis.

CONFLICTS OF INTERESTS

All authors have none to declare.

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