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

Prabhakar V. Raut[1*], Sudhakar L. Padwal[1], Madhusudhan T. Bachute[2] and Satish A. Polshettiwar[3]

[1]Department of Chemistry, K.B.P. Mahavidyalaya Pandharpur, Punyashlok Ahilyadevi Holkar Solapur University, Pandharpur, 413304, India.
[2]Department of Chemistry, Sangola College Sangola, Maharashtra, 413307, India.
[3]School 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.

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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.

**Corresponding author: E-mail: prabhakar_chem9484@rediffmail.com;*

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].

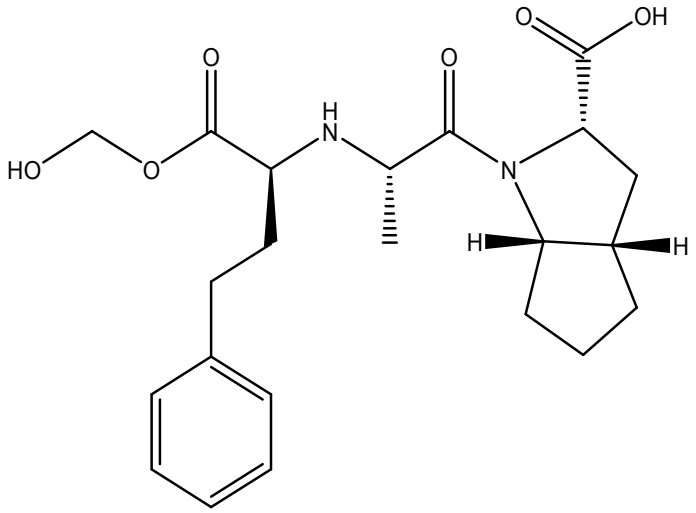


Fig 1. Structure of ramipril

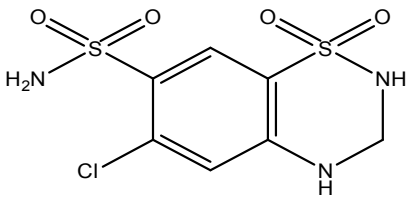


Fig. 2. Structure of hydrochlorothiazide

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:**Used0.1 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 dissolved 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 dissolved 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: 0.01mg/ml & Hydrochlorothiazide: 0.03mg/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: 0.01mg/ml & Hydrochlorothiazide: 0.03mg/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 be not more than 2.0%.

D. Calculations:

i. Content of Ramipril dissolved:

For Ramithiazide Tablet 10 mg/25 mg

$$\%\ \text{Drug Release} = \frac{\text{AT}}{\text{AS}} \text{X} \frac{\text{WS}}{250} \text{X} \frac{3}{25} \text{X} \frac{750}{1} \text{X} \frac{\text{P}}{\text{LC}}$$

Where,

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

ii. Content of Hydrochlorothiazide dissolved:

For Ramithiazide Tablets 10 mg/25 mg

$$\%\ \text{Drug Release} = \frac{\text{AT}}{\text{AS}} \text{X} \frac{\text{WS}}{250} \text{X} \frac{6}{25} \text{X} \frac{750}{1} \text{X} \frac{\text{P}}{\text{LC}}$$

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.

Table 1. Standard details of ramipril

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%

Table 2. Standard details of hydrochlorothiazide

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	
	RT	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

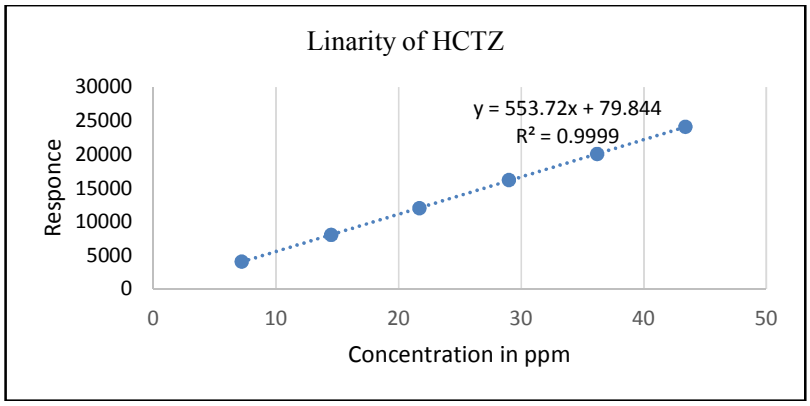
Observations/Results:

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 4098.371 4103.076	4103.275
2	50	3.00	25	14.5	8111.711 8058.520 8057.730	8075.987
3	75	4.50	25	21.7	12097.737 12006.775 12006.473	12036.995
4	100	6.00	25	29.0	16233.327 16166.768 16259.276	16219.790
5	125	7.50	25	36.2	20081.864 20099.317 20074.587	20085.256
6	150	9.00	25	43.4	24021.387 40480.045 40458.462	24123.775



