JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

Development and validation of analytical methods for estimation of calcium channel blocker

Rohit Kumar Dwivedi, Dr. Praveen Kumar Ashok, Ms. Arati Tamta, Dr. Divyanshi Rastogi
Research Student, Directorate, Associate Professor, Associate Professor
Gyani Inder Singh Institute of Professional Studies, Dehradun, uttarakhand

Abstract

In order to dete\rmine the amounts of cilnidipine and lisinopril dihydrate, the Dual Wavelength Spectroscopic approach that was proposed was successfully utilized. The method that was created was verified in accordance with ICH Q2 (R1) without any deviations, and based on the findings, it was determined that the current method has the potential to be utilized for the routine examination of raw materials as well as in the formulation of pharmaceuticals. According to the findings of the linearity investigation, the suggested approach was able to produce linear results throughout the concentration range of 4–24 and 10–60 µg/mL for lisinopril dihydrate and cilnidipine, respectively. It was determined that the proposed method was accurate based on the findings of the precision data and the low levels of the relative standard deviation (RSD). Both the LOD and LOQ values for cilnidipine and lisinopril dihydrate were determined and established. It was discovered that the recovery for lisinopril dihydrate was 98.82%, and the recovery for cilnidipine was 100.24%. The low values of the relative standard deviation (RSD) demonstrate that the procedure was accurate within the range that was defined. The suggested approach was found to be linear, sensitive, exact, and accurate for the simultaneous estimation of lisinopril dihydrate and cilnidipine in bulk and pharmaceutical formulations. This conclusion was reached as a consequence of the successful completion of the validation research and the findings that were discovered.

Introduction

Because of its high prevalence and important clinical impact, hypertension remains a leading contributor to the risk of cardiovascular disease and death [1,2,3,4]. In 2015, about 1.5 billion adults worldwide had a measured office blood pressure (BP) higher than 140 mmHg systolic or 90 mmHg diastolic [5]. According to a recent study, the number of subjects aged 30–79 years with a prior diagnosis of hypertension doubled from 331 million women and 317 million men in 1990 to 626 million women and 652 million men in 2019, despite a stable age-standardized prevalence worldwide [6]. It has been estimated that a systolic BP ≥140 mmHg explains about 70% of the burden of morbidity and mortality worldwide [7,8,9]. Cardiovascular diseases are

the leading cause of morbidity and mortality worldwide. Hypertension (HTN), despite considerable progress being made in recent decades, remains a major public health problem and the most frequent modifiable risk factor associated with considerable cardiovascular morbidity and mortality. The number of people aged 30–79 years with hypertension has doubled from 1990 to 2019, despite stable global age-standardized prevalence [1]. It might seem that the lowering of the threshold for the definition of hypertension to a systolic blood pressure of ≥130 mmHg and/or diastolic blood pressure of ≥80 mm in the 2017 American College of Cardiology and American Heart Association Guidelines could explain an increase in hypertension prevalence from 18.9 to 43.5% [2,3,4]. However, in the 2018 European Guidelines, and the most recent guidelines of the International Society of Hypertension in 2020, the definition remained unchanged (≥140/≥90 mmHg) [5,6]. Regardless of the difference in definition, the comprehensive worldwide analysis of hypertension prevalence, treatment, and control for the period from 1990 to 2019 has shown a significant increase in the number of people with hypertension [1]. Numerous trials have shown that sustained hypertension causes structural, functional, and neurohumoral abnormalities of the heart, involving the ventricular and atrial myocardium as well as the epicardial and intramural coronary arteries, a disease commonly termed hypertensive heart disease (HHD). However, the published definition and classifications of HHD do not always agree, and cardiology societies and scientists have not yet come to a consensus on the terminology of these morpho-functional alterations, using terms such as “hypertensive heart disease” [7,8,9], “hypertensive cardiomyopathy” [10], and “hypertensive heart failure” [11]

