Method development and Validation for Estimation of Irbesartan and Hydrochlorothiazide in Tablet Dosage form by using RPHPLC

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Abstract. In this study Irbesartan (IRN) and Hydrochlorothiazide (HRE) assay was done by utilizing stability indicating RP-HPLC, in which module was carried the water separation 2695, equipped with a detector and chromatographic separation Phenomenex column C18(250x4.mm,5µm) column was operated as stationary phase and mobile phase was combination of phosphate buffer (0.1M, pH 4.1) and methanol in 60% vol and 40% vol, respectively with isocratic elution type. By using 1.0 ml/min flow rate and effluents were observed at 230 nm. The HRE and IRN were eluted at 2.913 and 2.346 min, respectively. Linearity was 75-225 µg/ml (IRN) and 6.25-18.75 µg/ml (HRE). Limit of detection (LOD) and limit of quantification (LOQ) for Irbesartan are 1.173 For the purposes of μg/ml, and 3,911 μg/ml, 0.409 μg/ml and 1.364 μg/ml for Hydrochlorothiazide. The procedure was developed to suggest consistency by the subjection of medications to stress conditions like acid, alkaline, peroxide, dry heat and sunlight. Both validated criteria were appropriate. The Granry 150H tablet method can be used.

1. Introduction
Irbesartan (IRB) is used as a selective antihypertensive specialist its IUPAC name is 2-Butyl-3[p-(o-H-tetrazol5-ylphenyl) benzyl]-1, 3-diazaspiro[4.4], non-1-en-4-one 1. Inhibitor of angiotensin 2 receptor, inhibitor of Ca2+ channel, Benzene subsidiary, Tetrazoles, Biphenyls compound. It is targeted for elevated blood pressure and diabetic nephropathy in patients with high blood pressure and diabetes. Angiotensin II binds to AT1 receptor, inducing vasoconstriction and emitting aldosterone causing elevated pressure in the blood. IRN forests specifically official angiotensin II receptor in tissues such as vascular and adrenal muscles. IRN restraint of the authoritative of angiotensin II initiates smooth vascular muscle unwinding and turns away aldosterone discharge from adrenal organ, and in this way brings down circulatory strain. Hydrochlorothiazide TUPAC name is 6-Chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulphonamide 1,1-dioxide 2 and is used as for Diuretic mixes. The gathering of the HRE is Natriuretic specialist, Sulfonamides, Thiazides, Nephrotoxic Agent, Antihypertensive. It is intended for Edema aligned with congestive cardiovascular breakdown, liver cirrhosis, intense glomerulonephritis nephrotic disorder and constant kidney disappointment. HRE diminishes blood volume by working on the renals to limit Na+ re - take-up in the distal tubule. HRE follows up on the distally tangled tubule at proximal locale, restraining sodium-chloride symptom reabsorption. Symptom restraint diminishes the abundance of the fixation inclination between the distally tangled tubule and epithelial cell, in this manner diminishing water re – take-up. Writing overview of IRB and HRE uncovered not many techniques dependent on chromatography 3-38, electrochemical strategies 39, superior dainty layer chromatography 40, 41 and spectrophotometry 42-52 either in single or in consolidated structures. The current work depicts the turn of events and approval of converse stage superior fluid chromatographic (RPHPLC) technique, which can measure
these parts all the while. Affirmation of the relevance of the created strategy was approved by the International Conference on Harmonization (ICH) assurance of IRB is given in fig 1 and HRE is given in fig 2 in tablet measurement structure.

![Figure 1. Irbesartan (IRN) structure](image1.png)

![Figure 2. Hydrochlorothiazide (HRE) structure](image2.png)

2. Materials and Methods

2.1. Mobile phase and Diluent
Mobile phase and diluent are same solvent systems. Mobile phase was prepared by using 0.1 M potassium dihydrogen phosphate buffer, pH 4.1 and methanol in 60% vol. and 40% vol, respectively.

2.2. IRN & HRE Solutions

2.2.1. Preparation of Stock Solution
A suitable amount of IRN (150 mg) and HRE (12.5 mg) were precisely balanced and diluted with diluent to get a stock IRN & HRE solution. Concentration was 1500 µg/ml IRN and 125 µg/ml HRE.

