Development and Validation of RP-HPLC Chromatographic Dissolution Method for the Simultaneous Estimation of Ramipril and Hydrochlorothiazide from Solid Dosage Formulation

Prabhakar V. Raut1*, Sudhakar L. Padwal1, Madhusudhan T. Bachute2 and Satish A. Polshettiwar3

1Department of Chemistry, K.B.P. Mahavidyalaya Pandharpur, Punyashlok Ahilyadevi Holkar Solapur University, Pandharpur, 413304, India.
2Department of Chemistry, Sangola College Sangola, Maharashtra, 413307, India.
3School of Pharmacy, Dr. Vishwanath Karad, MIT World Peace University, Pune, India.

Authors’ contributions

This work was carried out in collaboration among all authors. Author PVR designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author SLP and Authors MTB, SAP managed the analyses of the study and done the plagiarism removal work. All authors read and approved the final manuscript.

ABSTRACT

The present study describes the dissolution method development and validation of Ramipril and Hydrochlorothiazide in tablet dosage form by HPLC Method. A simple, rapid, selective, reproducible and isocratic reversed-phase high performance liquid chromatographic (RP-HPLC) method has been developed and validated as per ICH guidelines. Analysis was performed on a Thermo, Sunniest C8 (150 mm x 4.6 mm, 5 µm) with the mobile phase consisting of mixing 500 mL of buffer solution and 500 mL of acetonitrile at a flow rate of 1.0mL/min. UV detection was performed at 210nm and the Run time for Ramipril and Hydrochlorothiazide were 10 minutes. The calibration curve was linear (correlation coefficient = 1.000) in the selected range for both analytes.
The optimized dissolution conditions include the USP Type 1 (Basket) rotation rate of 100 rpm and 750 mL of 0.1 N Hydrochloric acid as dissolution medium, at 37.0 ± 0.5°C. The method was validated for precision, linearity, specificity, accuracy, limit of quantitation and ruggedness. The system suitability parameters, such as theoretical plate, tailing factor and relative standard deviation (RSD) between six standard replicates were well within the limits. The stability result shows that the drug is stable in the prescribed dissolution medium.

Keywords: Ramipril; hydrochlorothiazide; dissolution; RP-HPLC.

1. INTRODUCTION

This medication is used to treat high blood pressure (hypertension). Lowering high blood pressure helps prevent strokes, heart attacks, and kidney problems [1-3].

This product contains 2 medications, Ramipril and Hydrochlorothiazide. Ramipril is called an ACE inhibitor and works by relaxing blood vessels so that blood can flow through the body more easily [4-10]. Hydrochlorothiazide (or "The thiazide diuretic") increases the amount of urine you make, removing extra water and salt from your body [11-14]. It also helps to relax the blood vessels so that blood can flow through the body more easily. Marketed formulation having two strength i.e. Ramithiazide 5 mg/12.5 mg and Ramithiazide 10 mg/25 mg [15-17].

Ramipril: Ramipril is a prodrug belonging to the angiotensin-converting enzyme (ACE) inhibitor class of medications. It is metabolized to ramiprilat in the liver and, to a lesser extent, kidneys. Ramiprilat is a potent, competitive inhibitor of ACE, the enzyme responsible for the conversion of angiotensin I (ATI) to angiotensin II (ATII). ATII regulates blood pressure and is a key component of the renin-angiotensin-aldosterone system (RAAS) [18-21]. Ramipril may be used in the treatment of hypertension, congestive heart failure, nephropathy, and to reduce the rate of death, myocardial infarction and stroke in individuals at high risk of cardiovascular events [22-24].

Hydrochlorothiazide: A thiazide diuretic always considered the prototypical member of this class. It reduces the reabsorption of electrolytes from the renal tubules [25,26]. This results in increased excretion of water and electrolytes, such as sodium, potassium, chloride, and magnesium. It has been used for the treatment of several disorders including edema, hypertension, diabetes insipidus, and hypoparathyroidism [27-29]. Hydrochlorothiazide is suddenly used for the treatment of hypertension, congestive heart failure, symptomatic edema, diabetes insipidus, renal tubular acidosis [30-34]. It is also used for the prevention of kidney stones in those who have high levels of calcium in their urine [35-39].

![Fig 1. Structure of ramipril](image-url)
2. MATERIALS AND METHODS

A. Instruments

HPLC (Agilent), Double beam UV-VIS spectrophotometer (UV-1800, Shimadzu, Japan) pH Meter (Lab India), Balance (Mettler Toledo), Sonicator (Rolex).

B. Reagents and Materials

Ramipril and Hydrochlorothiazide API, Formulation: Ramithiazide 10 mg/25 mg. Chemicals- Acetonitrile (HPLC Grade), Methanol (HPLC Grade), Potassium Dihydrogen Phosphate, Sodium Hydroxide, Distilled Water.