Cardiovascular diseases (CVD) represent the leading cause of death, accounting for one in three deaths in the United States (US) and worldwide [1–3]. One of their most potent risk factors, hypertension (also known as high blood pressure), is a common risk factor for CVD [3, 4]. Approximately 40% of adults aged 25 and over had elevated blood pressure in 2008 [3]. What is more, hypertension is responsible for at least 45% of deaths due to heart diseases and 51% of deaths due to stroke worldwide [3, 4]. In the US alone, the direct medical and indirect expenses from CVDs were estimated at approximately $329 billion in 2013 to 2014 [5]. Effective large-scale interventions to prevent or treat hypertension are therefore urgently needed to reverse this trend. Yet, as new and promising interventions are surfacing every day, the need for rigorous evaluation of these interventions to inform evidence-based policies and clinical practice is ever growing. To this effect, several randomized clinical trials (RCT) have been conducted to evaluate interventions used to prevent hypertension or improve its control [6–8]. However, although RCTs represent the gold standard for evaluating the efficacy (i.e., impact under ideal conditions) of most health interventions because of their *high internal validity* [9, 10], they are not always feasible, appropriate or ethical for the evaluation of certain types of interventions. Furthermore, results from RCTs are not always generalizable to populations or settings of interest due to the highly selected sample and because the intervention is generally conducted under more stringent conditions (*low external validity*) [11]. To evaluate the effectiveness of an intervention (i.e., impact under real conditions) and to increase the uptake and implementation of evidence-based health interventions in the communities of interests, other types of experimental designs have been proposed. One such example is natural and quasi-experiments. The terms “natural experiments” and “quasi-experiments” are sometimes used interchangeably. In this study, and as described by others [12], we will distinguish these two concepts.

MATERIALS AND METHODS

Lisinopril Dihydrate and Cilnidipine)

Preliminary Study

Lisinopril Dihydrate and Cilnidipine) were examined for physical and chemical properties; description, melting point determination and IR identification & results were summarized in chapter.

Simultaneous Estimation of Lisinopril Dihydrate and Cilnidipine in Pharmaceutical Formulation by Dual Wavelength Spectroscopy Method

Method development

Apparatus and Material

UV Spectrophotometer, CP224S analytical balance (Sartorius, Gottingen, Germany). Class 'A' volumetric glassware was used. Lisinopril Dihydrate and Cilnidipine) (Torrent pharmaceutical Ltd) and methanol were used.

Preparation of Stock Solutions

10 mg Lisinopril Dihydrate powder was weighed and transferred to a 100 ml volumetric flask. Then diluted up to 100 ml with methanol to get a concentration of 100 µg/ml. 10 mg Cilnidipine powder was weighed and diluted up to 100 ml with methanol to get a concentration of 100 µg/ml.

Preparation of Calibration Curve for Lisinopril Dihydrate and Cilnidipine

Series A, B, and C as follows were prepared from the standard stock solutions of Lisinopril Dihydrate and Cilnidipine). Series A; Lisinopril dihydrate (4–24 µg/ml) was prepared by diluting appropriate volume from the standard stock solution of Lisinopril Dihydrate with methanol to get the concentration in range of 4–24 µg/ml. Series B; Cilnidipine (10–60 µg/ml) was prepared by diluting appropriate volume from the standard stock solution of Cilnidipine with methanol to get the concentration 10-60 µg/ml. Series C consists of mixture of Lisinopril Dihydrate and Cilnidipine having concentration of Lisinopril dihydrate (4–24 µg/ml) and Cilnidipine (10–60 µg/ml).

Preparation of Sample Solution

Accurately weighed and powdered 20 tablets equivalent to 5 mg of Linsinopril dihydrate and 12.5 mg of cilnidipine and transferred in to a 100 ml volumetric flask. 50 ml methanol was added and sonicated for 20 min. The solution was then filtered through whatmann filter paper and the volume was adjusted up to the mark with methanol. This solution is expected to contain 50 µg/ml Linsinopril dihydrate and 125 µg/ml clinidipine. From his solution 2 ml was taken in to a 10 ml volumetric flask and mark up to the volume with methanol to get a final concentration of Linsinopril dihydrate (10 µg/ml) and cilnidipine (25 µg/ml).

Selection of Wavelength for Determination of Lisinopril Dihydrate and Cilnidipine

The dual wavelength spectroscopic method is used to calculate concentration of analyte in a mixture containing an unwanted components or excipients. For development of the method two wavelengths were selected as described below.

- First wavelength λ_1 (315.5 nm) at which absorbance of was observed and no any interference of Lisinopril dihydrate.
- Second wavelength λ_2 (286.0 nm) was selected where the absorbance of cilnidipine was same as at λ_1, and Lisinopril dihydrate was also give absorbance at this selected wavelength.

Method Validation

Method validation involved various validation parameters; Linearity, Precision, Recovery, LOD and LOQ, Assay of Lisinopril Dihydrate and Cilnidipine). All the validation parameters were successfully determined as per ICH Q2 (R1) and results were reported.

