International Journal of Pharmacy and Biological Sciences
ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online)
IJPBS™ | Volume 8 | Issue 4 | OCT-DEC | 2018 | 68-76
Research Article | Pharmaceutical Sciences | Open Access | MCI Approved| ज्ञान-विज्ञान विमुक्तये
|UGC Approved Journal |

SIMULTANEOUS ESTIMATION OF IMIDAPRIL HYDROCHLORIDE AND HYDROCHLOROTHIAZIDE BY RP-HPLC

Veditha.K[1]* and Sailaja.B[1]

[1*] Research Scholar, Institute of Pharmaceutical Technology, Sri Padmavati Mahila Visvavidyalayam, Tirupati-517502, Andhra Pradesh-India.

[1] Professor, Institute of Pharmaceutical Technology, Sri Padmavati Mahila Visvavidyalayam, Tirupati- 517502, Andhra Pradesh-India.

***Corresponding Author Email: k.veditha@yahoo.com**

ABSTRACT

A simple isocratic reverse phase high performance liquid chromatography (RP-HPLC) method has been developed and subsequently validated for simultaneous estimation of anti-hypertensive drugs, Imidapril(IMP) and Hydrochlorothiazide(HTZ) in tablet formulation. Quantification was carried out by using an isocratic mode on HPLC of Phenomenex Luna (C_{18} 250 X 4.6 mm, 5 microns) with flow rate of 1.0 ml/min and UV detection at 218nm. The separation was carried out using a mobile phase consisting of phosphate buffer: acetonitrile: methanol (58: 25: 17 v/v) and adjusted the pH to 2.5. The retention time for IMP and HTZ was found to be 6.243min and 2.370min respectively. A linear response was observed over the concentration range of 5-30 mcg/ml each for the assay of IMP and HTZ. The results of analysis were validated and recovery was found to be < 98%. Hence the proposed method was found to be accurate, precise, reproducible and specific and could be used for simultaneous analysis of these drugs in tablet formulation.

KEY WORDS

Imidapril, Hydrochlorothiazide, RP-HPLC method, tablet formulation.

INTRODUCTION:

Hypertension remains one of the most important risk factors for cardiovascular diseases and treatment of hypertension is challenging. The percentage of patients with controlled hypertension on treatment with single drugs is low. Therefore European guidelines and American guidelines recommend that, for some hypertensive patients, therapy should be initiated with two drugs for effective results. Imidapril (ACE inhibitor) is a first-choice anti-hypertensive. Used alone can control hypertension in ~50% patients and addition of diuretic (Hydrochlorothiazide)/ β blocker extend efficacy to ~90%. Requires relatively lower doses (2.5-10mg/day) which are well tolerated [1, 2].

Imidapril HCl is chemically named as (4S)-3-[(2S)-2-{[(2S)-1-ethoxy-1-oxo-4-phenylbutan-2-yl] amino} propanoyl]-1-methyl-2-oxoimidazolidine-4-carboxylic acid hydrochloride (Fig.1) and its molecular formula is $C_{20}H_{27}N_3O_6HCl$.

Hydrochlorothiazide chemically described as 6-chloro-1,1-dioxo-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide (Fig. 2). Molecular formula is $C_7H_8ClN_3O_4S_2$.

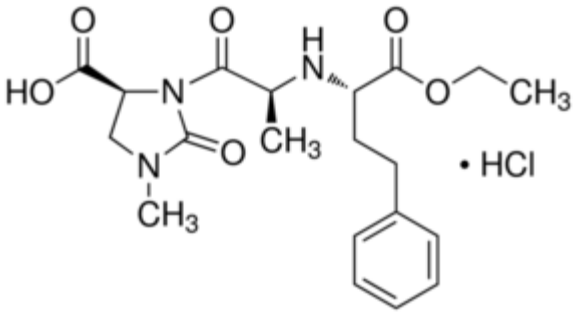


Fig.1 Imidapril hydrochloride

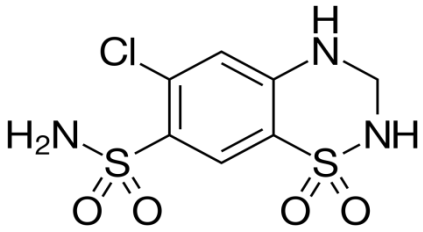


Fig. 2 Hydrochlorothiazide

A thorough literature survey reveals that analytical methods exist for the estimation of imidapril and hydrochlorothiazide in single and in combination with other categories of antihypertensive drugs [3-17]. There are no reported methods for the estimation of combination of imidapril and hydrochlorothiazide. Therefore, the present research work is designed to develop a possible validated new, simple, specific, reliable analytical method for quantitative determination of selected drugs in individual and in combination using RP-HPLC method.

