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Quantitative estimation of cilnidipine and valsartan in rat plasma by RP-HPLC: its pharmacokinetic application

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Abstract

Background: Cilnidipine (CLD) and valsartan (VAL) are antihypertensive agents used in the treatment of hypertension. So, pharmacokinetic study of CLD and VAL in rat plasma was carried out using chromatographic method. The chromatographic separation was performed on the Inertsil ODS column, using mobile phase methanol: water 85:15 v/v (pH 3.0) at the flow rate of 1.1 mL/min., detected at 254 nm.

Result: Cilnidipine (CLD) (1 mg/kg) and valsartan (VAL) (1 mg/kg) was administered orally in rats, and blood samples were collected at time intervals of 0, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, and 24 h after dosing. The retention time of plasma, CLD, and VAL was found to be 2.7, 6.6, and 4.3 min, respectively. The result was validated statistically and by recovery studies. Linearity was acceptable in the range of 1–5 and 8–40 μg/mL for CLD and VAL, respectively. Maximal concentration (C_{max}) of CLD and VAL was observed to be 338 ± 13.85 and 1282.21 ± 39.23 (ng/mL). The half-life of CLD and VAL was found to be 1.08 ± 0.21 and 1.43 ± 0.12 h, respectively.

Conclusion: The present method was successfully applied to the pharmacokinetic study of cilnidipine (CLD) and valsartan (VAL) in rat plasma after oral administration.

Keywords: Cilnidipine, Valsartan, RP-HPLC, Rat plasma, T_{max}, C_{max}

Background

Hypertension is one of the risk factors for cardiovascular diseases, including ischemic and hemorrhagic stroke, dementia, heart failure, vision loss, and kidney failure. Sign of multiple underlying physiological abnormalities in elevated vital signs. Cilnidipine (CLD, Fig. 1a) is a unique calcium channel blocker used for hypertension which inhibits sympathetic voltage channels additionally to vascular dihydropyridine channel. Intracellular calcium influx is prevented by cilnidipine leading to vasodilatation. Cilnidipine possesses superior selectivity for vascular smooth muscle cells [1, 2]. Valsartan (VAL, Fig. 1b) belongs to a category of medicine called angiotensin receptor blockers (ARBs). It works by relaxing blood vessels so that blood can flow more easily. Valsartan blocks the vasoconstrictor and aldosterone-secreting effects of angiotensin II by selectively blocking the binding of angiotensin II to the AT1 receptor in many tissues, like vascular smooth muscle and therefore the adrenal gland [3, 4] just in case with treatment of hypertension target blood pressures can't be achieved enough by use of one antihypertensive agent, and thus combination therapy is used having a different mechanism of action. Additionally, there are many patients with hypertension who take other medicines concomitantly with antihypertensive agents [3, 5–7]. The literature survey of CLD and VAL revealed various methods are used for analysis of CLD alone and together with other antihypertensive agents such as LC-MS [8, 9], UPLC [10], and RP-HPLC [11], while for VAL alone or together with other antihypertensive agents, methods like UPLC

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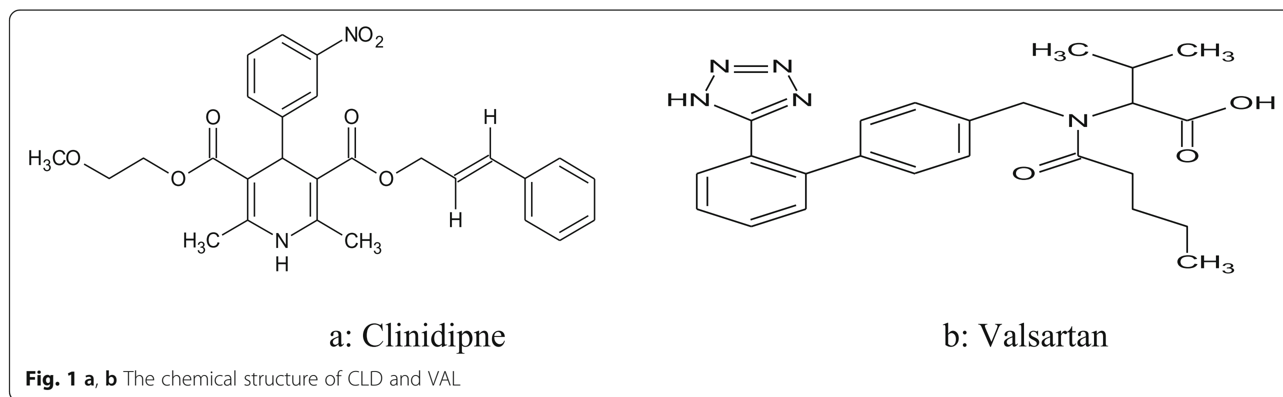