RESULTS AND DISCUSSION

Lisinopril Dihydrate and Cilnidipine:

Preliminary Study

Table Physical Properties of Lisinopril Dihydrate and Cilnidipine

Physical Property	Lisinopril Dihydrate		Cilnidipine	
	Observed	Standard	Observed	Standard
Appearance	Crystalline Powder	Fine Crystalline Powder	White fine Powder	White fine Powder

Melting Point Determination

The melting point of both API was taken and compared with reported melting point, the melting points were found to be in the range of reported melting point.

Table Melting Point Study of Drug Sample

Drug Sample	**Observed Melting Point**	**Reported Melting Point**
Lisinopril Dihydrate	222-230 ˚C	220-240 ˚C
Cilnidipine	272-274 ˚C	273-275 ˚C

IR Identification

IR Spectral Analysis of Lisinopril Dihydrate

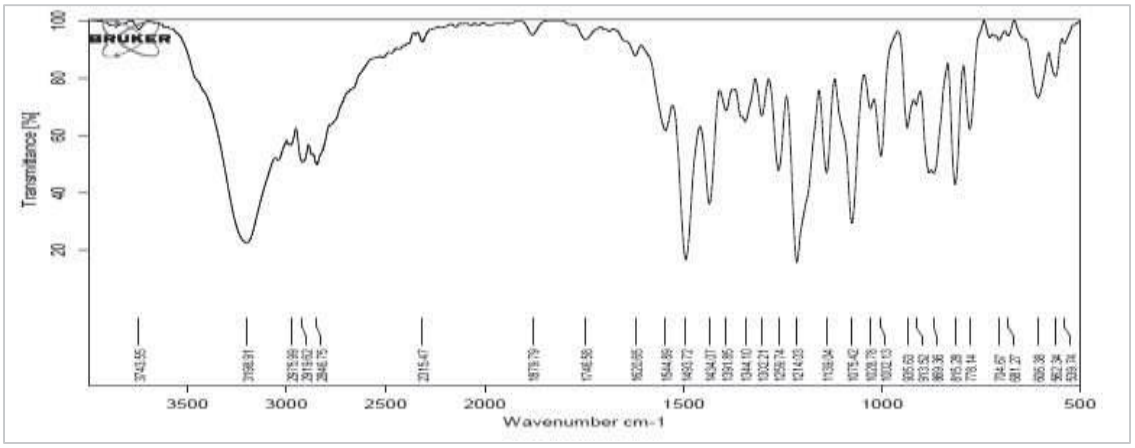

Figure IR Spectra of Lisinopril Dihydrate

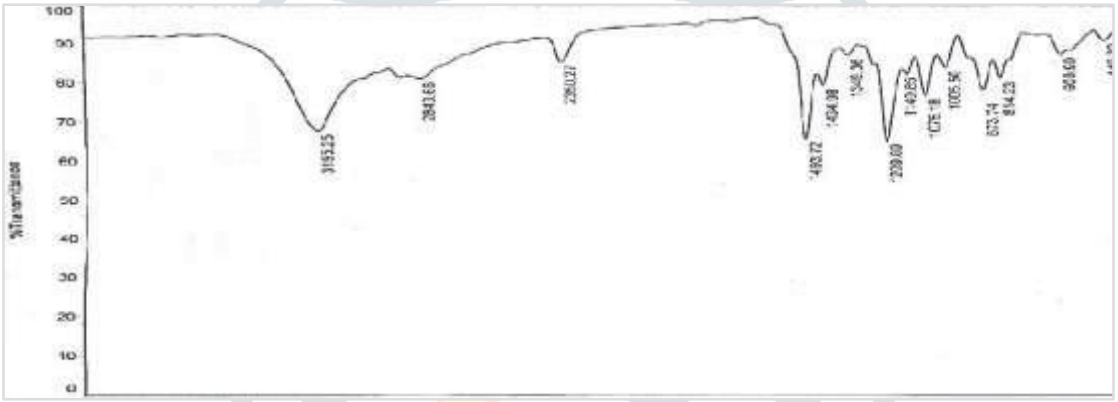

Figure IR Spectra of Lisinopril Dihydrate **Standard**

The IR spectrum of Lisinopril Dihydrate was taken by KBr pellets technique and compared with reference spectrum, the transmittance peaks were found to be identical in API spectra.

