Simultaneous Estimation and Quantification of Telmisartan and Cilnidipine Using High Performance Thin Layer Chromatography in Pharmaceutical Dosage

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Abstract- This study presents streamlined, highly precise, and efficient HPTLC method for the simultaneous assessment of Telmisartan and Cilnidipine in fixed-dose combination. The chromatographic process utilized aluminium-backed plates coated with silica gel 60 F254 as the stationary phase. A mobile phase consisting of dichloromethane, toluene, and methanol in 7:2:1 (v/v/v) ratio was used. Chromatograms were developed in 10 X 20 cm twin-trough TLC chamber, pre saturated with mobile phase vapor for 20 minutes to ensure optimal separation. Detection was performed at 260 nm, where Distinct and compact spots were observed at Rf values of 0.43 ± 0.05 and 0.55 ± 0.05 for Telmisartan and Cilnidipine respectively, ensuring good resolution. Precision studies were conducted by analyzing the variations in peak responses, expressed as RSD, under both intraday and interday conditions. Accuracy was measured by percentage recovery for Telmisartan and Cilnidipine, and the method was validated according to ICH standards for optimized performance.

Keywords: Telmisartan, Cilnidipine, HPTLC, ICH guidelines, Assay.

I.INTRODUCTION

Affecting a large number of people worldwide, hypertension is persistent condition that plays a critical role in the onset of cardiovascular diseases, stroke and kidney failure. [1].As such, the combination of active ingredients in the appropriated dosages effectively aids in reducing blood pressure and managing hypertension.[2] Telmisartan, a oral non-peptide angiotensin II antagonist, with terminal elimination half-life of 24 hours, ultimately improving its bioavailability.[3] Telmisartan (**Fig.1)** is chemically referred to as 2-[4-[[4-methyl-6-(1-methylbenzimidazol-2-yl)-2-propylbenzimidazol-1-yl]methyl]phenyl]benzoic acid, and is utilized in the treatment of hypertension, diabetic nephropathy and congestive heart failure. Cilnidipine (**Fig.2)** is chemically, Methyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4- dihydro-3-(2-methoxyethyl)-5-propenylpyridine-3,5- dicarboxylate. It is dual channel blocker that acts on both N- and L- type calcium channels and is effective in hypertension. Telmisartan ability to be absorbed and utilized by the body varies with dosage. [4] Cilnidipine has a low bioavailability of about 13%, Its low solubility is considered the main factor behind its poor absorption, even though it readily pass through cell membranes.[5] Combining Cilnidipine and Telmisartan at fixed, human- equivalent dose has been found to lower blood pressure without causing any changes in heart rate. This combination provides various therapeutic benefits like blood pressure reduction, a reduced likelihood of heart rate increase, cardio protection, vascular protection, and improved glycemic control. [6]