MATERIALS AND METHODS:

Chemicals:

Active pharmaceutical ingredients of Imidapril HCl and Hydrochlorthiazide were received as gift samples from Hetero labs Hyderabad Telangana, India. Triple distilled water from milliq, acetonitrile, methanol, ethanol and hydrochloric acid of HPLC grade bought from Merck 50, Pvt ltd (Mumbai), potassium dihydrogen phosphate, ortho-phosphoric acid of AR grade obtained from S.D.Fine chemicals(Mumbai). The sample mixture containing Imidapril (10mg) and HTZ (12.5mg) was prepared in laboratory.

Instrumentation

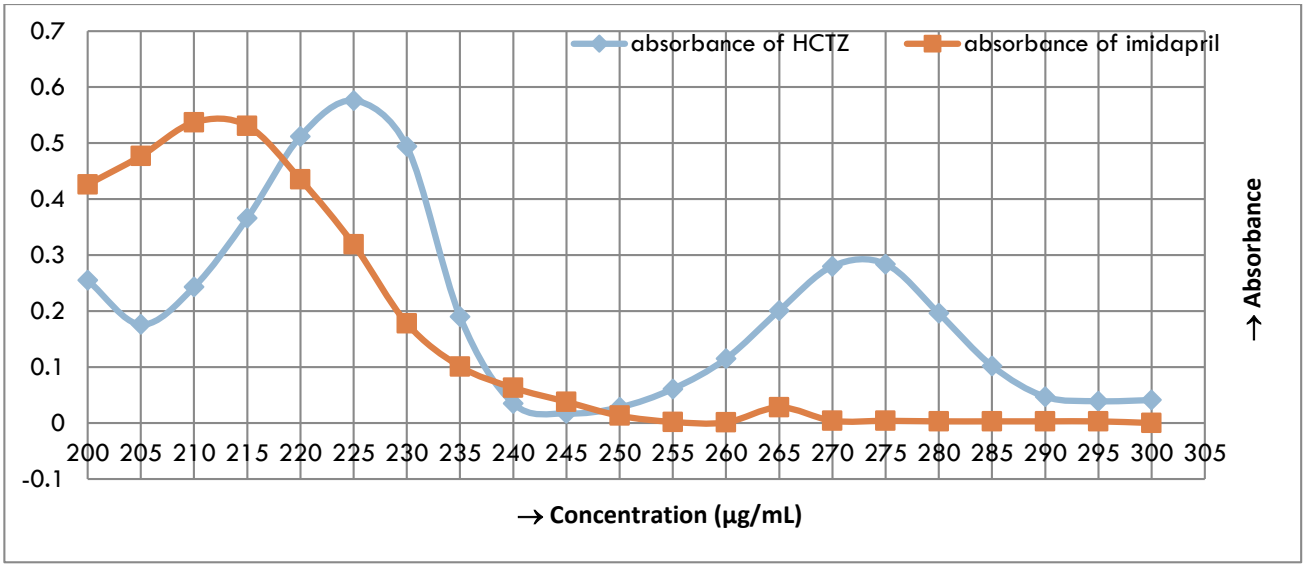
HPLC instrument used was of Shimadzu HPLC system. Software used is Spinchrome, Systronics UV-VIS spectrophotometer, Essar Digital analytical balance, Ultra sonic bath sonicator (PCI), Elico Digital pH meter model: LI120.

Solubility:

Solubility was checked in different solvents like water, phosphate & citro phosphate buffers (3.0, 4.0, 5.0, 6.8, 9.0), methanol, ethanol, acetonitrile, DMF individually and in combination. Solubility of combination of imidapril and HTZ was found to be best in phosphate buffer, methanol and acetonitrile (55:10:35).

Scanning of drugs for λ_{max} determination:

10mg each of Imidapril and Hydrochlorothiazide was weighed on ESSAR electronic balance. The drugs were transferred to 10 ml volumetric flasks. The drugs were dissolved in phosphate buffer: methanol: ACN (55:10:35) and the volume was made up to the mark with the same solvent. The stock solutions were diluted with the same solvent to obtain 10µg/ml. The resulting solutions were scanned from 210- 400nm (Fig.3).