Table IR Spectral Interpretation of Lisinopril Dihydrate

Sr. No.	**Observed Frequency (cm^{-1})**	**Mode of Vibration**	**Frequency Range (cm^{-1})**
1.	873.74	Aromatic	800-900
2.	1493.72	N-N Stretching	1350-1500
3.	2843.66	C-H Stretching	2800-2900
4.	3195.25	OH Stretching	3300-3500

IR Spectral Analysis of Cilnidipine

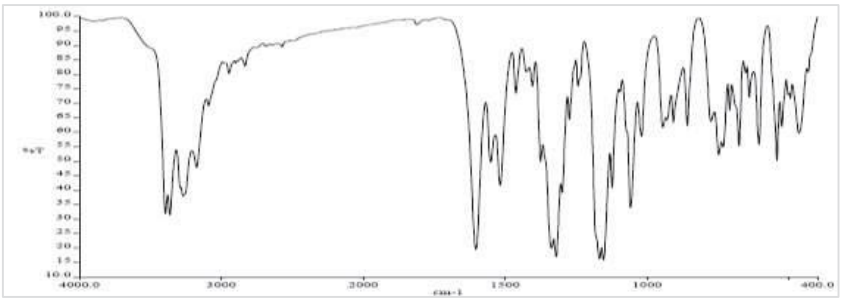

Figure IR Spectra of Cilnidipine

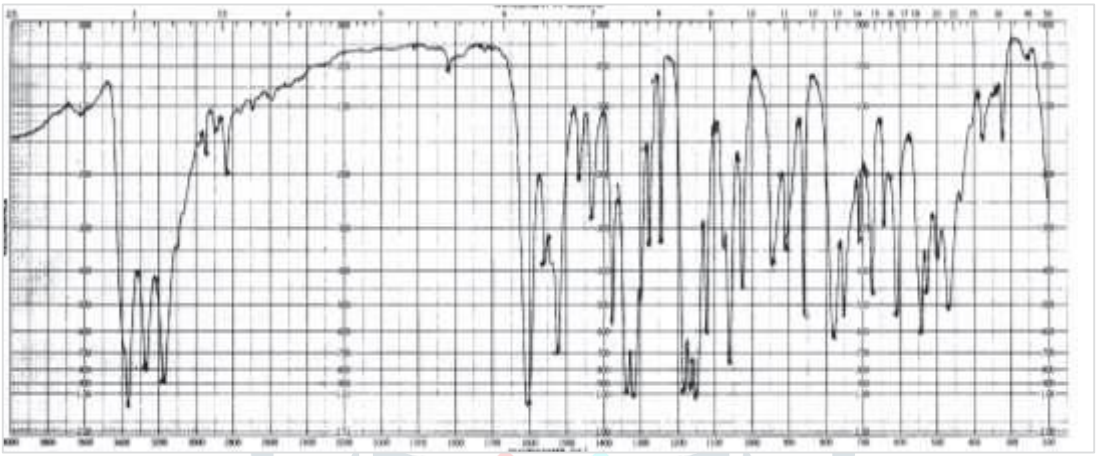

Figure IR Spectra of Cilnidipine **Standard**

The IR spectrum of Cilnidipine was taken by KBr pellets technique and compared with reference spectrum, the transmittance peaks were found to be identical in API spectra.

Table IR Spectral Interpretation of Cilnidipine

Sr. No.	**Observed Frequency (cm^{-1})**	**Mode of Vibration**	**Frequency Range (cm^{-1})**
1.	3471	N-H amine (ring)	3200-3500
2.	1486, 1591	C=C (aromatic)	1405-1550
3.	1339.88	C-N Stretching	1250-1280
4.	2843.66	C-H Stretching	2800-2900
5.	3195.25	OH Stretching	3300-3500
6.	3436	N-H (free)	3350-3500

Simultaneous Estimation of Lisinopril Dihydrate and Cilnidipine **in Pharmaceutical Formulation by Dual Wavelength Spectroscopy Method**

Method Development

For development of the method, two wavelengths were selected as described below.

Figure Overlain Spectra of Lisinopril Dihydrate and Cilnidipine

The difference in absorbance at these two wavelengths (A286.0–A315.5) cancels out the contribution of absorbance of Lisinopril Dihydrate and Cilnidipine in the mixture.