2.3. Working Solution
10 ml of stock IRN & HRE solution was diluted to 100 ml with diluent. Concentration was 150 µg/ml IRN and 12.5 µg/ml HRE.

2.4. Conditions for IRN and HRE
Assay Temperature in column 25ºc, Temp at sample injector 25ºc, Rate of flow 1ml/min, PH maintained 4.1, wavelength 230nm, Detector Photo Diode Array (PDA), Runtime 6 min, vol. of injection sample 10µl.

2.5. Quantification of IRN & HRE in GRANRY 150H Tablets
The 100 ml flask was taken with 282 mg homogenised packets of Granry 150 H (equivalent to 150 mg IRN and 12.5 mg HRE). 70 ml of diluent and ultrasound was used (15 min). The blend has been filtered with diluent and made up to 100 ml. In shop the IRN & HRE tablet solution was diluted to 100ml with a diluent to preparation of IRN & HRE test tablet solution at a concentration of 1500µg/ml IRN and 125µg/ml HRE.10ml. In a column for IRN & HRE evaluation conditions as explained in section "CONDITIONS FOR IRN & HRE ASSAY" were theoretical concentration 150 µg/ml IRN and the test tablet IRN &HRE solution 12.5 µg/ml HRE10 µl and SST is given in table 1.

3. Stability Studies for IRN & HRE
These studies were operated on 10 ml of stock IRN & HRE tablet solution applying guidelines of ICH [50].
• For acid-based degradation: Stock IRN & HRE tablet solution (10 ml) was sonicated with HCl (0.1N, 10 ml) in ultra sonicator at 25°C for nearly 30 min.
• For alkali-based degradation: Stock IRN & HRE tablet solution (10 ml) was sonicated with NaOH (0.1N, 10 ml) in ultra sonicator at 25°C for nearly 30 min.
• For peroxide-based degradation: Stock IRN & HRE tablet solution (10 ml) was sonicated with peroxide (30%, 10 ml) in ultra sonicator at 25°C for nearly 30 min.
• For sunlight-based degradation: Stock IRN & HRE tablet solution (10 ml) was placed for nearly 6 hr in sun light.
• For dry heat-based degradation: Stock IRN & HRE tablet solution (10 ml) was placed for nearly 6 hr in oven set at 105°C.

The degraded IRN & HRE tablet solutions were diluted to 100 ml with diluent to prepare degradation IRN & HRE sample solutions. Theoretical concentration of IRN was 150 µg/ml and 12.5 µg/ml HRE. For assessment of IRN & HRE, 10 μl of the degradation IRN & HRE sample solutions were injected into the column which is shown in chromatogram is given in fig 3 and remained by conditions explained in section “CONDITIONS FOR IRN & HRE ASSAY”

![Chromatogram](image)

**Figure 3.** Irbesartan and Hydrochlorothiazide chromatogram

| Name | Retention Time | Area | % Area | Height | USP Resolution | USP Tailing | USP Plate Count |
|------|----------------|------|--------|--------|----------------|-------------|-----------------|
| IRN  | 2.346          | 2238253 | 81.51  | 252164 | -              | 1.49        | 3366            |
| HRE  | 2.913          | 507730 | 18.49  | 72451  | 3.41           | 1.12        | 5261            |

### 4. Validating the Method

#### 4.1. Linearity
The calibration curves for IRN and HRE fig 4, fig 5 ranged from 75 to 225 µg/ml and 6.25 to 18.75 µg/ml, respectively using five calibration solution standards. The regression equations were

\[ y = 15655x - 141168 \] for IRN
\[ y = 40761x - 2660.6 \] for HRE

We prepared and injected all the concentrations into the device. The curve of linearity was built by plotting peak area versus analyte concentration. The suggested procedure was discovered to be linear from the data gathered. The correlation value of coefficient \( r^2 \) was 0.9993 for IRN and 0.9999 for HRE. These results essentially confirmed linearity are given in the Table 2.