C. Method

i. Diluent: Used 0.1 N hydrochloric acid as blank.

ii. Blank: Used diluent as blank.

iii. Preparation of buffer: Dissolved 12.2 g of Sodium perchlorate in 1000 mL water and adjust the pH to 2.5 with diluted orthophosphoric acid. Filter through 0.45 µ membrane filter and degas.

iv. Preparation of mobile phase: Mixed 500 mL of buffer solution and 500 mL of acetonitrile.

v. Ramipril standard stock solution: Weigh accurately and transferred 25.0 mg of Ramipril working standard into 250 mL of volumetric flask, add 150 mL of mobile phase, sonicate to dissolve and diluted to 250 mL with mobile phase and mixed well.

vi. Hydrochlorothiazide standard stock solution: Weigh accurately and transfer 30.0 mg of Hydrochlorothiazide working standard into 250 mL of volumetric flask, add 150 mL of mobile phase, sonicate to dissolve and diluted to 250 mL with mobile phase and mixed well.

vii. Standard solution: (For Ramithiazide Tablets 10 mg/25 mg)

Diluted 3.0 mL of Ramipril standard stock solution and 6.0 mL of Hydrochlorothiazide standard stock solution to 25 mL with diluent and mixed well. (Concentration of Ramipril: 0.01 mg/ml & Hydrochlorothiazide: 0.03 mg/ml)

Sample solution: Placed 750 mL of dissolution medium in the vessel of the apparatus, assemble the apparatus equilibrated the dissolution medium to 37 ± 0.5°C. Place one tablet in each vessel and immediately operate the apparatus at 100 rpm. After 45 minutes withdraw sample from zone midway between the surface of dissolution medium and top of the rotating basket. Filtered the sample through 0.45µ nylon filter. (Concentration of Ramipril: 0.01 mg/ml & Hydrochlorothiazide: 0.03 mg/ml)

viii. Procedure: Separately inject 10 µL of the blank solution, standard solution in five replicate and sample solution into the chromatographic system, record the chromatograph and measure the peak response for the major peaks.

ix. System Suitability Parameters: The relative standard deviation for five replicate injections of standard solution should not be more than 2.0%.

D. Calculations:

i. Content of Ramipril dissolved:

For Ramithiazide Tablet 10 mg/25 mg

\[
\% \text{ Drug Release} = \frac{\text{AT} \times \text{WS} \times 3 \times 750 \times \text{P}}{\text{AS} \times 25 \times 25 \times 1 \times \text{LC}}
\]

Where,

\(\text{AT}\) = Area of Ramipril peak obtained from sample preparation.

\(\text{AS}\) = Average area of Ramipril peak obtained from standard solution.

\(\text{WS}\) = Weight of Ramipril working standard in mg.

\(\text{P}\) = % Potency of Ramipril working standard on as is basis.

\(\text{LC}\) = Label claim of Ramipril per tablet in mg.
ii. Content of Hydrochlorothiazide dissolved:
For Ramithiazide Tablets 10 mg/25 mg

\[
\% \text{ Drug Release} = \frac{AT \times WS}{AS^2} \times \frac{6 \times 750 \times P}{25 \times 1 \times LC}
\]

Where,

- AT = Area of Hydrochlorothiazide peak obtained from sample preparation.
- AS = Average area of Hydrochlorothiazide peak obtained from standard solution.
- WS = Weight of Hydrochlorothiazide working standard in mg.
- P = %Potency of Hydrochlorothiazide working standard on as is basis.
- LC = Label claim of Hydrochlorothiazide per tablet in mg

3. RESULTS AND DISCUSSION

Validation

1. Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present.

Acceptance criteria:

- Relative standard deviation for five replicate injections of standard solution should be not more than 2.0%.
- There should not be any interference of peak due to blank and placebo at the retention time of the Ramipril and Hydrochlorothiazide.

Observations/Results:

Standard Details: (Ramipril 10 mg & Hydrochlorothiazide 25 mg).

Summary:

No interference found due to blank and placebo at the retention time of the Hydrochlorothiazide and Ramipril.

2. Linearity And Range:

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

| Sr. No. | RT  | Area   |
|---------|-----|--------|
| 1       | 3.937 | 2089.654 |
| 2       | 3.937 | 2082.810 |
| 3       | 3.937 | 2084.558 |
| 4       | 3.937 | 2085.380 |
| 5       | 3.937 | 2089.446 |
| Average | 3.937 | 2086.370 |
| SD      | 0.00  | 3.05   |
| %RSD    | 0.00  | 0.15   |
| System Suitability Results |                |
| % RSD of Area | 0.15 | NMT 2.0% |