Fig. 1 a, b The chemical structure of CLD and VAL

[12–14], stability indicating assay method by HPLC-DAD [15], LC-MS/MS [16–19], and LC-florescence detector [20] were reported. The tactic was developed to offer an easy, reliable, and validated bioanalytical method for the pharmacokinetic study of CLD and VAL in synthetic mixture. In the present research work, a successful attempt was made for determination of cilnidipine and valsartan in pharmaceutical tablet dosage form by reversed-phase high-performance liquid chromatography (RP-HPLC) in rat plasma. The method was developed by experimentation, based on literature survey and ascertained by statistical parameter of sampling. The simplicity, rapidity, and reproducibility of the proposed method completely fulfilled the objective of this research work. The developed method was applied successfully for pharmacokinetic studies of cilnidipine and valsartan in rats. The applicability of method suggests its further application for bioequivalence, bioavailability, and drug interaction studies. A simple and inexpensive liquid-liquid extraction procedure and an isocratic chromatography condition using a reversed phase column provided an assay well suited for real-time analyses. The developed RP-HPLC method has many advantages such as simple, accurate, sensitive, and precise for quality control and routine analysis of CLD and VAL combination. The method was compared with the previous pharmacokinetic method developed by Lee et al. [7] and located to be reliable.

Method

Chemicals and reagents

Cilnidipine and valsartan gift samples were obtained from J. B. Chemicals and Pharmaceuticals, Hyderabad, India, and Lupin Pharmaceutical Ltd., India, respectively. The purity of CLD is 99% and VAL is 98–102%. HPLC-grade acetonitrile and methanol were procured from Merck (Mumbai, India). Analytical reagent-grade (AR) triethylamine and orthophosphoric acid were also procured from SD fine chemicals (Mumbai, India).