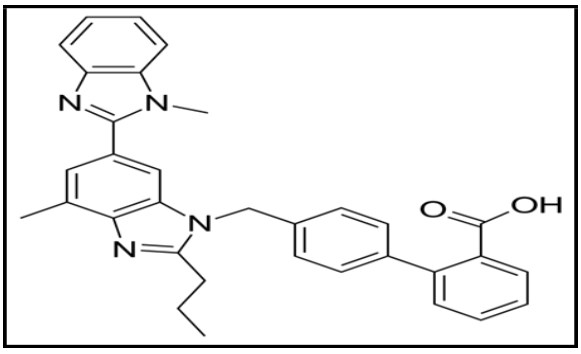

Fig. 1: Telmisartan

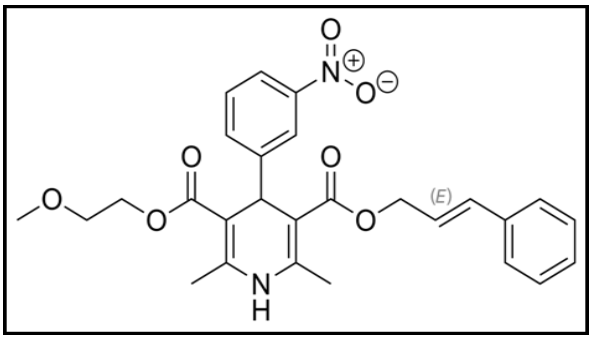

Fig. 2: Cilnidipine

II.MATERIALS & METHODS

Chemical, Reagents and Solutions

IP standards of Telmisartan and Cilnidipine were purchased from Indian Pharmacopeia Commission (Ghaziabad, Uttarpradesh). A fixed-dose combination of Cinod-T containing 40 mg Telmisartan and 20 mg Cilnidipine was purchased from a local market in Goregaon west, Mumbai. Methanol (HPLC grade), Toluene, Methylene Dichloride which were AR grade, were purchased from Merck chemicals. All dilutions were performed in a standard volumetric flask.

Standard preparation Stock Solution:

Weighed 200 mg of Telmisartan reference standard and 100 mg of Cilnidipine reference standard, transfer both to 50 ml volumetric flask, added Methylene chloride, then sonicated to complete dissolution and finally, diluted with methylene chloride to the 50ml mark and mixed well

Standard Solution preparation:

Pipette 1.0 ml of the prepared stock solution into 10 ml volumetric flask, diluted to volume with methanol and mixed well to obtain a homogeneous solution.

Sample preparation:

20 tablets were weighed, and the average tablet weight was calculated. The powdered form of the tablet, weighing approximately 40 mg of Telmisartan and 20 mg of Cilnidipine, was transferred into 100 ml flask, about 10 ml of Methylene chloride was added, and the mixture was sonicated for 20 minutes with intermediate shaking. After cooling, the volume was made up to 100 ml with methanol and mixed thoroughly. The resulting solution was filtered through 0.45 micron nylon filter paper.

Instrumentation and Chromatographic Conditions

TLC analysis was carried out on aluminium-backed plates (10 x 10cm and 20 x 20 cm) coated with silica gel 60 F254, sourced from Merck. Among the solvent systems evaluated a combination of methylene chloride, toluene and methanol in a 7:2:1 ratio provided the best resolution for Telmisartan and Cilnidipine dissolved in methanol. [7] A 5- microlitre of both reference standard and sample solution was applied to the TLC plates in 6 mm wide bands, 10 mm from the bottom and 11.9 mm apart using CAMAG Linomat 5 sample applicator. The plates were then scanned at 260 nm using CAMAG TLC scanner 4, utilizing Vision CATS software for analysis.

III.METHOD VALIDATION

The HPTLC method developed for the simultaneous analysis of Cilnidipine and Telmisartan was validated in accordance with ICH guidelines. [8], the method underwent validation in terms of precision, linearity accuracy and robustness. [9]

IV.RESULTS AND DISCUSSION

Chromatographic separation studies were executed using standard solutions of Cilnidipine and Telmisartan. Various mobile phase systems were evaluated to achieve the effective separation of Cilnidipine and Telmisartan. After numerous trials, a mobile phase comprising Methylene chloride, Toluene and methanol in a 7:2:1 ratio was chosen. This mobile phase system provided complete peak resolution and distinct baseline separation at 260 nm. Cilnidipine and Telmisartan exhibited Rf values of 0.55 and 0.43 respectively. Fig 3 presents the chromatograms for the

separation of Telmisartan and Cilnidipine in the standard and sample solutions.

Peak #	Name		Area	
		R_F	A	%
1	Telmisartan	0.432	0.00942	36.13
2	Cilnidipine	0.551	0.01666	63.87

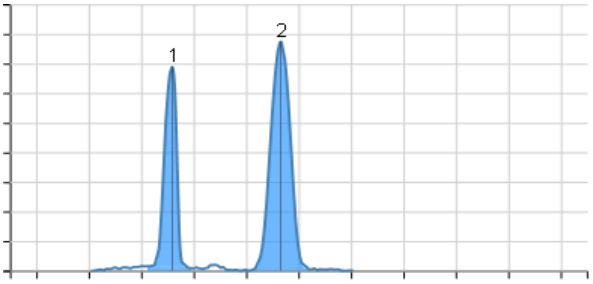

Fig 3: Chromatogram of Separation of Telmisartan and Cilnidipine in standard solution and sample solution.