| Sr. No. | RT  | Area   |
|---------|-----|--------|
| 1       | 2.153 | 7760.560 |
| 2       | 2.153 | 7758.658 |
| 3       | 2.153 | 7762.480 |
| 4       | 2.153 | 7763.766 |
| 5       | 2.153 | 7765.055 |
| Average | 2.153 | 7762.104 |
| SD      | 0.00  | 2.54   |
| %RSD    | 0.00  | 0.03   |
| System Suitability Results |                |
| % RSD of Area | 0.03 | NMT 2.0% |
Table 3. Interference details

| Sample No.      | Area of Hydrochlorothiazide | Area of Ramipril |
|-----------------|-----------------------------|------------------|
|                 | Area RT | Area | Area RT | Area |
| Blank Solution  | Not Detected | Not Detected | Not Detected | Not Detected |
| Placebo Solution| Not Detected | Not Detected | Not Detected | Not Detected |
| Sample Solution | 2.157 | 7546.123 | 3.940 | 2212.454 |

The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

Acceptance Criteria:

- Relative standard deviation for five replicate injections of standard solution should be not more than 2.0%.
- The value obtained for correlation coefficient (r2) should not be less than 0.98

Observations/Results:

Table 4. Standard details for hydrochlorothiazide

| Injection No. | Retention Time | Area of Standard |
|---------------|----------------|------------------|
| 1             | 2.213          | 16393.6          |
| 2             | 2.213          | 16300.7          |
| 3             | 2.213          | 16265.5          |
| 4             | 2.213          | 16273            |
| 5             | 2.213          | 16318.8          |
| Average       | 2.213          | 16310.4          |
| SD            | 0.000          | 51.1             |
| %RSD          | 0.000          | 0.314            |

System Suitability Results

- % RSD of Area: 0.31 NMT 1.5%
- Tailing Factor: 1.19 NMT 2.0
- Theoretical Plates: 4323 NLT 2000

Table 4 (A). Linearity for Hydrochlorothiazide

| Level | Linearity level (%) | ml of Standard Stock Solution added | Diluted to volume (mL) | Actual Concentration (ppm) | Area of sample | Average area |
|-------|---------------------|-------------------------------------|------------------------|-----------------------------|----------------|--------------|
| 1     | 25                  | 1.50                                | 25                     | 7.2                         | 4108.377       | 4103.275     |
|       |                     |                                     |                        |                             | 4098.371       |              |
|       |                     |                                     |                        |                             | 4103.076       |              |
|       |                     |                                     |                        |                             | 8111.711       | 8075.987     |
|       |                     |                                     |                        |                             | 8058.520       |              |
|       |                     |                                     |                        |                             | 8057.730       |              |
|       |                     |                                     |                        |                             | 12097.737      | 12036.995    |
|       |                     |                                     |                        |                             | 12006.775      |              |
|       |                     |                                     |                        |                             | 12006.473      |              |
|       |                     |                                     |                        |                             | 16233.327      | 16219.790    |
|       |                     |                                     |                        |                             | 16166.768      |              |
|       |                     |                                     |                        |                             | 16259.276      |              |
| 2     | 50                  | 3.00                                | 25                     | 14.5                        | 20081.864      | 20085.256    |
|       |                     |                                     |                        |                             | 20099.317      |              |
|       |                     |                                     |                        |                             | 20074.587      |              |
|       |                     |                                     |                        |                             | 24021.387      | 24123.775    |
|       |                     |                                     |                        |                             | 40480.045      |              |
|       |                     |                                     |                        |                             | 40458.462      |              |
| 3     | 75                  | 4.50                                | 25                     | 21.7                        | 16233.327      | 12036.995    |
|       |                     |                                     |                        |                             | 16166.768      |              |
|       |                     |                                     |                        |                             | 16259.276      |              |
| 4     | 100                 | 6.00                                | 25                     | 29.0                        | 20081.864      | 20085.256    |
|       |                     |                                     |                        |                             | 20099.317      |              |
|       |                     |                                     |                        |                             | 20074.587      |              |
| 5     | 125                 | 7.50                                | 25                     | 36.2                        | 20081.864      | 20085.256    |
|       |                     |                                     |                        |                             | 20099.317      |              |
|       |                     |                                     |                        |                             | 20074.587      |              |
| 6     | 150                 | 9.00                                | 25                     | 43.4                        | 24021.387      | 24123.775    |
|       |                     |                                     |                        |                             | 40480.045      |              |
|       |                     |                                     |                        |                             | 40458.462      |              |
Calibration curve of Hydrochlorothiazide

Table 4(B). Standard details for Ramipril

| Slope               | 553.72 |
|---------------------|--------|
| y-intercept         | 79.844 |
| Correlation coefficient | 0.9999 |

Table 4(C). Linearity for Ramipril

| Injection No. | Retention Time | Area of Standard |
|---------------|----------------|------------------|
| 1             | 4.300          | 4413.1           |
| 2             | 4.300          | 4391.8           |
| 3             | 4.300          | 4387.0           |
| 4             | 4.295          | 4383.7           |
| 5             | 4.295          | 4394.0           |
| Average       | 4.298          | 4393.9           |
| SD            | 0.003          | 11.4             |
| %RSD          | 0.06           | 0.26             |