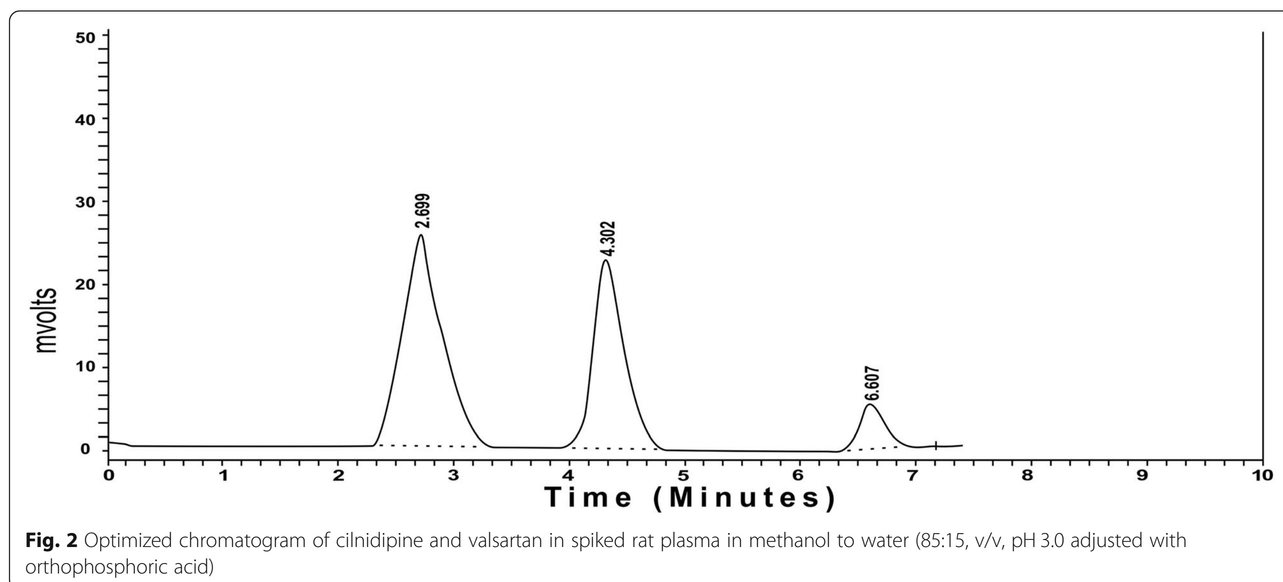

Fig. 2 Optimized chromatogram of cilnidipine and valsartan in spiked rat plasma in methanol to water (85:15, v/v, pH 3.0 adjusted with orthophosphoric acid)

Table 1 The calibration curves for cilnidipine and valsartan

Parameters	CLD	VAL
Slope	110.1	36.92
Y-intercept	0.74	− 8.916
Correlation coefficient	0.999	0.999
Regression equation	$y = 110.1 \times + 0.74$	$y = 36.92 \times - 8.916$
Linearity range	1–5 μg/mL	8–40 μg/mL
LOD	0.023 μg/mL	0.069 μg/mL
LOQ	0.078 μg/mL	0.235 μg/mL

Table 3 Recovery study

Drug	Amount took (μg/mL)	Amount added (μg/mL)	Amount found (μg/mL)	% Recovery
CLD	2.0	1.6	1.582 ± 0.03	98.87 ± 2.08
		2.0	2.008 ± 0.05	100.4 ± 1.58
		2.4	2.396 ± 0.03	99.83 ± 1.54
VAL	16	12.8	12.62 ± 0.26	98.59 ± 2.07
		16	16.02 ± 0.04	100.12 ± 0.29
		19.2	19.16 ± 0.07	99.79 ± 0.40

Instrumentation

HPLC (Waters 600 controllers) instrument equipped with a model code 6CE In-Line Degasser Af, reciprocating pump, Rheodyne 7725i Manual Injector with a 20-μl fixed loop and HPLC syringe of 100 μl, using a UV-visible detector, was used for separation and quantitation.

Chromatographic condition

Chromatographic separation was achieved on RP-18, Inertsil ODS column, (250 mm × 4.6 mm × 5 μ), particle size 5 μm. The mobile phase used for separation of analytes was methanol to water, adjusted at pH 3.0 with orthophosphoric acid in the ratio 85:15 (v/v) used throughout the analysis. The flow rate of the mobile phase was 1.1 mL/min, using injection volume 20 μl of analyte and detected at 254 nm, as shown in Fig. 2.

Preparation standard solutions

The standard calibration curve was carried out by preparing primary stock solutions using QC samples which were weighed separately and prepared for a concentration of cilnidipine (1 mg/mL) and valsartan (1 mg/mL) using methanol as solvent. Working standard solutions were prepared by transferring different volumes of 0.1–0.5 mL stock of CLD and 0.8–4 mL stock of VAL using a mixture of methanol and water pH 3.0 (85:15 v/v) as a diluent.

Preparation of CC standards and QC samples

Working standard solutions of 1–5 ppm of CLD and 8–40 ppm of VAL were prepared from the stock solution. Volumes of 20 μl of working standard solution were added to 500 μl of plasma to obtain drug concentration levels of 1–5 μg/mL and 8–40 μg/mL for cilnidipine and valsartan, respectively. Quality control samples were prepared separately and pooled at three different concentration levels of 1, 3, and 5 μg/mL and 8, 24, and 40 μg/mL for cilnidipine and valsartan as low, medium, and high, respectively.