1. Specificity:

Specificity of the developed method was assessed by determining the analytes in the presence of potential interfering substances, including impurities, degradants and excipients. It was verified by monitoring peak purity at the selected wavelength. [10]

2. Linearity:

The concept of linearity in analytical methods reflects the direct proportionality between the measured response and the analyte concentration within a given range. This is commonly demonstrated by evaluating different analyte concentrations over the intended range and representing the data graphically. [11]

A standard mixture solution of Telmisartan (200 to 600 µg/mL) and Cilnidipine (100 to 300 µg/mL) was spotted onto a chromatographic plate and developed according to the method described in the proposed procedure. The developed plates were subjected to densitometric evaluation at 260 nm in absorbance mode using a CAMG TLC Scanner. The analytical data for Telmisartan and Cilnidipine is represented in Table No. 1 and Table No.2 respectively. The linearity curve for the same represented in Fig.4 and Fig 5 respectively. The three dimensional representation of the linearity curve is shown in Fig. 6

Table No. 1: Analysis performanance data of Telmisartan

	Telmisartan
Linear working range	200 to 600 µg/mL
Slope	0.00001
Intercept	0.00493
Correlation coefficient	0.99325

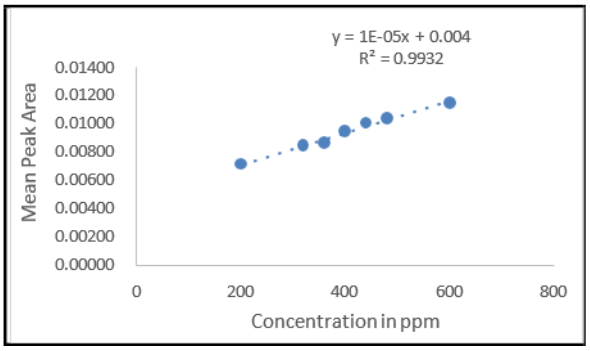

Fig 4: Linearity curve for Telmisartan

Table No. 2: Analysis performanance data of Cilnidipine

	Cilnidipine
Linear working range	100 to 300 µg/mL
Slope	0.00005
Intercept	0.00600
Correlation coefficient	0.99440

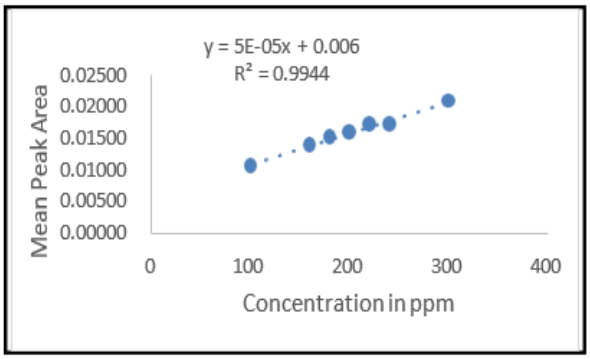

Fig 5: Linearity curve for Cilnidipine

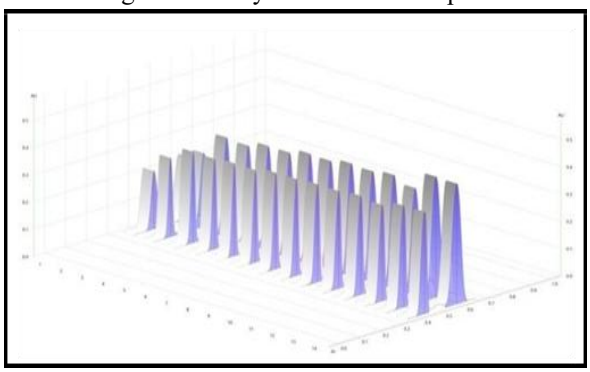

Fig 6: The three dimensional representation of linearity curve

3. Precision

The precision of an analytical method reflects the consistency of individual test results when the method is repeatedly applied to various samples of a homogeneous material. It quantifies the random error in the results and is usually expressed as the coefficient of variation (CV) or Relative standard Deviation (RSD).