Table Determination of Lisinopril Dihydrate alone and Lisinopril Dihydrate in the presence of cilnidipine

SERIES A				**SERIES C**			
Concentration of mixture (µg/ml)		**Absorbance at 286.0 nm ± S.D (n = 5)**	**% RSD**	**Concentration of mixture (µg/ml)**		**Absorbance at 286.0 nm – 315.5 nm ± S.D (n = 5)**	**% RSD**
Lisinopril Dihydrate	**Cilnidipine**			Lisinopril Dihydrate	**cilnidipine**		
4	0	0.057 ± 0.001	1.75	4	10	0.057 ± 0.001	1.75
8	0	0.109 ± 0.001	0.91	8	20	0.108 ± 0.002	1.92
12	0	0.162 ± 0.003	1.85	12	30	0.163 ± 0.002	1.22
16	0	0.218 ± 0.001	0.45	16	40	0.218 ± 0.003	1.60
20	0	0.259 ± 0.004	1.73	20	50	0.264 ± 0.003	1.32
24	0	0.314 ± 0.005	0.18	24	60	0.323 ± 0.003	1.08

Table Determination of cilnidipine alone and cilnidipine in the presence of Lisinopril Dihydrate

SERIES B				**SERIES C**			
Concentration of mixture (µg/ml)		**Absorbance at 315.5 nm ± S.D (n = 5)**	**% RSD**	**Concentration of mixture (µg/ml)**		**Absorbance at 315.5 nm ± S.D (n = 5)**	**% RSD**
Lisinopril Dihydrate	**Cilnidipine**			Lisinopril Dihydrate	**cilnidipine**		

0	10	0.105 ± 0.001	0.95	4	10	0.097 ± 0.0005	0.59
0	20	0.204 ± 0.0015	1.74	8	20	0.211 ± 0.003	1.44
0	30	0.298 ± 0.0005	0.19	12	30	0.313 ± 0.005	1.75
0	40	0.393 ± 0.003	0.77	16	40	0.404 ± 0.0005	0.14
0	50	0.494 ± 0.004	0.88	20	50	0.496 ± 0.005	1.00
0	60	0.603 ± 0.002	0.33	24	60	0.617 ± 0.003	0.48

Method Validation

Linearity

The linearity range for Lisinopril Dihydrate and cilnidipine were confirmed to be 4–24, and 10–60 µg/mL, respectively. The specificity of the method was established from the standard and sample of Lisinopril Dihydrate and cilnidipine . The correlation coefficient of standard Lisinopril Dihydrate and cilnidipine were 0.9987 and 0.9981 respectively.

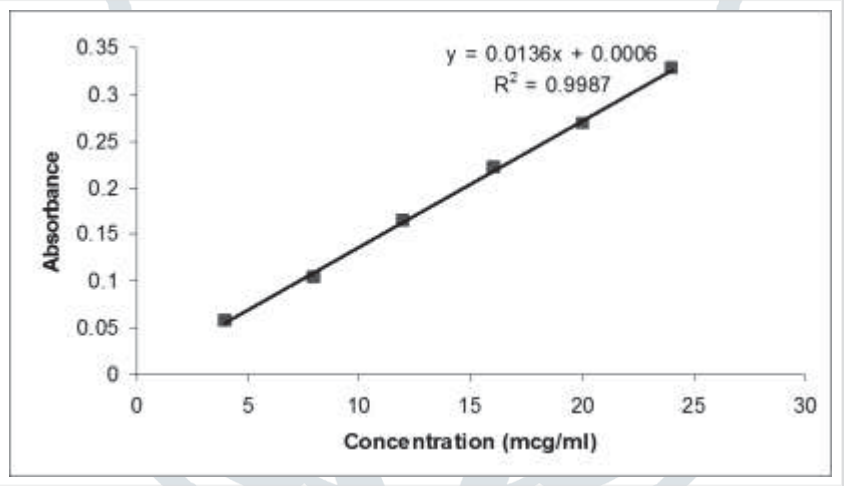

Figure Calibratio curve of cilnidipine

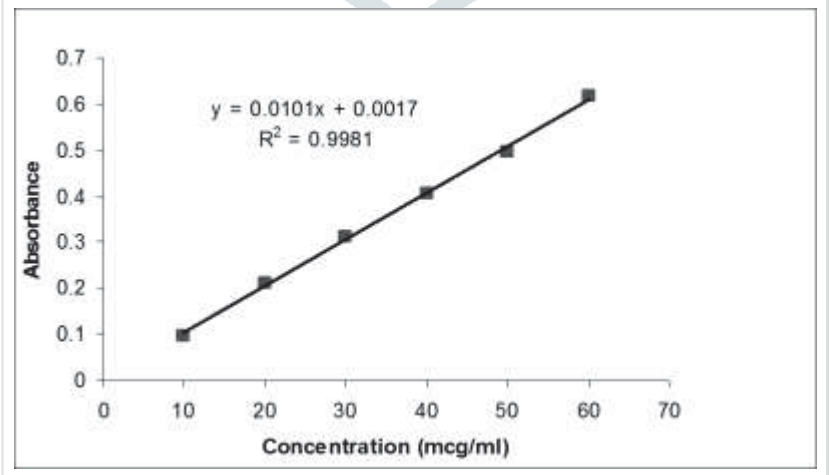

Figure Calibration curve of Precision

RSD was found to be less than 2%, which indicates that the DW Spectroscopic method is repeatable.