The drugs has exhibited the maximum absorbance at wavelength of 218nm

Fig. 3 Absorption spectra of Imidapril and Hydrochlorothiazide

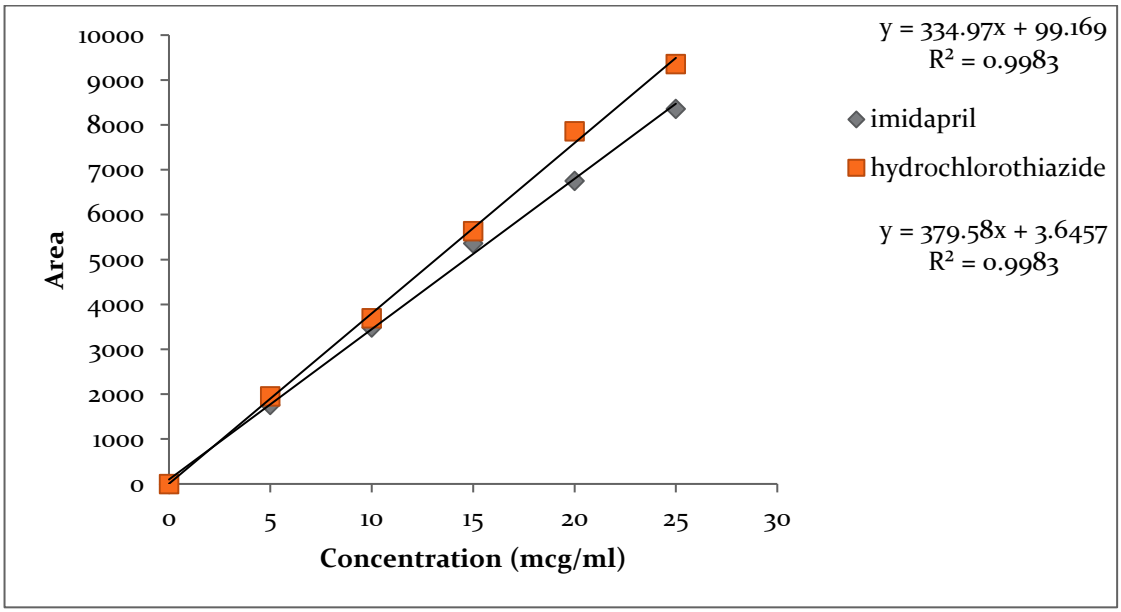


Fig. 4 Linearity plots of Imidapril & Hydrochlorothiazide

Chromatographic conditions:

To develop a suitable and robust HPLC method for the determination of IMP and HTZ, different mobile phases like Acetonitrile: Water (10:90); Methanol: Water (10:90); Acetonitrile: Methanol: 3.0 pH phosphate buffer (20:10:70); Acetonitrile: Methanol: 6.8pH phosphate buffer (10:20:70); Acetonitrile: 6.8pH phosphate buffer (10:90, 20:80, 30:70), were used at flow rate of 1ml/min. The detection is performed at the wave length 225nm.

The chromatographic separation was achieved on Phenomenex Luna (C_{18} 250 X 4.6 mm, 5 microns) column using mobile phase consisting of phosphate buffer: acetonitrile: methanol (58: 25: 17 v/v) and adjusted the pH to 2.5±0.1 and filtered through 0.45µm membrane filter and diluent used was same for standard and sample preparation. The column was maintained at room temperature (25°C) and the flow rate is 1ml/min. Preceding to inject the solutions, the column is stabilized for 30 minutes with the mobile phase flowing through the system. 20µl of sample was injected in HPLC at UV-Visible at a detective wavelength of 218nm. Under defined experimental conditions; all the peaks were well resolved and free from tailing with good

resolution. A typical chromatogram of IMP and HTZ sample is shown in Fig.5.