Intra and inter day precision

The precision of the method was demonstrated through interday and intraday variation studies. In these studies, six replicate injections of the mixed sample were made, and percentage RSD was calculated. From the data obtained, the developed HPTLC method found to be precise. The mean, standard deviation and relative standard deviation (RSD) of all these parameters were calculated, the results of intraday precision are given in table 3 for Telmisartan and table 4 for Cilnidipine, and the results of interday precision for Telmisartan are tabulated in Table 5 and for Cilnidipine results represented in Table 6.

Table No. 3: Assay Results of Method Precision

Sample	Telmisartan (Assay)
Sample-1	102.4
Sample-2	100.4
Sample-3	100.0
Sample-4	99.8
Sample-5	100.5
Sample-6	100.2
Mean	100.6
STD DEV	0.9296
% RSD	0.9

Table No. 4: Assay Results of Method Precision

Sample	Cilnidipine (Assay)
Sample-1	99.6
Sample-2	98.8
Sample-3	100.1
Sample-4	101.1
Sample-5	100.6
Sample-6	103.5
Mean	100.0
STD DEV	1.6253
% RSD	1.6

Table No. 5: Assay Results of Intermediate Precision for Telmisartan

Sample	Telmisartan (Assay)
Sample-1	99.4
Sample-2	101.1
Sample-3	99.4
Sample-4	100.3
Sample-5	99.6
Sample-6	100.6
Mean	100.0
STD DEV	0.6880
% RSD	0.7

Table No. 6: Assay Results of Intermediate Precision for Cilnidipine

Sample	Cilnidipine (Assay)
Sample-1	100.8
Sample-2	102.3
Sample-3	99.6
Sample-4	101.1
Sample-5	100.5
Sample-6	106.6
Mean	**100.9**
STD DEV	**2.5019**
% RSD	**2.5**

4. Accuracy

Accuracy was established by applying the method to simulated mixtures of drug product components where known amounts of each drug were added, corresponding to 50%, 100% and 150% based on individual label claims for each drug. Put simply, accuracy indicates the percentage of original analytes amount that the assay was able to detect and recover. Mean recoveries for Telmisartan and Cilnidipine from the specific formulations. The findings indicate that the method offers excellent accuracy for the concurrent determination of the drug combination. The results from recovery analysis of Telmisartan and Cilnidipine are given in Table 7 and Table 8 respectively.

Table No. 7: Accuracy Results for Telmisartan

Accuracy level (%)		Telmisartan
50	Mean	99.81
100		99.43

150		100.07
50	SD	0.1
100		0.3
150		0.2
50	% RSD	0.1
100		0.2
150		0.1

Table No. 8: Accuracy Results for Cilnidipine

Accuracy level (%)		Telmisartan
50	Mean	99.53
100		99.68
150		99.15
50	SD	0.3
100		0.05
150		1.1
50	% RSD	0.3
100		0.05
150		1.1
Sample-2		98.8

5. Robustness

The Robustness of the proposed method was evaluated by altering the wavelength and by variation in the mobile phase ratio. The wavelength of the detection was changed by 258 nm and 262 nm and variation of the mobile phase ratio changed by Methylene chloride: toluene: Methanol (7.1:2:0.9 v/v/v and 6.9:2:1.1 v/v/v).At both deliberate changes in the system, there was no significant difference in the peak resolution and Rf factor of Telmisartan and Cilnidipine. The results indicated that the separation of two active substances is achievable under the given conditions using the method developed, which is satisfactory for simultaneous determination of Telmisartan and Cilnidipine in tablet dosage form.