**Table 2.** Concentrations and peak areas of IRN & HRE

|         | IRN          | HRE          |
|---------|--------------|--------------|
| Area values obtained | µg/ml conc. | Area values obtained | µg/ml conc. |
| 1001495 | 75           | 252065       | 6.25         |
| 1647031 | 112.5        | 379429       | 9.375        |
| 2230816 | 150.0        | 506775       | 12.5         |
| 2791528 | 187.5        | 634682       | 15.625       |
| 3364565 | 225          | 761336       | 18.75        |
Figure 4. Linearity curves for IRN and HRE

Figure 5. Linearity study chromatograms
4.2 Limit of Detection
The LOD for IRN and HRE has been defined as the lowest concentration of IRN and HRE at which their output is almost 3 times greater than the noise of the background baseline. Limit of detection value and signal to noise ratio for IRN – 1.173 µg/ml, 3.4, HRE – 0.409 µg/ml, 3.6 is given in fig. 6.

![Figure 6. Limit of detection study chromatogram](image)

4.3 Limit of Quantification
The limit of quantification for IRN and HRE has been established as the lowest IRN and HRE concentration at which their output is nearly 10 times larger than with the background baseline noise. Limit of quantification value and signal to noise ratio for IRN – 3.911 µg/ml, 10.5, HRE-1.364 µg/ml, 10.9 is given in fig 7.

![Figure 7. Limit of quantification study chromatogram](image)

4.4 Precision
Six replicates (n=6) of both normal and test solutions of the same concentration were inserted into the process accuracy and device accuracy tests. The resulting RSD values for IRN and HRE peak areas did not overdo 2% confirming precision is given in Figure 8 and Precision outcomes and study chromatograms for IRN and HRE are given in the table 3.

Table 3. Precision outcomes for IRN and HRE

| IRN area values obtained | HRE area values obtained |
|-------------------------|-------------------------|
| 2233267                 | 506394                  |
| Mean                    | Mean                    |
| 2239214                 | 2232350                 |
| 505884                  | 505818                  |
| Deviation               | Deviation               |
| 2229101                 | 5509.799                |
| 506118                  | 474.960                 |
| 2237086                 | 506003                  |
| RSD                     | RSD                     |
| 2223981                 | 505382                  |
| 0.247                   | 0.094                   |
| 2231451                 | 505127                  |

Figure 8. Precision study chromatograms
4.5 Accuracy
Accuracy was concentrated by spiking unadulterated IRN and HRE to tablet IRN and HRE arrangement ((IRN – 150 µg/ml; HRE – 12.5 µg/ml). Spiking was finished by adding IRN groupings of 74.25 µg/ml, 148.50 µg/ml, and 222.75 µg/ml and HRE centralizations of 6.18 µg/ml, 12.375 µg/ml and 18.63 µg/ml at 50, 100, and 150%, separately levels of fixation. The spiked examples were broke down and percent recuperation of IRN and HRE were determined at all levels. The subsequent mean recuperation esteems for IRN and HRE at all levels didn’t exaggerate acknowledgment limits affirming selectivity of strategy for testing IRN and HRE in tablets. Accuracy levels of, 100%, 150% examination chromatograms are given in fig 10, fig 11, fig12 and Recoveries of IRN and HRE measure are given in table 5 and table 6.

Table 4. Accuracy outcomes for IRN and HRE

| Drug | µg/ml Concentration taken | µg/ml Concentration Analyzed | Percentage Concentration assayed | Mean Percentage Concentration value |
|------|--------------------------|-----------------------------|---------------------------------|-----------------------------------|
| IRN  | 150                      | 148.14                      | 98.76                           | 98.72                             |
|      | 150                      | 148.55                      | 99.03                           |                                   |
|      | 150                      | 147.87                      | 98.58                           |                                   |
|      | 150                      | 148.40                      | 98.93                           |                                   |
|      | 150                      | 147.53                      | 98.35                           |                                   |
|      | 150                      | 148.02                      | 98.68                           |                                   |
| HRE  | 12.5                     | 12.39                       | 99.12                           | 99.00                             |
|      | 12.5                     | 12.38                       | 99.02                           |                                   |
|      | 12.5                     | 12.38                       | 99.06                           |                                   |
|      | 12.5                     | 12.38                       | 99.04                           |                                   |
|      | 12.5                     | 12.37                       | 98.92                           |                                   |
|      | 12.5                     | 12.36                       | 98.87                           |                                   |
Figure 9. Accuracy study chromatograms