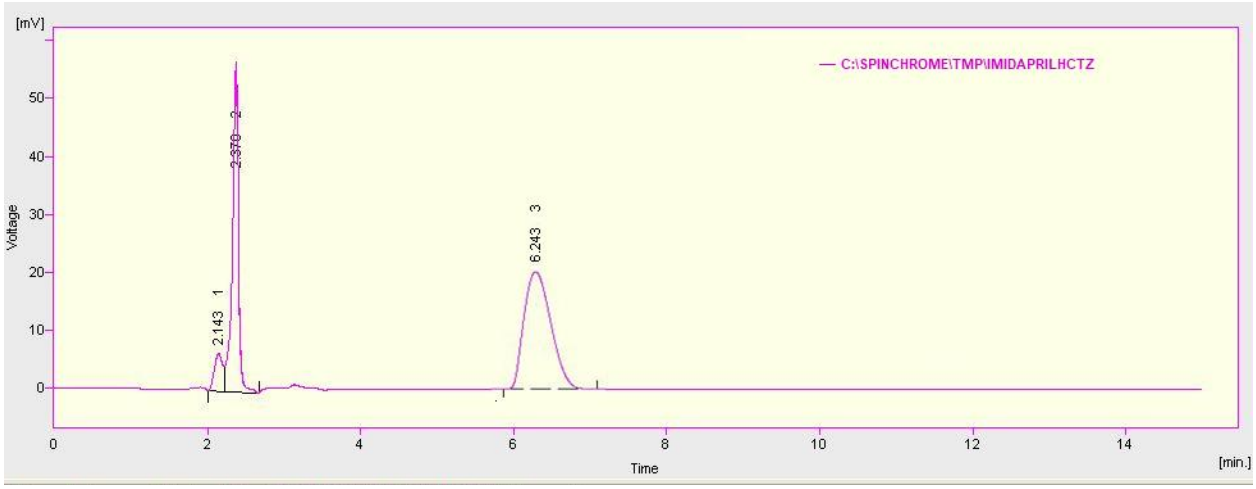


Fig. 5 Typical Chromatogram of Hydrochlorothiazide & Imidapril HCl

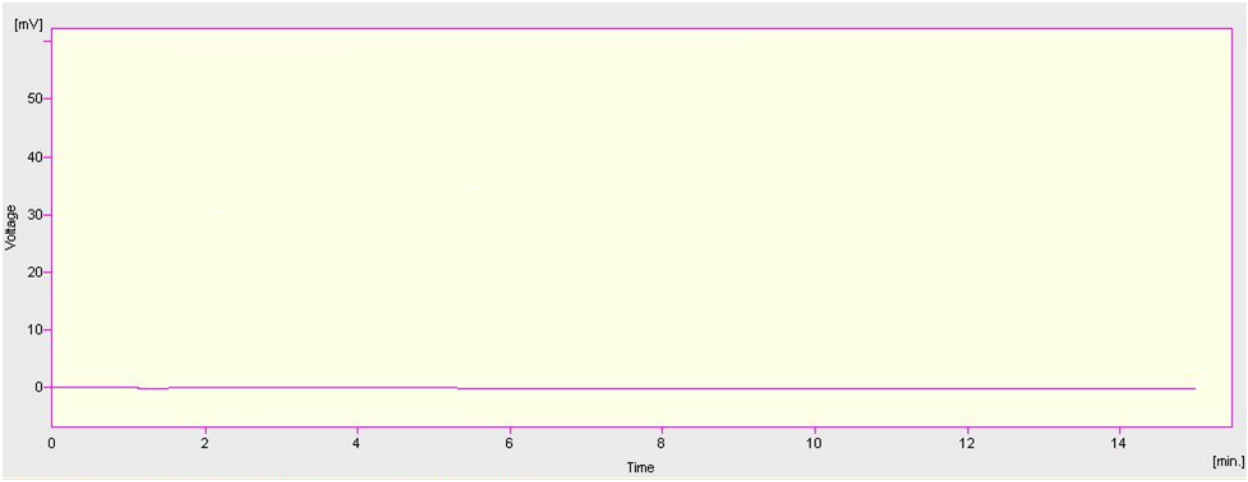


Fig. 6 Chromatogram of Blank

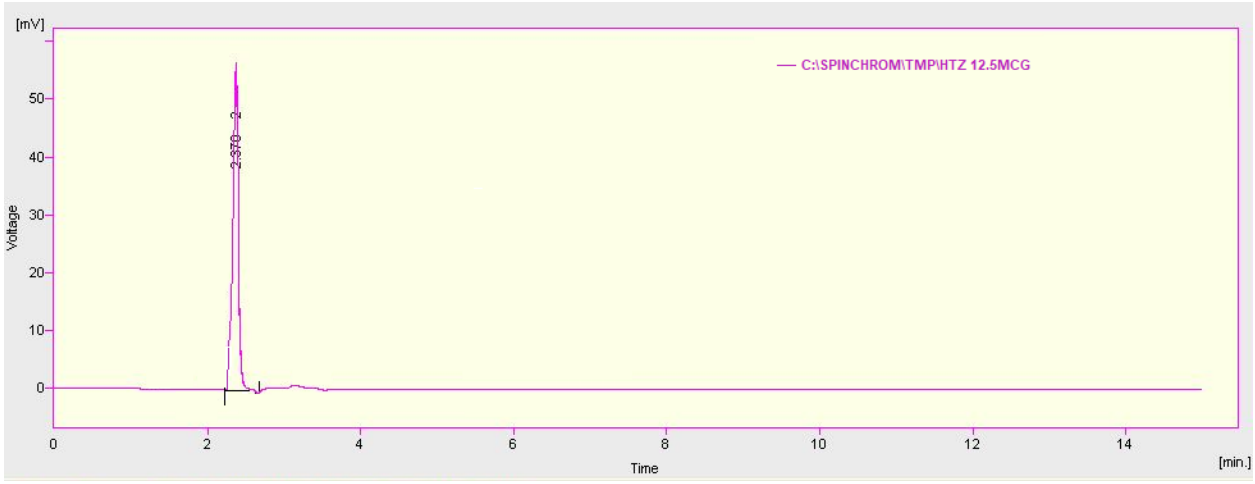


Fig. 7 Chromatogram of Hydrochlorothiazide

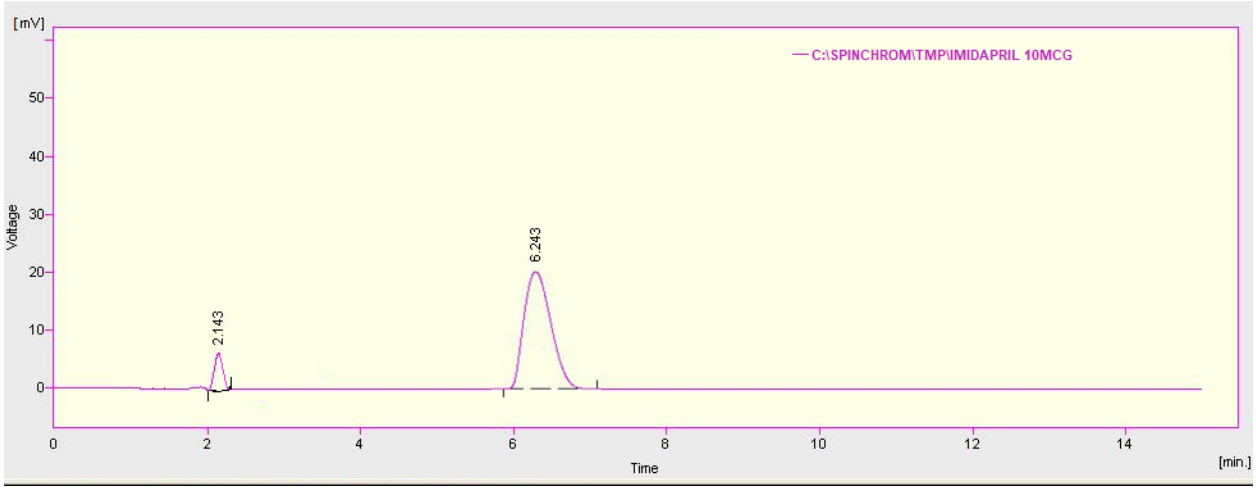


Fig. 8 Chromatogram of Imidapril HCl

Preparation of buffer*:*

Preparation of 5mM potassium di hydrogen phosphate buffer: Dissolved 0.68g of potassium di hydrogen phosphate in 1000ml of water and adjusted the pH to 2.5 with 25% ortho- phosphoric acid and 10M potassium hydroxide.

Preparation of tablets:

Tablets were formulated by employing direct compression technique. The required quantities of the drug, diluent, and disintegrant were mixed in a poly bag for five minutes. The required quantities of glidant and lubricant were incorporated and then the blend was compressed to form a tablet with 9mm compression tool. The composition of the formulated tablets is given in Table 1.

Table 1: Composition of tablet

Ingredients	Quantity mg per tablet
Imidapril hydrochloride equivalent to Imidapril	10
Hydrochlorothiazide	12.5
Mannitol	170
Crospovidon	6
Magnesium stearate	0.5
Talc	1
Tablet weight	200

Standard and sample preparation:

Preparation of IMP and HTZ stock solutions*:* *(*10 mg/ml Imidapril, 12.5 mg/ml HTZ): Accurately weighed and transferred 10mg of IMP, 12.5mg of HTZ working standards into a 10.0 mL clean dry volumetric flask. Then a small amount of diluent was added and the flask was sonicated for 30 min and diluted up the mark with diluent.