Table Precision Data for Lisinopril Dihydrate and cilnidipine

Lisinopril Dihydrate and cilnidipine **(10 : 25 µg/ml)**	**Absorbance at 286.0 nm**	**Absorbance at 315.5 nm**	**Absorbance at 286.0–315.5 nm**
1	0.390	0.255	0.135
2	0.392	0.256	0.136
3	0.390	0.257	0.133
4	0.393	0.255	0.134
5	0.390	0.256	0.138
6	0.388	0.251	0.137
MEAN	0.390	0.255	0.135
S.D.	0.001	0.002	0.001
% RSD	0.450	0.820	1.380

Limit of detection (LOD) & limit of Quantitation (LOQ)

Parameter	Lisinopril Dihydrate	cilnidipine
LOD	0.50 µg/mL	0.60 µg/mL
LOQ	1.5 µg/mL	2.0 µg/mL

Table Regression Analysis Data for DW Spectroscopic method

Parameters	Lisinopril Dihydrate **estimation at 286.0 nm**	**Cilnidipine estimation At 315.5 nm**
Wavelength for measurement	286.0 nm	315.5 nm
Linearity	4–24 µg/ml	10–60 µg/ml
Regression line equation (Y)	Y=0.0136X + 0.0006	Y=0.0101X + 0.0017
Slop (b)	0.0136	0.0101
Intercept (a)	0.0006	0.0017
Correlation coefficient (r)	0.9987	0.9981
%Recovery (n = 5)	98.82%	100.24%

Recovery Study

The RSD values confirmed that the developed DW Spectroscopic method is accurate.

Table Recovery data for the DW Spectroscopic Method

Drug	Level	Amount of sample taken (µg/mL)	Amount of standard spiked (µg/mL)	Amount of recovered (µg/mL)	% Recovery ± %RSD
Lisinopril Dihydrate	I	10	7.5	17.23	98.48 ± 0.041
	II	10	10	19.66	98.30 ± 0.077
	III	10	12.5	22.08	98.16 ± 0.005
Cilnidipine	I	25	18.75	43.89	100.32 ± 0.025
	II	25	25	50.32	100.65 ± 0.028
	III	25	31.25	56.56	100.55 ± 0.028

Analysis of Pharmaceutical Formulation

DW Spectroscopic method was used to determine Lisinopril dihydrate and cilnidipine in tablet dosage forms. There was no any interference of the excipient observed; hence the proposed method is applicable for the routine estimation of Lisinopril dihydrate and cilnidipine in pharmaceutical formulations.

Table Estimation of Lisinopril dihydrate and cilnidipine in Tablet Dosage Form

Formulations (Tablet)	Amount taken (µg/mL)		Amount found (µg/mL)		%Assay ± %RSD	
	Lisinopril dihydrate	cilnidipine	Lisinopril dihydrate	cilnidipine	Lisinopril dihydrate	cilnidipine
Set-1 Set-2	10 10	25 25	9.88 9.88	25.07 25.27	98.82 ± 0.045 98.82 ± 0.045	100.31 ± 0.025 101.10 ± 0.018

Conclusion

The proposed Dual Wavelength Spectroscopic method was used for the estimation of Lisinopril dihydrate and cilnidipine. The developed method was validated as per ICH Q2 (R1) without any deviation and from the results it was concluded that the present method may be used for the routine analysis of the raw materials and in the pharmaceutical formulation. The linearity study was revels that the proposed method was give the linear results in the concentration range of 4–24 and 10–60 µg/mL for Lisinopril dihydrate and cilnidipine, respectively. From the results of precision data and low levels of % RSD, it was concluded that the proposed method was precise. The LOD and LOQ values were established for the Lisinopril dihydrate and cilnidipine. The %recovery for lisinopril dihydrate was found to be 98.82% and for cilnidipine were 100.24% and the low values of %RSD revels that the method was accurate in the established range. From the successful completion of validation study and from the results found, it was concluded that the proposed method was linear, sensitive, precise and accurate for the simultaneous estimation of Lisinopril dihydrate and cilnidipine in bulk and Pharmaceutical formulations.