Table 2 Mean value of system suitability study

Parameters	CLD	VAL
HETP	0.1024	0.2372
Retention time	6.6 ± 0.01	4.3 ± 0.02
Tailing factor	1.37 ± 0.02	1.45 ± 0.02
Resolution	13.66 ± 0.15	9.36 ± 0.14

Preparation of plasma sample

Before processing the stored plasma samples, they were allowed to soften at room temperature. A liquid-liquid extraction procedure was used for the extraction of drugs from plasma. ACN is used as an extracting solvent. Plasma was centrifuged at 4000–4500 rpm. For 10 min, an aliquot (0.5 mL) was pipetted into a 10-mL polypropylene tube and acetonitrile (2.0 mL) was added. The mixture was vortex mixed briefly and after standing for 5 min at room temperature the mixture was centrifuged at 7000–7500 rpm for 10 min. The supernatant was carefully transferred into vials filtered through a syringe filter 0.2 μm and then injected into the HPLC system.

Method validation

The proposed analytical method was validated according to US FDA guidelines [21]. The assay was validated for specificity, linearity, accuracy, and precision. Six different lots of rat plasma samples were taken and the specificity of the method was determined. The interfering substances or background noises responses at the retention time of cilnidipine and valsartan were acceptable less than 20% of the response of the lower limit of quantification (LLOQ). Linearity was performed in the concentration range for cilnidipine and valsartan from 1000 to 5000 ng/mL and 8000 to 40,000 ng/mL, respectively. For

Table 4 Intraday precision for cilnidipine

Statistical data	CLD	VAL
Mean	100.03	99.60
S.D.	0.56	0.076
%R.S.D.	0.569	0.0764

Table 5 Intermediate precision

Statistical data	CLD		VAL	
	Day to day	Analyst to analyst	Day to day	Analyst to analyst
Mean	99.82	99.25	99.75	99.56
S.D.	0.06	0.268	0.12	0.20
%R.S.D.	0.0656	0.271	0.126	0.208

the determination of linearity, standard calibration curves containing five points were plotted and checked. The volume of biological matrix precision was carried out using five independent sample preparations of a single lot of bulk drug and formulation. Minimum three concentrations in the range of excepted study were recommended. The precision is determined at each concentration level which does not exceed 15% of the coefficient of variance (CV) except for the LLOQ, where it should not exceed 20% of the CV.

Result

Selectivity

Selectivity is the ability of an analytical method to differentiate and quantify the analyte in the presence of other component in the sample. Analysis of blank samples of the appropriate biological matrix of six samples. Each blank sample should be tested for interference and selectivity should be ensured at the LLOQ. If the method is intended to quantify, more than one analyte should be tested to ensure that there is no interference. Six individual rat blank plasma samples were analyzed for investigation of selectivity of the method. Each blank sample was tested for interference using the present analytical method and it was compared with spiked samples whose concentration of the analyte was at the LLOQ.

Linearity

The linearity was performed by making appropriate working stock solutions with the mobile phase to obtain concentration ranging from 1 to 5 μg/mL of cilnidipine and 8 to 40 μg/mL of valsartan. The calibration curves were plotted with observed peak areas against concentration followed by the determination of regression equations and calculation of the correlation coefficients. Limit of detection and quantification of CLD and VAL were found to be 0.023 μg/mL and 0.069 μg/mL, and 0.078 μg/mL and 0.235 μg/mL, respectively. The result is shown in Table 1.

System suitability study

System suitability parameters such as retention time, HETP, resolution, and peak tailing are determined. The results obtained are shown in Table 2.

Recovery study

The recovery study was performed by comparing the analytical result for extracted samples at three different concentrations (low, medium, and high) with an unextracted standard that represents 100% recovery, as shown in Table 3.