4.6 Recovery

Table 5. Recoveries of IRN assay

| Spiking Level | Area value obtained | µg/ml Conc. added | µg/ml Conc. found | % Conc. Recovery | Mean Conc. Recovery value |
|---------------|---------------------|-------------------|-------------------|------------------|---------------------------|
| 50%           | 1104524             | 74.250            | 73.27             | 98.68            |                           |
| 50%           | 1098736             | 74.250            | 72.88             | 98.16            |                           |
| 50%           | 1104597             | 74.250            | 73.27             | 98.68            |                           |
| 100%          | 2230392             | 148.500           | 147.95            | 99.63            |                           |
| 100%          | 2221860             | 148.500           | 147.39            | 99.25            |                           |
| 100%          | 2237694             | 148.500           | 148.44            | 99.96            |                           |
| 150%          | 3360100             | 222.750           | 222.89            | 100.06           |                           |
| 150%          | 3358724             | 222.750           | 222.80            | 100.02           |                           |
| 150%          | 3363621             | 222.750           | 223.13            | 100.17           |                           |
Recovery was concentrated by spiking unadulterated IRN and HRE to tablet IRN and HRE arrangement ((IRN – 150 µg/ml; HRE – 12.5 µg/ml). Spiking was finished by adding IRN groupings of 74.25 µg/ml, 148.50 µg/ml, and 222.75µg/ml, and HRE centralizations of 6.18 µg/ml, 12.375 µg/ml and 18.63 µg/ml at 50, 100, and 150%, separately levels of focus. The spiked examples were dissected and percent recuperation of IRN and HRE were determined at all levels. The subsequent mean recuperation esteems for IRN and HRE at all levels didn’t exaggerate acknowledgment limits affirming selectivity of strategy for examining IRN and HRE in tablets. Recuperation levels of half , 100%, 150% investigation chromatograms are given in fig 10, fig 11,fig12 and Recoveries of IRN and HRE measure are given in table 5 and table 6.

Table 6. Recoveries of HRE assay

| Spiking Level | Area value obtained | µg/ml Conc. added | µg/ml Conc. found | % Conc. Recovery | Mean Conc. Recovery value |
|---------------|---------------------|------------------|------------------|-----------------|-------------------------|
| 50%           | 252582              | 6.188            | 6.18             | 99.87           | 99.67                   |
| 50%           | 251174              | 6.188            | 6.15             | 99.32           |                         |
| 50%           | 252402              | 6.188            | 6.18             | 99.80           |                         |
| 100%          | 505714              | 12.375           | 12.37            | 99.98           | 100.01                  |
| 100%          | 505857              | 12.375           | 12.38            | 100.01          |                         |
| 100%          | 506045              | 12.375           | 12.38            | 100.05          |                         |
| 150%          | 761412              | 18.563           | 18.63            | 100.36          | 100.33                  |
| 150%          | 760494              | 18.563           | 18.61            | 100.24          |                         |
| 150%          | 761701              | 18.563           | 18.64            | 100.40          |                         |

Figure 10. Recovery (50% level) study chromatograms
Figure 11. Recovery (100% level) study chromatograms

Figure 12. Recovery (150% level) study chromatograms
4.7 Robustness
Robustness was determined on working IRN and HRE arrangement (IRN – 150 μg/ml; HRE – 12.5 μg/ml) by hardly altering the normalized test conditions. Results assessment was directed by finding any varieties in framework reasonableness esteems for IRN and HRE. The subsequent framework appropriateness esteems for IRN and HRE in totally changed conditions are given in Table-7 didn't exaggerate acknowledgment limits affirming vigor. Change in stream rate was 0.9ml/min, 1.1ml/min, in nm of recognition was 228nm, 232nm, in PH was 4.0, 4.2units, in temp esteem was 23ºc, 27ºc.

Table 7. System aptness outcomes for robustness
### Table 8. Stability test outcomes of IRN and HRE

| Degradation | IRN | HRE |
|-------------|-----|-----|
|             | Area values obtained | Remained percent | Degraded percent | Area values obtained | Remained percent | Degraded percent |
| Acid        | 1997984 | 88.36 | 11.64 | 460304 | 90.10 | 9.9 |
| Alkali      | 2114693 | 93.52 | 6.48 | 466482 | 91.30 | 8.7 |
| Peroxide    | 2143977 | 94.81 | 5.19 | 499842 | 97.83 | 2.17 |
| Dry heat    | 2038671 | 90.16 | 9.84 | 450179 | 88.11 | 11.89 |
| Sun light   | 2163156 | 95.66 | 4.34 | 486312 | 95.19 | 4.81 |
5. Conclusion