V.CONCLUSION

HPTLC is a versatile and widely used method in various industries. For there several applications, one of which is its unique capability to display results as an image, making it distinct from other chromatographic techniques. Other benefits include its ease of use, affordability, ability to process multiple samples simultaneously, high sample capacity, quick result turnaround and the option for diverse detection approaches.[6] Accelerated analysis with HPTLC aims to quickly analyze large quantities of numerous compounds. The advancement of this field has been fueled by need for analytical support for various drug targets arising from genetic biology, human genetics and genomic research.[7] Moreover, the development has been propelled by demand for analyzing large chemical compound repositories developed via parallel and combinatorial chemistry, coupled with the economic pressure to speed up timeframe for launching new drug candidates.[8] The aim of this study was to evaluate Cilnidipine and Telmisartan using High Performance Thin layer Chromatography (HPTLC) coupled with UV absorbance analysis. In the current study, Rf values for Telmisartan and Cilnidipine were obtained 0.43 and 0.53 respectively. The correlation values for Telmisartan and Cilnidipine were recorded as 0.9932 and 0.9944 respectively. The recovery of drugs was consistently within ideal range of 98% to 102% demonstrating strong accuracy in the assay. Thus, the method presented is of considerable value, with substantial industrial applicability for quality control and in the examination of raw bulk drug and pharmaceutical preparations. [10]

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Conflict of Interest Declaration

The authors affirm that there are no known conflicts of interest associated with this publication.

REFERENCE

[1] Pooja Kadu, Amar Zalte and Vishal Gulecha (2023), "Pharmacokinetic Evaluation of Telmisartan and Cilnidipine Bilayer Tablet in A Rabbit Model." Int J life Sci Pharma Res, Volume 13, No.4 page .87-94

[2] Kyu-Hyun Lee, Hong-Jun Park, Chang-Yong Shin, Chang-Seon Myung(,2015), Pharmaceutical composition comprising Telmisartan and

Cilnidipine", International application published under Patent Cooperation Treaty(PCT).

[3] Rahul Sawant, Sachin Suryawanshi, Mayur Jadhav, Hanmant Barkate, Sumit Bhushan, and Tanmay Rane (2023) "A Prospective, Randomized Open-Label Study for Assessment of Antihypertensive Effect of Telmisartan versus Cilnidipine Using Ambulatory Blood Pressure Monitoring (START ABPM Study)",PubMed, 14(3),211- 220.

[4] Mohsen Imenshahidi, Ali Roohbaksh, Hussein Hosseinzadeh (2024) "Effects of Telmisartan on Metabolic syndrome components; a comprehensive review," Biomedicine and Pharmacotherapy, Volume 171,116169

[5] Farhat JahanShaikh, Meenakshi Patel, Vandana Patel, Ashwini Patel, Gajanan Shinde, Santosh Shelke, Inayat Pathan (2022), "Formulation and optimization of Cilnidipine loaded nanosuspension for the enhancement of solubility, dissolution and bioavailability", Journal of Drug Delivery Science and Technology, Volume 69.

[6] Jun-Hwan Jo, Do-Hyung Lee, Joo-Hui Han, MijiLee,Keun-woo Jang and Chang-seon Myung (2021), "Effects of combination treatment with Cilnidipine and Telmisartan on hypertension, cardiovascular injury, and high blood glucose" Volume 51, pages 337–346.

[7] Anuj Misra, Mohammad Sajid Alam, Pankaj Kumar Mishra (2020), "A comparative study of Cilnidipine and Telmisartan in tablets by high performance thin-layer chromatography with ultraviolet absorption densitometric detection", International Journal of Health and Clinical Research (IJHCR), Volume 3.0 Issue 8.

[8] Q2 (R1), Validation of Analytical Procedures: Text and Methodology. 2006. International Conference on Harmonization, EMEA. Geneva, Switzerland.

[9] Wicharm Janwitavanuchit, Puangkaew Lukkanatinaporn (2014) "Development of HPTLC Method For Determination of Brompheniramine Maleate and Phenylnephrine Hydrochloride Tablets", International Journal of Pharmacy and Pharmaceutical science,6(6), 106-109.

[10] B. Mehta, P. Patel, V. Yadav and K. Detholia, (2020), "Development and Validation of Stability-Indicating HPTLC Method For Estimation of Celecoxib and Amlodipine Besylate from Synthetic Mixture", International Journal of Pharmaceutical Sciences and Research,Vol 12(10); 5476-5485

[11] Manuel Aboal-Somoza, Rosa M.Crujeiras, "Misuse of Linear regression technique in analytical chemistry?" Journal of Chemical Education, Volume 101, Issue1, 1062-1070