References

1. Unger T, Borghi C, Charchar F, Khan NA, Poulter NR, Prabhakaran D, et al. 2020 . international society of hypertension global hypertension practice guidelines. *Hypertension.* (2020). 75:1334–57. 10.1161/HYPERTENSIONAHA.120.15026

2. Mills KT, Stefanescu A, He J. The global epidemiology of hypertension. *Nat Rev Nephrol.* (2020) 16:223–37. 10.1038/s41581-019-0244-2

3. Sudharsanan N, Theilmann M, Kirschbaum TK, Manne-Goehler J, Azadnajafabad S, Bovet P, et al. Variation in the proportion of adults in need of blood pressure–lowering medications by hypertension care guideline in low- and middle-income countries. *Circulation.* (2021) 143:991–1001. 10.1161/CIRCULATIONAHA.120.051620

4. Fang J, Chang T, Wang G, Loustalot F. Association between cost-related medication nonadherence and hypertension management among us adults. *Am J Hypert.* (2020) 33:879–86. 10.1093/ajh/hpaa072

5. Parati G, Kjeldsen S, Coca A, Cushman WC, Wang J. Adherence to single-pill versus free-equivalent combination therapy in hypertension. *Hypertension.* (2021) 77:692–705. 10.1161/HYPERTENSIONAHA.120.15781

6. Campana E, Cunha V, Glaveckaite S, Gruev I, Lamirault G, Lehmann E, et al. The use of single-pill combinations as first-line treatment for hypertension: translating guidelines into clinical practice. *J Hypertens.* (2020) 38:2369–77. 10.1097/HJH.0000000000002598

7. Tsioufis K, Kreutz R, Sykara G, van Vugt J, Hassan T. Impact of single-pill combination therapy on adherence, blood pressure control, and clinical outcomes: a rapid evidence assessment of recent literature. *J Hypert.* (2020) 38:1016–28. 10.1097/HJH.0000000000002381

8. Segers P, Rietzschel ER, Chirinos JA. How to measure arterial stiffness in humans. *Arterioscl Throm Vasc Biol.* (2020) 40:1034–43. 10.1161/ATVBAHA.119.313132

9. Kaess BM, Rong J, Larson MG, Hamburg NM, Vita JA, Levy D, et al. Aortic stiffness, blood pressure progression, and incident hypertension. *JAMA.* (2012) 308:875–81. 10.1001/2012.jama.10503

10. Dumor K, Shoemaker-Moyle M, Nistala R, Whaley-Connell A. Arterial stiffness in hypertension: an update. *Curr Hypertens Rep.* (2018) 20:72. 10.1007/s11906-018-0867-x

11. Chirinos JA, Segers P, Hughes T, Townsend R. Large-artery stiffness in health and disease: jacc state-of-the-art review. *J Am Coll Cardiol.* (2019) 74:1237–63. 10.1016/j.jacc.2019.07.012

12. Wilson J, Webb AJS. Systolic blood pressure and longitudinal progression of arterial stiffness: a quantitative meta-analysis. *J Am Heart Assoc.* (2020) 9:e017804–017804. 10.1161/JAHA.120.017804

13. Zanoli L, Briet M, Empana JP, Cunha PG, Mäki-Petäjä KM, Protogerou AD, et al. Vascular consequences of inflammation: a position statement from the esh working group on vascular structure and function and the artery society. *J Hypertens.* (2020) 38:1682–98. 10.1097/HJH.

14. Tocci G, Battistoni A, Passerini J, Musumeci MB, Francia P, Ferrucci A, et al. Calcium channel blockers and hypertension. *J Cardiovas Pharmacol Ther.* (2014) 20:121–30. 10.1177/1074248414555403

15. Kraehling JR, Sessa WC. Contemporary approaches to modulating the nitric oxide–cgmp pathway in cardiovascular disease. *Circ Res.* (2017) 120:1174–82. 10.1161/CIRCRESAHA.117.303776

16. Garcia V, Sessa WC. Endothelial nos: perspective and recent developments. *Br J Pharmacol.* (2019) 176:189–96. 10.1111/bph.14522

17. Farah C, Michel LYM, Balligand JL. Nitric oxide signalling in cardiovascular health and disease. *Nat Rev Cardiol.* (2018) 15:292–316. 10.1038/nrcardio.2017.224

18. Sanyour HJ, Li N, Rickel AP, Childs JD, Kinser CN, Hong Z. Membrane cholesterol and substrate stiffness co-ordinate to induce the remodelling of the cytoskeleton and the alteration in the biomechanics of vascular smooth muscle cells. *Cardiovasc Res.* (2019) 115:1369–80. 10.1093/cvr/cvy276