System suitability results

| % RSD of Area | 0.26 | NMT 2.0% |
| Tailing factor | 1.02 | NMT 2.0 |
| Theoretical plates | 5757 | NLT 2000 |

| Level | Linearity level (%) | ml of Standard Stock Solution added | Diluted to volume (mL) | Actual Concentration (ppm) | Area of sample | Average area |
|-------|----------------------|-------------------------------------|------------------------|-----------------------------|----------------|--------------|
| 1     | 25                   | 0.75                                | 25                     | 3.1                         | 1106.260       | 1104.932     |
|       |                      |                                     |                        |                             | 1104.019       |              |
|       |                      |                                     |                        |                             | 1104.517       |              |
| 2     | 50                   | 1.50                                | 25                     | 6.1                         | 2242.621       | 2238.404     |
|       |                      |                                     |                        |                             | 2235.066       |              |
|       |                      |                                     |                        |                             | 2237.525       |              |
| 3     | 75                   | 2.25                                | 25                     | 9.2                         | 3288.544       | 3279.522     |
|       |                      |                                     |                        |                             | 3276.074       |              |
|       |                      |                                     |                        |                             | 3273.948       |              |
| 4     | 100                  | 3.00                                | 25                     | 12.3                        | 4396.359       | 4393.220     |
|       |                      |                                     |                        |                             | 4380.366       |              |
|       |                      |                                     |                        |                             | 4402.936       |              |
| 5     | 125                  | 3.75                                | 25                     | 15.3                        | 5455.809       | 5458.614     |
|       |                      |                                     |                        |                             | 5461.386       |              |
|       |                      |                                     |                        |                             | 5458.646       |              |
| 6     | 150                  | 4.50                                | 25                     | 18.4                        | 6544.987       | 6560.594     |
|       |                      |                                     |                        |                             | 6568.676       |              |
|       |                      |                                     |                        |                             | 6568.118       |              |
Fig. 3. Calibration curve of ramipril

**Chart (A). Linearity values**

| Parameter       | Value  |
|-----------------|--------|
| Slope           | 354.95 |
| y-intercept     | 29.368 |
| Correlation coefficient | 0.9999 |

Summary:

- The relative standard deviation for peak area of Ramipril and Hydrochlorothiazide of standard solution is within acceptance criteria.
- The correlation coefficient obtained from the graph for Hydrochlorothiazide and Ramipril is 0.9999.

3. Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

A) System Precision

Acceptance criteria:

Relative standard deviation for five replicate injections of standard solution should be not more than 2.0%.

Observations/Results:

| Injection No. | Retention Time | Area of Standard | Retention Time | Area of Standard |
|---------------|----------------|------------------|----------------|------------------|
| 1             | 2.169          | 8305.110         | 4.053          | 2238.676         |
| 2             | 2.165          | 8293.775         | 4.048          | 2233.925         |
| 3             | 2.165          | 8308.217         | 4.053          | 2235.174         |
| 4             | 2.165          | 8275.966         | 4.053          | 2224.596         |
| 5             | 2.169          | 8267.204         | 4.057          | 2227.548         |
| **Average**   | **2.167**      | **8288.405**     | **4.054**      | **2231.669**     |
| **SD**        | **0.002**      | **16.57**        | **0.003**      | **5.21**         |
| **%RSD**      | **0.10**       | **0.20**         | **0.08**       | **0.23**         |

Table 5 (A).

**System suitability results**

|                  | % RSD          | Acceptance criteria |
|------------------|----------------|---------------------|
| % RSD of Ramipril| 0.19           | NMT 2.0%            |
| % RSD of HCTZ    | 0.10           | NMT 2.0%            |
Summary:

Relative standard deviation for five replicate injections of standard solution is within acceptance criteria.

B) Method precision

Acceptance criteria:

1. Relative standard deviation for five replicate injections of standard solution should be not more than 2.0%.
2. % Release of all six units should not be less than 75% of labeled amount.
3. The relative standard deviation for % release of six units should not be more than 5.0%.

Summary:

1. The relative standard deviation for peak area of Ramipril and Hydrochlorothiazide of standard solution is within acceptance criteria.
2. % Release of all six units is within acceptance criteria.
3. The relative standard deviation for % release of six units is within acceptance criteria.

C) Intermediate Precision:

Intermediate precision of the method will be established by carrying out the analysis on different day, different column with different analyst and different instrument.