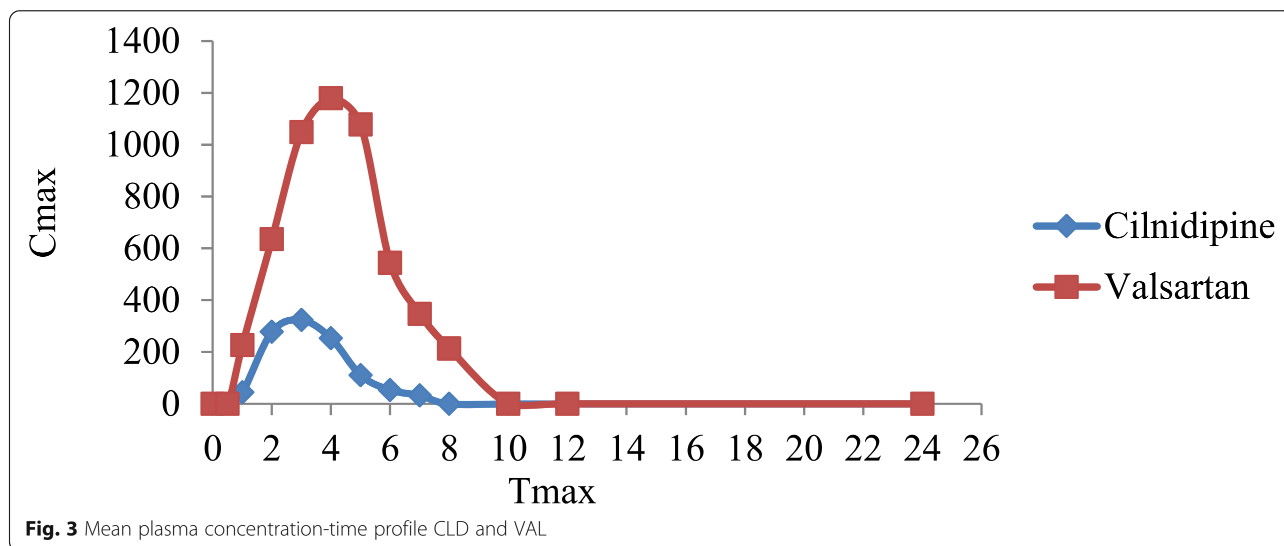

Fig. 3 Mean plasma concentration-time profile CLD and VAL

Table 6 Pharmacokinetic parameters

Parameters	CLD	VAL
C_{max} (ng/mL)	338 ± 13.85	1282.21 ± 39.23
T_{max} (h)	3.0 ± 0.81	4.0 ± 0.89
AUC_{last} (ng/mL h)	1069.04 ± 36.93	5132.8 ± 76.76
AUC_{extra} (ng/mL h)	51.94 ± 22.28	452.19 ± 84.32
$AUC_{0-\infty}$ (ng/mL h)	1117.84 ± 54.90	5584.89 ± 125.7
Half-life (h)	1.08 ± 0.21	1.43 ± 0.12

Precision

Repeatability

The repeatability was performed for three replicate at five concentrations in linearity range 1–5 μg/mL for cilnidipine and 8–40 μg/mL for valsartan. Results of repeatability for cilnidipine and valsartan are reported in Table 4.

Intermediate precision

It was performed within laboratory variation on a different day in three replicates at three different concentrations. A result of day to day variation and analyst to analyst variation was performed. Cilnidipine and valsartan are reported in Table 5.

Robustness

The capacity of a method to remain unafected by small delibrate varitations in method parameters, the RSD of peak areas of Cilnidipine and Valsartan were found to be well within the acceptable limit of 2%. The tailing factor for both the peaks was found to be (Symmetrical) < 1.5.

LOD and LOQ

The developed method has calculated the limit of detection and quantification based on the standard deviation response and slope of the linearity curve.

Plasma estimation of cilnidipine and valsartan in rat plasma

The plasma concentrations of cilnidipine and valsartan vs time are shown in Fig. 3. The C_{max} and T_{max} after oral administration of cilnidipine and valsartan were 338 ± 13.85 and 1282.21 ± 39 ng/ml, 3 and 6 h, respectively. The biological half-life ($t_{1/2}$) of cilnidipine and valsartan was 1.08 ± 0.21 and 1.43 ± 0.12 h, respectively. The result is shown in Table 6.

Discussion

Pharmacokinetic applications

The developed method has been successfully applied to quantify cilnidipine and valsartan concentrations in rat plasma [23–33]. Six healthy rats (200–400 g) were used for conducting in vivo studies. After an initial period of acclimatization for 1 week to laboratory conditions, the rats were randomly selected and administered the dose. In this study, a single dose was designed as, one-way cross over study with a washout period of 14 days. The protocol followed in experimental was under Animal Ethical Guidelines (reg. No.-1153/PO/Re/S/08/CPCSEA) for investigations in laboratory animals and approved by the Animal Ethics Committee. Animals have fasted for 2 h after dose administration. A synthetic mixture of CLD (1 mg/kg) and VAL (1 mg/kg) dose was administered orally. A total of 13 blood collection time points including the pre-dose sample (0, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, and 24 h). Vacutainers containing K_2EDTA as an anticoagulant was used for collecting blood samples separately. The obtained plasma sample was stored until analysis. The sample was separated from plasma by using a cooling centrifuge at 4000–7500 rpm for CLD and VAL. After analysis, the pharmacokinetic parameters were computed using Kinetica software version 5.0.