Preparation of Standard working solution **(**10 µg/ml IMP, 12.5µg/ml HTZ): From the above stock solutions, 0.5 ml was pipetted out to a 50ml volumetric flask and the final volume was made up with diluent to obtain final concentrations.

Preparation of Stock sample solution*:* Twenty tablets were accurately weighed and powered. Accurately weighed and transferred the tablet powder equivalent to 10mg of IMP and 12.5mg of HTZ into a 10 ml volumetric flask that contained approximately 3 ml of diluent, sonicated for 30 mins. Finally, the volume was made up to the mark with diluent and labeled as sample stock solution. Sample stock solution was filtered by using 0.45µm membrane filter paper.

*Preparation of Sample working solutions***:** 0.5 ml of filtered sample stock solution was transferred to 50 ml volumetric flask and made up to the mark with diluent to obtain final concentrations.

Analytical method validation:

The analytical method was validated as per ICH guidelines [18] with respect to parameters such as linearity, accuracy, precision, assay, ruggedness, robustness, limit of detection and limit of quantification as follows.

Linearity

Linearity of this method was assessed by linear regression analysis, calculated by least square method and studied by preparing standard solutions of IMP and HTZ at different concentrations. The peak areas of IMP and HTZ were plotted against their respective concentrations. The response was found to be linear over the concentration range of 5-25µg/ml for IMP and HTZ shown in figure 4. Typically, the regression equation were y = 334.97x + 99.169 for IMP and y = 379.58x + 3.6457for HTZ. The correlation coefficient (r^2) for IMP and HTZ was found to be greater than 0.998. The data is given in Table 2.

Table 2: Linearity regression data

Concentration (µg/mL)	Imidapril	Hydrochlorothiazide
5	1761.7	1954.8
10	3487.6	3690.9
15	5362.9	5632.3
20	6748.4	7856.3
25	8356.6	9356.2
Correlation coefficient	0.998	0.998
Intercept	99.16	3.64
Slope	334.91	379.58

Accuracy

Accuracy was performed in triplicate for various concentrations of IMP and HTZ equivalent to 80%, 100% and 120% of standard amount, was injected into the HPLC system as per test procedure. The average % recovery of IMP and HTZ was calculated. The data was given in the Table 3.

Table 3: Results of recovery studies (Accuracy)

Drug	Drug amount (µg/mL)	Level of addition (%)	Amount added (µg/mL)	Amount recovered (µg/mL)	% recovery	Average % recovery
Imidapril	10	80	8	17.89	98.6%	98.4%
	10	100	10	19.91	99.1%	
	10	120	12	21.84	98.6%	
HTZ	12.5	80	10	22.10	98.2%	98.2%
	12.5	100	12.5	24.51	98.0%	
	12.5	120	15	27.13	98.5%	

Precision

A) Method precision (Intraday) Six sample solutions of the same concentration were prepared and injected into the HPLC system as per test procedure.

B) Intermediate precision (Inter day) Two analysts as per test procedure conducted the study. For Analyst-1 Method Repeatability and for Analyst-2 six sample solutions of same concentration were prepared and injected into the HPLC system as per test procedure. The results were given in Table 4.

Table 4: Data for Intra and Interday precision

Type of precision	Intraday Precision		Interday precision			
Drug mixture (10/12.5 mg/ml of IMP&HTZ)	Area of IMP	Area of HTZ	Sample area of IMP		Sample area of HTZ	
			Analyst 1	Analyst 2	Analyst 1	Analyst 2
Mixture 1	3488.6	4599.8	3512.5	4610.1	3488.5	4460.9
Mixture 2	3501.7	4612.1	3408.9	4526.2	3424.4	4624.5
Mixture 3	3475.4	4605.2	3474.1	4638.7	3472.1	4532.1
Mixture 4	3492.1	4619.4	3489.4	4492.4	3463.6	4547.7
Mixture 5	3467.3	4585.5	3415.7	4573.2	3415.7	4682.3
Mixture 6	3513.5	4623.8	3436.6	4654.5	3497.2	4639.4
Mean	3489.7	4607.6	3456.2	4582.5	3460.2	4581.1
SD	16.87	13.9	42.08	63.99	33.42	81.83
% RSD	0.48	0.30	1.21	1.39	0.96	1.78

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The limit of quantification (LOQ) and the limit of detection (LOD) were calculated on the basis of the standard deviation of the response and the slope obtained from the linearity plot as described in the relevant ICH guideline. LOD and LOQ were calculated.