Table 6. Precision sample details (Ramipril 10 mg/Hydrochlorothiazide 25 mg)

| Sample No. | Sample Weight (mg) | Area of Sample Hydrochlorothiazide | % Release Hydrochlorothiazide | Ramipril | Area of Sample Ramipril |
|------------|--------------------|-----------------------------------|------------------------------|---------|------------------------|
| 1          | 198.94             | 19198.409                         | 4609.030                     | 101.7   | 94.1                   |
| 2          | 195.06             | 18912.346                         | 4668.720                     | 100.2   | 95.3                   |
| 3          | 192.33             | 18907.745                         | 4611.656                     | 100.2   | 94.2                   |
| 4          | 195.52             | 19545.561                         | 4897.433                     | 103.6   | 100.0                  |
| 5          | 198.97             | 18810.796                         | 4524.970                     | 99.7    | 92.4                   |
| 6          | 196.19             | 19192.810                         | 4728.588                     | 101.7   | 96.6                   |

Average: 101.2  95.4
SD: 1.4  2.6
%RSD: 1.4  2.8

Table 7. Standard details (Ramipril 10 mg & Hydrochlorothiazide 25 mg)

| Injection No. | Retention Time | Area of Standard Hydrochlorothiazide | Retention Time | Area of Standard Ramipril |
|---------------|----------------|-------------------------------------|----------------|--------------------------|
| 1             | 2.181          | 8018.445                            | 4.064          | 5671.704                 |
| 2             | 2.182          | 8039.233                            | 4.069          | 5666.834                 |
| 3             | 2.182          | 8030.953                            | 4.069          | 5685.921                 |
| 4             | 2.182          | 8037.891                            | 4.066          | 5676.504                 |
| 5             | 2.181          | 8032.151                            | 4.068          | 5670.239                 |

Average: 2.182  8031.735  4.187  5674.240
SD: 0.001  8.24  0.002  7.40
%RSD: 0.03  0.10  0.05  0.13

Table 7 (A): System Suitability Results

| % RSD of Ramipril | NMT 2.0% |
|-------------------|----------|
| % RSD of HCTZ     | NMT 2.0% |

Table 7(A). System Suitability Results

| % RSD of Ramipril | NMT 2.0% |
|-------------------|----------|
| % RSD of HCTZ     | NMT 2.0% |
Acceptance criteria:

1. Relative standard deviation for five replicate injections of standard solution should be not more than 2.0%.
2. % Release of all six units should not be less than 75% of labeled amount.
3. The relative standard deviation for % release of six units should not be more than 5.0%.
4. The difference in the mean value between the % release at method precision and intermediate precision should not be more than 5.0%.

Table 8. Intermediate precision sample details (Ramipril 10 mg & Hydrochlorothiazide 25 mg)

| Sample No. | Sample Weight (mg) | Area of Sample | % Release |
|------------|--------------------|----------------|-----------|
|            |                    | HCTZ           | Ramipril  | HCTZ     | Ramipril  |
| 1          | 201.07             | 9646.399       | 6057.965  | 105.1    | 97.2      |
| 2          | 187.60             | 9248.398       | 5925.331  | 100.8    | 95.1      |
| 3          | 200.63             | 9429.118       | 5943.174  | 102.7    | 95.4      |
| 4          | 198.11             | 9322.973       | 5928.674  | 101.6    | 95.2      |
| 5          | 198.05             | 9347.578       | 5931.141  | 101.9    | 95.2      |
| 6          | 192.33             | 9361.114       | 5934.695  | 102.0    | 95.3      |

Average 102.4 95.6

Table 9 Comparison of Method Precision and Intermediate precision:

i) For Ramipril 10 mg & Hydrochlorothiazide 25 mg

| Sample No. | Parameter     | % Release |
|------------|---------------|-----------|
|            | Method precision | Hydrochlorothiazide | Ramipril |
| 1          | 101.7         | 94.1      |
| 2          | 100.2         | 95.3      |
| 3          | 100.2         | 94.2      |
| 4          | 103.6         | 100       |
| 5          | 99.7          | 92.4      |
| 6          | 101.7         | 96.6      |
| 1          | Intermediate precision | 105.1 | 97.2 |
| 2          | 100.8         | 95.1      |
| 3          | 102.7         | 95.4      |
| 4          | 101.6         | 95.2      |
| 5          | 101.9         | 95.2      |
| 6          | 102           | 95.3      |
| Mean       | 101.8         | 95.5      |
| SD         | 1.52          | 1.86      |
| %RSD       | 1.50          | 1.95      |

Fig. 4. Typical chromatograph of blank
4. CONCLUSION

1. The relative standard deviation for peak area of Ramipril and Hydrochlorothiazide of standard solution is less than 2.0%.
2. % Release of all six units is more than 75% of labeled amount.
3. The relative standard deviation for % release of six units is less than 5.0%.
4. The difference in the mean value between the % releases at method precision and intermediate precision is less than 5.0%.

Accuracy (Recovery)

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.

Acceptance criteria:

% Recovery for each accuracy level should be between 95.0% to 105.0%.