In this current study, a solidity demonstration of the RP-HPLC technique was developed and accepted for the simultaneous evaluation of Irbesartan and Hydrochlorothiazide in the tablet structure. The pressure testing contemplated uncovered that the technique was effectively utilized to determine the debased items from the example. From the top immaculateness profile, it was seen that there was no impedance of the debasement artefacts and that the virtue of the point was not exactly the virtue of the boundary. The effects of the acceptance are palatable and acceptable. This technique can be used to monitor the consistency of tablets containing an IRN and HRE mixture with accuracy and precision.

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References

[1] Benjamin E J and Virani S S, et al. 2018 American Heart Association Council on Epidemiology and Prevention Statistics Committee and Stroke Statistics Subcommittee. Coronary illness and Stroke Statistics-2018 Update: A Report from the American Heart Association. Flow 137 (12), p 67-492.
[2] Farley A, McLaflerty E and Hendry C 2012 The cardiovascular framework., 27 (9), 35-39.
[3] Briggs M A, Petersen K S and Kris-Etherton P M 2017 Immersed Fatty Acids and Cardiovascular Disease: Replacements for Saturated Fat to Reduce Cardiovascular Risk. Medical care (Basel) 5 (2), p 29.
[4] Rosiek A and Lekowski K 2016 The danger elements and anticipation of cardiovascular infection: The significance of electrocardiogram in the analysis and treatment of intense coronary disorder. Remedial and Clinical Risk Management 12,p 1223-29.
[5] Key realities about cardiovascular illnesses, WHO, Accessed on June 2020.
[6] Cardiovascular specialist, MedGen UID: 2847, Concept ID: C0007220, Pharmacologic Substance, 2020.
[7] Alquwaizani M, Buckley L, Adams C and Fanikos J 2013 Anticoagulants: A Review of the Pharmacology, Dosing, and Complications. Current Emergency and Hospital Medicine Reports, 1 (2), p 83-97.
[8] Das P, Oliphant CS, Beach E and Thapa R. 2010 Arising antiplatelet specialists, differential pharmacology, and clinical utility. Diary of Blood Medicine, 1,p 79-91.
[9] Ali MR, Salim Hossain M and Islam MA, et al. 2014 Part of thrombolytic treatment: an audit. Logical World Journal, 586510.
[10] Herman L L, Padala S A and Annamaraju P et al. 2020 Angiotensin Converting Enzyme Inhibitors (ACEI) Stat Pears Publishing
[11] Maggioni A P 2006 Viability of Angiotensin receptor blockers in cardiovascular infection. Cardiovascular Drugs Therapy, 20 (4), p 295-308.
[12] Franciosa J A 1999 Beta-adrenergic impeding specialists: past, present, and future points of view. Coronary Artery Disease, 10 (6) p 369-76.
[13] Elliott W J and Ram C V 2011 Calcium channel blockers. Diary of Clinical Hypertension 13 (9),p 687-89.
[14] Roush G C, Kaur R and Ernst M E. 2014 Diuretics: an audit and update. Diary of Cardiovascular Pharmacology and Therapeutics 19 (1) p 5-13.
[15] Hariri L and Patel J 2020 Vasodilators. StatPears Publishing
[16] Virgadamo S, Charnigo R, Darrat Y, Morales G and Elayi C S 2015 Digoxin: A deliberate survey in atrial fibrillation, congestive cardiovascular breakdown and post myocardial localized necrosis. World Journal of Cardiology 7 (11), p 808-816.
[17] Ramkumar S, Raghunath A and Raghunath S 2016 Statin Therapy: Review of Safety and Potential Side Effects. Acta Cardiologica Sinica 32 (6), p 631-39
[18] Ananya M 2020 Cardiovascular medications, new clinical life sciences.
[19] FDA endorsement 2020.
[20] Bramlage P 2009 Fixed blend of irbesartan and hydrochlorothiazide in the administration of hypertension. Vascular Health and Risk Management 5 (1), p 213-224.
[21] Irbesarta n/HCTZ RBX 300/12.5 Tablets 2020 NPS Medicine wise