Assay

The validated method was applied for the determination of IMP and HTZ in tablets which were prepared in our institution. The results of assay undertaken yielded 98.4% of IMP and 98.0 % of HTZ. The chromatogram representing the sample is shown in Fig 5.

Table 5: observations of chromatogram in robustness study

Parameter		Flow volume			pH			Wavelength		
		0.8	1.0	1.2	2.3	2.5	2.7	216	218	220
Retention time	IMP	3.225	2.371	2.351	2.356	2.372	2.354	2.342	2.375	2.370
	HZT	7.368	6.246	6.239	6.238	6.245	6.263	6.268	6.243	6.254
Theoretical plates	IMP	3523	3472	3412	3427	3485	3471	3457	3462	3475
	HZT	4499	4575	4527	4563	4582	4571	4526	4591	4548
Tailing factor	IMP	1.2	0.9	1.3	1.3	1.1	1.1	1.0	0.8	1.0
	HZT	1.0	0.9	1.0	1.0	1.1	1.0	1.0	0.9	1.2
% RSD of peak area	IMP	0.50	0.48	0.72	0.46	0.51	0.98	1.20	0.49	0.56
	HZT	1.34	0.94	1.17	0.37	0.68	1.45	1.41	0.83	0.76

Robustness

Robustness was done by minor deliberate changes in chromatographic conditions and retention time of IMP and HTZ was noted. The factors selected were wavelength, pH and temperature and the results remained unaffected. Ruggedness of method was checked by using different instruments. The relative standard deviation of the results obtained from different instruments was <2.0%. The results were given in Table 5.

System Suitability

System suitability and chromatographic parameters were validated such as number of theoretical plates, tailing factor and % RSD of peak area was calculated. The results are given in the Table 6.

Table 6: System suitability

Parameter	Acceptance criteria	Observed values of Imidapril	Observed values of HTZ
Tailing factor	NMT 2.0	1.5	0.68
% RSD 0f peak area	NMT 2.0	1.27	1.42
Theoretical plates	NLT 3000	3534	4548

RESULTS AND DISCUSSION:

The proposed method was found to be linear over concentration range of 5-25µg/ml for IMP and HTZ respectively. System suitability parameters indicate good number of theoretical plates and resolution and %RSD was less than 2. The method was found to be accurate and precise as indicated by the results of recovery studies and precision studies whose percent relative standard deviation is not more than 2%. The LOD and LOQ were found to be 0.000734 µg mL-1, 0.002446 µg mL-1 and 0.000794 µg mL-1, 0.002649 µg mL-1 for IMP and HTZ respectively. There were no marked changes in the chromatograms which confirmed the ruggedness of the method. The standard deviation of %assay for sample was calculated, for each parameter in robustness studies the relative standard deviation was found less than 2%. The low percent relative standard deviation (%RSD) value confirms the robustness of method. The summary of results were given in Table 7.

Table 7: Summery of Validation Parameters.

Parameters	Imidapril HCl	Hydrochlorothiazide
Linearity (µg/mL)	5-25	5-25
Correlation coefficient	0.998	0.998
LOD(µg/mL)	0.000734	0.000794
LOQ(µg/mL)	0.002446	0.002649
% Recovery	98.6-99.1	98.0-98.5
Intraday (%RSD)	<2.0	<2.0
Interday/ Ruggedness (%RSD)	<2.0	<2.0
Robustness	Robust	Robust
Assay	98.4	98.2

CONCLUSION:

The proposed method was found to be robust, rapid, precise, accurate and sensitive. It makes use of fewer amounts of solvents and has shorter retention times than existing methods. Hence the developed method could be used for routine analysis of IMP and HTZ in tablet dosage form.

ACKNOWLEDGEMENTS:

The authors are thankful to Hetero labs (Hyderabad, Telangana, India) for providing API of Imidapril and HTZ.