Calculation:

\[
\% \text{ Recovery} = \frac{\text{Amount recovered}}{\text{Amount added}} \times 100
\]

Accuracy for Ramipril 10 mg & Hydrochlorothiazide 25 mg

| Sr.No. | RT  | Area   |
|--------|-----|--------|
| 1      | 2.232 | 17285.3 |
| 2      | 2.228 | 17282.2 |
| 3      | 2.228 | 17295.8 |
| 4      | 2.230 | 17285.7 |
| 5      | 2.232 | 17282.3 |
| Average| 2.230 | 17286.3 |

| SD     | 0.00 | 5.57  |
| %RSD   | 0.09 | 0.03  |

System Suitability Results

| % RSD of Area | NMT 2.0% |
| Tailing factor | NMT 2.0% |
| Theoretical plates | NLT 2000 |
### Chart 2. Accuracy of hydrochlorothiazide

| Accuracy Level (%) | Placebo Weight (mg) | mL of Std Added | mL of Volume | Amount Added (ppm) | Area | Amount Recovered (ppm) | % Recovery | Mean % Recovery | % RSD |
|--------------------|---------------------|-----------------|--------------|-------------------|------|------------------------|------------|-----------------|-------|
| 25                 | 165.07              | 1.2             | 750          | 6.9               | 4312.542 | 7.18                   | 103.75     | 103.7           | 0.01  |
|                    | 166.34              | 1.2             | 750          | 6.9               | 4311.647 | 7.18                   | 103.73     |                 |       |
|                    | 166.60              | 1.2             | 750          | 6.9               | 4311.853 | 7.18                   | 103.73     |                 |       |
| 50                 | 168.66              | 2.5             | 750          | 14.4              | 8853.362 | 14.75                  | 102.24     | 102.2           | 0.03  |
|                    | 167.30              | 2.5             | 750          | 14.4              | 8854.541 | 14.75                  | 102.25     |                 |       |
|                    | 166.96              | 2.5             | 750          | 14.4              | 8850.059 | 14.74                  | 102.20     |                 |       |
| 100                | 167.09              | 5.0             | 750          | 28.8              | 17595.550 | 29.31                  | 101.60     | 101.5           | 0.14  |
|                    | 166.57              | 5.0             | 750          | 28.8              | 17547.427 | 29.23                  | 101.32     |                 |       |
|                    | 167.06              | 5.0             | 750          | 28.8              | 17569.828 | 29.26                  | 101.45     |                 |       |
| 150                | 165.74              | 7.5             | 750          | 43.3              | 25971.796 | 43.26                  | 99.97      | 99.9            | 0.09  |
|                    | 166.21              | 7.5             | 750          | 43.3              | 25966.558 | 43.25                  | 99.95      |                 |       |
|                    | 165.37              | 7.5             | 750          | 43.3              | 25930.706 | 43.19                  | 99.81      |                 |       |
|                   | Mean                |                 |              |                   |        |                        | 101.2      |                 | 1.18  |
|                   | SD                  |                 |              |                   |        |                        | 1.18       |                 |       |
|                   | % RSD               |                 |              |                   |        |                        | 1.16       |                 |       |

### Chart 3. Accuracy of ramipril

| Accuracy Level (%) | Placebo Weight (mg) | mL of Std Added | mL of Volume | Amount Added (ppm) | Area | Amount Recovered (ppm) | % Recovery | Mean % Recovery | % RSD |
|--------------------|---------------------|-----------------|--------------|-------------------|------|------------------------|------------|-----------------|-------|
| 25                 | 165.07              | 1.2             | 750          | 3.0               | 1046.315 | 2.93                   | 98.5       | 97.8            | 0.77  |
|                    | 166.34              | 1.2             | 750          | 3.0               | 1039.863 | 2.91                   | 97.9       |                 |       |
|                    | 166.60              | 1.2             | 750          | 3.0               | 1030.405 | 2.89                   | 97.0       |                 |       |
| 50                 | 168.66              | 2.5             | 750          | 6.2               | 2235.645 | 6.27                   | 101.0      | 101.0           | 0.19  |
|                    | 167.30              | 2.5             | 750          | 6.2               | 2238.134 | 6.27                   | 101.1      |                 |       |
|                    | 166.96              | 2.5             | 750          | 6.2               | 2229.837 | 6.25                   | 100.8      |                 |       |
| 100                | 167.09              | 5.0             | 750          | 12.4              | 4477.165 | 12.55                  | 101.2      | 100.4           | 0.96  |
|                    | 166.57              | 5.0             | 750          | 12.4              | 4456.113 | 12.49                  | 100.7      |                 |       |
|                    | 167.06              | 5.0             | 750          | 12.4              | 4394.682 | 12.32                  | 99.3       |                 |       |
| Accuracy Level (%) | Placebo Weight (mg) | mL of Std Added | mL of Volume | Amount Added (ppm) | Area | Amount Recovered (ppm) | % Recovery | Mean % Recovery | % RSD |
|---------------------|---------------------|----------------|--------------|-------------------|------|------------------------|------------|-----------------|-------|
| 150                 | 165.74              | 7.5            | 750          | 18.6              | 6506.724 | 18.23                  | 98.0       | 98.6            | 0.53  |
|                     | 166.21              | 7.5            | 750          | 18.6              | 6571.839 | 18.42                  | 99.0       |                 |       |
|                     | 165.37              | 7.5            | 750          | 18.6              | 6560.354 | 18.38                  | 98.8       |                 |       |
| Mean                |                     |                |              |                   |       |                        | 100.0      |                 |       |
| SD                  |                     |                |              |                   |       |                        | 1.23       |                 |       |
| % RSD               |                     |                |              |                   |       |                        | 1.23       |                 |       |
Chart 4. Robustness of hydrochlorothiazide