Calibration curve of Hydrochlorothiazide

Table 4(B). Standard details for Ramipril

Slope	553.72
y-intercept	79.844
Correleation 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 1104.019 1104.517	1104.932
2	50	1.50	25	6.1	2242.621 2235.066 2237.525	2238.404
3	75	2.25	25	9.2	3288.544 3276.074 3273.948	3279.522
4	100	3.00	25	12.3	4396.359 4380.366 4402.936	4393.220
5	125	3.75	25	15.3	5455.809 5461.386 5458.646	5458.614
6	150	4.50	25	18.4	6544.987 6568.676 6568.118	6560.594

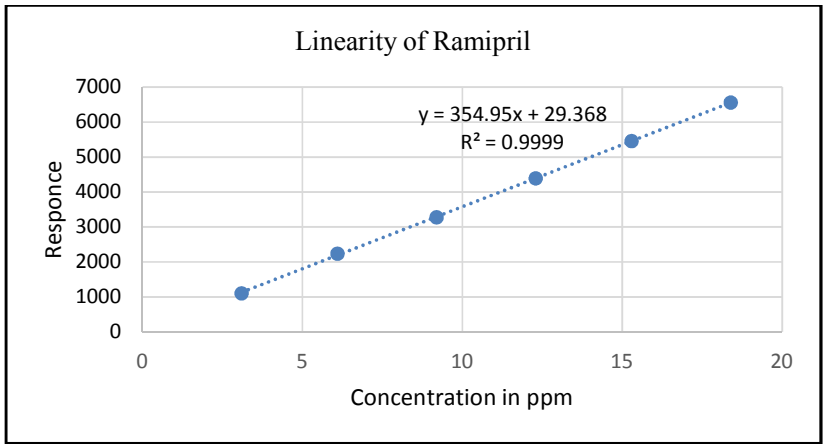


Fig. 3. Calibration curve of ramipril

Chart (A). Linearity values

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:

Table 5. Standard details (Ramipril 10 mg & Hydrochlorothiazide 25 mg)

Injection No.	Hydrochlorothiazide		Ramipril	
	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 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		% Release	
		Hydrochlorothiazide	Ramipril	Hydrochlorothiazide	Ramipril
1	198.94	19198.409	4609.030	101.7	94.1
2	195.86	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.	Hydrochlorothiazide		Ramipril	
	Retention Time	Area of Standard	Retention Time	Area of Standard
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	0.13	NMT 2.0%	
	% RSD of HCTZ	0.10	NMT 2.0%	

Table 7(A).

	System Suitability Results		
	% RSD of Ramipril	0.13	NMT 2.0%
	% RSD of HCTZ	0.10	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**
			SD	**1.5**	**0.8**
			%RSD	**1.5**	**0.9**

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	105.1	97.2
2	precision	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**

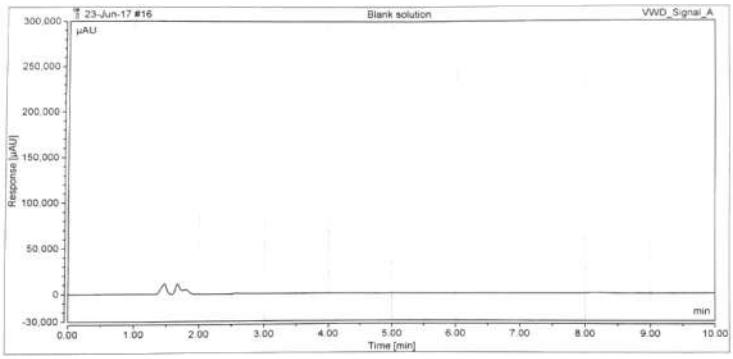


Fig. 4. Typical chromatograph of blank

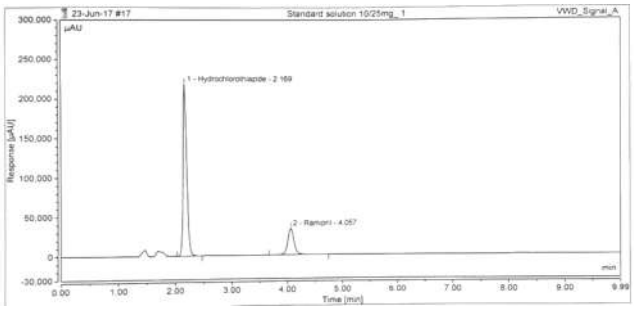


Fig. 5. Typical chromatograph of standard

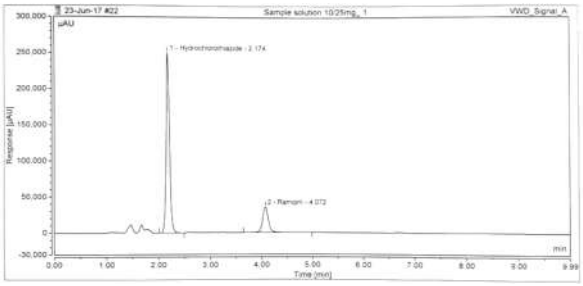


Fig. 6. Typical chromatograph of sample

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:

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

Accuracy for Ramipril 10 mg & Hydrochlorothiazide 25 mg

Chart 1. Standard details: Hydrochlorothiazide

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	0.03	NMT 2.0%
Tailing factor	1.17	NMT 2.0%
Theoretical plates	4075	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	
							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.

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