REFERENCES:

1. Lawes CM., Vander Hoorn S., Rodgers A., International Society of Hypertension Global burden of blood-pressure–related disease. Lancet, 371: 1513-1518 (2008)
2. Tripathi KD., Essential medical of Pharmacology, 5th Edn, 38th chapter, 2008; pp:502-520.
3. Sirisha N., Haripriya A., SwethaBhavani N., Bhagirath R., Satyanarayana M., Panikumar D Anumolu., Simultaneous quantification of nebivolol hydrochloride and hydrochlorothiazide by first derivative UV-Spectroscopy. Der Pharmacia Lettre, 5 (2): 78-84 (2013)
4. Hapse SA, Wagh VS., Kadaskar PT., Dokhe MD., Shirsath AS. Spectrophotometric estimation and validation of hydrochlorothiazide in tablet dosage forms by using different solvents. Der Pharma Chemica, 4 (1): 10-14 (2012)
5. Maha Abdel-Monem Hegazy., Maya Shaaban Eissa., Osama Ibrahim AbdEl-Sattar, Mohamed Mohamed Abd El-Kawy., Determination of a novel ACE inhibitor in the presence of alkaline and oxidative degradation products using smart spectrophotometric and chemometric methods. Journal of Pharmaceutical Analysis, 4(2): 132–143, (2014)
6. Sunandamma Y., Single RP-HPLC method for the quantification of candesartan and hydrochlorothiazide in formulation. The Experiment, 7(4): 428-437, (2013)
7. Qutab SS., Razzaq SN., Ashfaq M., Shuja ZA, Khan IU., Simple and sensitive LC–UV method for simultaneous analysis of hydrochlorothiazide and candesartan cilexetil in pharmaceutical formulations. Acta Chromatographica (2007)
8. Khushboom Gondaliya., Pankaj Kapupara P., Ketan Shah V., Development and validation of RP-HPLC method for simultaneous estimation of clonidine hydrochloride and hydrochlorothiazide in pharmaceutical formulation. International Bulletin of Drug Research, 4(6): 106-115, (2014)
9. Kakumani Kishore Kumar., Chimalakonda Kameswara Rao., Madhusudan G., Rapid Simultaneous Determination of Olmesartan —Amlodipine and Hydrochlorothiazide in Combined Pharmaceutical

Dosage Form by Stability-Indicating Ultra Performance Liquid Chromatography. American Journal of Analytical Chemistry, 3: 50-58, (2012)

10. Urvesh Patel M., Avani Chokshi B., Pritesh Desai R., Development and validation of RP-HPLC method for determination of hydrochlorothiazide, olmesartan medoxomil and their related substances in combined tablet dosage form. International Journal of Pharmacy and Pharmaceutical Sciences, 6(9), (2014)
11. Jabir Aboobacker O., Venkatachalam T., Senthilkumar N., Vijayamiruthraj R., Kalaiselvi P., Development and Validation of Hydrochlorothiazide, Amlodipine Besylate and Telmisartan in Tablet Dosage Form by RP-HPLC Method. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 3(3): 509, (2012)
12. Santaji nalwade., Vangala ranga reddy., Dantu durga rao., Inabathina koteswara rao., Rapid Simultaneous Determination of Telmisartan, Amlodipine Besylate and Hydrochlorothiazide in a Combined Poly Pill Dosage Form by Stability-Indicating Ultra Performance Liquid Chromatography. Scientia pharmaceutica, 3(2):47-54, (2013)
13. Manjulatha YB., Gowrisankar D., Development and validation of a reversed phase hplc method for simultaneous estimation of olmesartan and hydrochlorothiazide in combined tablet dosage form. An International Journal of Advances in Pharmaceutical Sciences, 5(5): 2331-2333, (2014)
14. Gaurang Pandya P., Hitendra Joshi S., Development and validation of stability indicating HPLC assay method for simultaneous determination of amlodipine besylate, olmesartanmedoxomil and hydrochlorothiazide in tablet formulation. Der Pharmacia Sinica, 4(2): 145-152, (2013)
15. AshutoshKumar S., Manidipa Debnath., SeshagiriRao JVLN., GowriSankar D., A new and rapid analytical method development & validation for simultaneous estimation of hydrochlorothiazide, amlodipine &olmesartan in tablet dosage form by using RP-HPLC. Journal of Chemical and Pharmaceutical Research, 6(5):1208-1213, (2014)
16. Tahir MS., RP-HPLC method development and validation for the simultaneous estimation of imidapril hcl and amlodipine besylate in bulk and tablet. Journal of the Chemical Society of Pakistan, 35: 49-51, (2013)
17. Waelabudayyih. Development and validation of a Simultaneous estimation of some prills in drug forms. International Journal of Pharmacy and Pharmaceutical Sciences, 5(1), (2013)
18. ICH harmonised tripartite guideline, Validation of analytical procedures: Text and methodology q2(r1), 1994.

Received:05.08.18, Accepted: 08.09.18, Published:01.10.2018

***Corresponding Author:**
Veditha.K*
Email: k.veditha@yahoo.com