| Robustness Parameter          | % Release | % Difference (NMT 2.0 %) |
|-------------------------------|-----------|--------------------------|
| Precision                     | 100.8     | NA                       |
| Flow Rate 0.9 mL/min          | 101.8     | 1.0                      |
| Flow Rate 1.1 mL/min          | 101.9     | 1.1                      |
| Column temperature 23         | 102.0     | 1.2                      |
| Column temperature 27         | 101.8     | 1.0                      |
| Buffer pH 2.3                 | 101.3     | 0.5                      |
| Buffer pH 2.7                 | 100.4     | 0.3                      |
| Low RPM                       | 100.2     | 0.6                      |
| High RPM                      | 100.1     | 0.7                      |
| Low Medium Volume             | 100.7     | 0.1                      |
| High Medium Volume            | 98.4      | 2.4                      |

Chart 5. Robustness of ramipril

| Robustness Parameter          | % Release | % Difference (NMT 2.0 %) |
|-------------------------------|-----------|--------------------------|
| Precision                     | 99.0      | NA                       |
| Flow Rate 0.9 mL/min          | 100.2     | 1.2                      |
| Flow Rate 1.1 mL/min          | 100.4     | 1.4                      |
| Column temperature 23         | 99.1      | 0.1                      |
| Column temperature 27         | 99.4      | 0.4                      |
| Buffer pH 2.3                 | 100.0     | 1.0                      |
| Buffer pH 2.7                 | 98.2      | 0.6                      |
| Low RPM                       | 99.6      | 0.6                      |
| High RPM                      | 100.1     | 1.1                      |
| Low Medium Volume             | 97.2      | 1.8                      |
| High Medium Volume            | 96.7      | 2.3                      |

Summary:

% Recovery at each level is between 95.0 to 105.0%

4. Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Acceptance criteria:

The difference in the mean value between the dissolution results at method precision and robustness should not more than 5%.

Summary:

The difference in the mean value between the dissolution results at method precision and robustness is less than 5.0%.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Masoom R, Zeid A, AlOthman A, Nafisur R. Analytical techniques in pharmaceutical analysis: A review, Arabian Journal of Chemistry. 2013;1878-5352.

2. Panchumarthy R, Naga N, Pravallika D, Navya S. A review on step-by-step analytical method validation, IOSR Journal of Pharmacy. 2015;5(10):07-19.

3. Skoog D, Holler J, Crouch S. Principles of instrumental analysis. 6th ed. Belmont, CA: Thomson Brooks/Cole. 2007;169–173.

4. Michael I, Schartz J, Krull. Analytical method development and validation. Goel Publishing House: Meerut. 2004;25-46.

5. ICH – Q2B. Analytical guidelines on conference on analytical method validation. International Conference on Harmonization (ICH). 1996;1518.
6. British Pharmacopoeia. Appendices-III. Chromatographic Separation Techniques. The Stationary Office behalf of the Medicines and Healthcare Products Regulatory Agencies: London. 2007;5:189-190.

7. Juran JM. Juran on quality by design: The new steps for planning quality into goods and services. The Free Press: USA. 1992:287-300.

8. Daenens P, Mannaert E, Tytgat J. Detection of 2-Amino-5-Chloropyridine in Urine as a Parameter of Zopiclone (Imovane Intake using HPLC with Diode Array Detection. Journal of Analytical Toxicology. 1997;21:208-12.

9. Blanchard JC, Julou L. Suriclone: A new cyclopyrroline derivative recognizing receptors labeled by benzodiazepines in rat hippocampus and cerebellum. JNeurochem. 1983;40(3):601–7.

10. Skerritt J, Johnston G. Enhancement of GABA binding by benzodiazepines and related anxiolytics. European Journal of Pharmacology. 1983;89(3-4):193–8.

11. Sethi PD. HPLC Quantitative analysis of pharmaceutical formulations, 1st ed. CBS Publishers and distributors. New Delhi. 2001:94-105.