19. Chattopadhyay A, Kwartler CS, Kaw K, Li Y, Kaw A, Chen J, et al. Cholesterol-induced phenotypic modulation of smooth muscle cells to macrophage/fibroblast-like cells is driven by an unfolded protein response. *Arterioscler Thromb Vasc Biol.* (2021) 41:302–16. 10.1161/ATVBAHA.120.315164

20. Yap C, Mieremet A, de Vries CJM, Micha D, de Waard V. Six shades of vascular smooth muscle cells illuminated by klf4 (krüppel-like factor 4). *Arterioscler Thromb Vasc Biol.* (2021) 41:2693–707. 10.1161/ATVBAHA.121.316600

21. Chandran RR, Xie Y, Gallardo-Vara E, Adams T, Garcia-Milian R, Kabir I, et al. Distinct roles of klf4 in mesenchymal cell subtypes during lung fibrogenesis. *Nat Commun.* (2021) 12:7179. 10.1038/s41467-021-27499-8

22. Sun D, Ding D, Li Q, Xie M, Xu Y, Liu X. The preventive and therapeutic effects of aav1-klf4-shrna in cigarette smoke-induced pulmonary hypertension. *J Cell Mol Med.* (2021) 25:1238–51. 10.1111/jcmm.16194

23. Sun D, Li Q, Ding D, Li X, Xie M, Xu Y, et al. Role of krüppel-like factor 4 in cigarette smoke-induced pulmonary vascular remodeling. *Am J Transl Res.* (2018) 10:581–91.

24. Roush GC, Sica DA. Diuretics for hypertension: a review and update. *Am J Hypert.* (2016) 29:1130–7. 10.1093/ajh/hpw030

25. Morris RC, Schmidlin O, Sebastian A, Tanaka M, Kurtz TW. Vasodysfunction that involves renal vasodysfunction, not abnormally increased renal retention of sodium, accounts for the initiation of salt-induced hypertension. *Circulation.* (2016) 133:881–93. 10.1161/CIRCULATIONAHA.115.017923

26. Kawarazaki W, Fujita T. Kidney and epigenetic mechanisms of salt-sensitive hypertension. *Nat Rev Nephrol.* (2021) 17:350–63. 10.1038/s41581-021-00399-2

27. Ellison DH, Welling P. Insights into salt handling and blood pressure. *New England J Med.* (2021) 385:1981–93. 10.1056/NEJMra2030212

28. Terker AS, Zhang C, McCormick JA, Lazelle RA, Zhang C, Meermeier NP, et al. Potassium modulates electrolyte balance and blood pressure through effects on distal cell voltage and chloride. *Cell Metab.* (2015) 21:39–50. 10.1016/j.cmet.2014.12.006

29. Cuevas CA, Su XT, Wang MX, Terker AS, Lin DH, McCormick JA, et al. Potassium sensing by renal distal tubules requires kir4.1. *J Am Soc Nephrol.* (2017) 28:1814–25. 10.1681/ASN.2016090935

30. Marques FZ, Mackay CR, Kaye DM. Beyond gut feelings: how the gut microbiota regulates blood pressure. *Nat Rev Cardiol.* (2018) 15:20–32. 10.1038/nrcardio.2017.120

31. Muralitharan RR, Jama HA, Xie L, Peh A, Snelson M, Marques FZ. Microbial peer pressure. *Hypertension.* (2020) 76:1674–87. 10.1161/HYPERTENSIONAHA.120.14473

32. Yan X, Jin J, Su X, Yin X, Gao J, Wang X, et al. Intestinal flora modulates blood pressure by regulating the synthesis of intestinal-derived corticosterone in high salt-induced hypertension. *Circ Res.* (2020) 126:839–53. 10.1161/CIRCRESAHA.119.316394

33. Ferguson JF, Aden LA, Barbaro NR, Van Beusecum JP, Xiao L, Simmons AJ, et al. High dietary salt-induced dendritic cell activation underlies microbial dysbiosis-associated hypertension. *JCI Insight.* (2019) 5:e126241. 10.1172/jci.insight.126241

34. Wilck N, Matus MG, Kearney SM, Olesen SW, Forslund K, Bartolomaeus H, et al. Salt-responsive gut commensal modulates th17 axis and disease. *Nature.* (2017) 551:585–9. 10.1038/nature24628

35. Marques FZ, Nelson E, Chu P-Y, Horlock D, Fiedler A, Ziemann M, et al. High-fiber diet and acetate supplementation change the gut microbiota and prevent the development of hypertension and heart failure in hypertensive mice. *Circulation.* (2017) 135:964–77. 10.1161/CIRCULATIONAHA.116.024545