12. Glajch L, Kirkal J, Snyder R. Practical HPLC method development.2nd ed. John Wiley and sons.Inc. New York. 1999;234-260.

13. Sethi PD. HPLC- high performance liquid chromatography- Quantitative analysis of pharmaceutical formulations.1st ed. CBS Publishers and Distributors. New Delhi. 2001;3-72, 116-120.

14. Satinder A, Michael D. Handbook of pharmaceutical analysis by HPLC Series, editor.separation sciences and technology. Elsevier. 2009;3-5, 8, 35, 43, 62, 97, 125, 146-166, 197.

15. Kar A. Pharmaceutical drug analysis. 2nd ed. New age international limited publishers. 2005;452-474.

16. Sethi PD. Quantitative analysis of drugs in pharmaceutical formulations. 3rd ed. CBS publishers and distributors, New Delhi. 1997;17-19.

17. Bassett J, Denney RC, Jeffery GH, Mendham J. Vogel’s textbook of quantitative inorganic analysis. 4th ed. Longman Group Ltd: England. 1978;1-9.

18. Available:www.mournetrainingservices.co.uk/book_introduction_HPLC.html

19. Snyder LR, Joseph JK, Joseph LG. Practical HPLC method development, 2nd ed. A Wiley- Inter science Publication; New York. 1997;1- 9,8-13, 21-37, 72-76,685-712.

20. Code (Q2B). Validation of analytical procedures. Methodology. ICH Harmonized Tripartite Guidelines. Geneva: Switzerland. 1996;1-8.

21. Analytical method development, http://www.pharmainfo.net

22. Kalsi PS. Spectroscopy of organic compounds. 6th ed. New age International publisher. 9-54.

23. Vidyasagar G. Instrumental method of drug analysis. Pharma med press editor. 2012;106.

24. The Merck Index. Merck & Co. Inc. 14th ed. White house station. NJ. 2006;4781, 491, 4935, 6528.

25. Beckett AH, Stenlake JB. Practical pharmaceutical chemistry. 4th ed. Part 2. Publication of New Delhi. 2004;275-300.

26. Lampman Pavia, Kriz Vyvyan. Spectroscopy. 4th ed. Cengage Learning. 379-406.

27. De DP, Macdonald RL. Effects of non-sedative anxiolytic drugs on responses to GABA and on diazepam-induced enhancement of these responses on mouse neurones in cell culture. British Journal of Pharmacology. 1988;95(1):109–20.

28. ICH, Q2 (R1) validation of analytical procedures: Harmonized tripartie guideline text and methodology current step 4 version.

29. ICH, Q2 (R1) validation of analytical procedures: international conference on harmonization; 1994.

30. Mendham J, Denney RC, Barnes JD, Thomas MJ. Vogel’s textbook of quantitative chemical analysis. 6th ed. Publication of Pearson Education. 172-173, 301-308.

31. Christian G. Analytical chemistry. 6th ed. Publication of John Wiley and Sons. 2003;1-15, 92-114, 126-132, 604-619.

32. Sharma BK. Instrumental methods of chemical analysis. 25th ed. Goel Publication Co.; Meerut. 2002;3:6.

33. Chatwal GR, Anand SK. Instrumental methods of chemical analysis. 5th ed. Himalaya Publishing House; Delhi. 2007;2:150, 2.566-2.585, 2.624-2.630.
34. Sharma YR. Introduction of organic spectroscopy. 4th ed CBS Publishers and Distributors; New Delhi. 1991;5-25.

35. Indian pharmacopoeia: Government of India, Ministry of Health and Family Welfare; Vol. 3, Published by the Controller of Publications: Delhi. 1996;25-27.

36. Mistri HN, Jangid AG, Pudage A, Shrivastav P. HPLC-ESI-MS/MS validated method for simultaneous quantification of zopiclone and its metabolites, N-desmethylzopiclone and zopiclone-N-oxide in human plasma. J Chromatogr B AnalyTechol Biomed Life Sci. 2008;137-148.

37. Kumar HK, Vijaya BD, Jagadeesh B, Ravindranath L K, Veera K N and Venkateswarulu V: A rapid LC-MS/MS method for quantitation of eszopiclone in human plasma: Application to a human pharmacokinetic study. Biomedical Chromatography. 2011;26 (2):225-31.

38. Rao B, Nagendrakumar MV, Sivanadh AVD, Bramhachari PV. Development of new Reverse phase -HPLC method for analysis and assay of Zopiclone in Formulation. Journal of Pharmacy Research. 2011;4(1):248.

39. Souri E, Shirvin A, Shabani RN, Alvandifar F, Tehrani MB: Validated stability indicating HPLC method for determination of zolpidem in the presence of its degradation products. The Open Conference Proceedings Journal. 2012;3:13-17.

© 2021 Raut et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle4.com/review-history/73330