In vivo data analysis

To determine the various pharmacokinetic parameters, the plasma concentration of cilnidipine and valsartan at different time intervals was subjected to quantitative analysis shown in Fig. 3. Pharmacokinetic parameters like maximum plasma concentration (C_{max}), time to reach maximum concentration (T_{max}), and area under the plasma concentration-time curve ($AUC_{0\to\infty}$) was calculated using the Kinetica software version 5.0 [22].

Comparative study of the developed method

The developed method compared with the previously reported method by Lee et al., [7] the C_{max} observed by the previous method were 8.3 ± 4.5 and 4612.6 ± 2302.4

Table 7 Comparative study of pharmacokinetic parameters

Parameters	Developed pharmacokinetic method		Previous pharmacokinetic method [7]	
	CLD	VAL	CLD	VAL
C_{max} (ng/mL)	338 ± 13.85	1282.21 ± 39.23	8.3 ± 4.5	4612.6 ± 2302.4
T_{max} (h)	3.0 ± 0.81	4.0 ± 0.89	2.5	4
AUC_{last} (ng h/mL)	1069.04 ± 36.93	5132.8 ± 76.76	38.7 ± 19.7	25,864.5 ± 12,611
$AUC_{0-\infty}$ (ng h/mL)	1117.84 ± 54.90	5584.89 ± 125.7	43.1 ± 22.5	27,498.1 ± 13,676

ng/mL, T_{max} were found to be 2.5 and 4 h, AUC_{last} were reported as 38.7 ± 19.7 and 25,864.5 ± 12,611 ng/mL, and AUC_{∞} were reported as 43.1 ± 22.5 and 27,498.1 ± 13,676 ng × h/mL for CLD and VAL, respectively [7].

In the developed method, C_{max} were found to be 338 ± 13.85 and 1282.21 ± 39.23 ng/mL, T_{max} were found to be 3.0 ± 0.81 and 4.0 ± 0.89 h, AUC_{last} were 1069.04 ± 36.93 and 5132.8 ± 76.76 ng × h/mL, and AUC_{∞} were 1117.84 ± 54.90 and 5584.89 ± 125.7 ng × h/mL for CLD and VAL, respectively. The result is shown in Table 7.

Conclusion

A simple, accurate, precise, and rapid UV-liquid chromatographic method for simultaneous estimation of cilnidipine and valsartan in rat plasma is validated as per the USFDA guidelines. The method is found to be suitable for the study of pharmacokinetic application in rats. The cost-effectiveness, simplicity of the assay, and usage of liquid-liquid extraction make it an attractive procedure in high-throughput bioanalysis of cilnidipine and valsartan. From the results of all the validation parameters, we can conclude that the developed method can be useful routinely for bioavailability and bioequivalence studies and therapeutic drug monitoring with the desired precision and accuracy.

Abbreviations

RP-HPLC: Reversed-phase high-performance liquid chromatography; ODS: Octa-decyl silane; v/v: Volume by volume; C_{max}: Concentration maxima; T_{max}: Therapeutic maxima; CLD: Cilnidipine; VAL: Valsartan; CV: Coefficient of variance; LOD: Limit of detection; LOQ: Limit of quantitation; DAD: Diode array detector

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Authors' contributions

RNK was design and optimized method. The manuscript was drafted by RNK. The method was performed and validated by SSY. AGM have contributing in grammatically molding and writing of manuscript and gives their scientific suggestion. All authors have read and approved the manuscript.

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Availability of data and materials

All data and materials are available upon request.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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References

1. Chandra SK, Ramesh G (2013) The fourth-generation calcium channel blocker cilnidipine. Ind. Heart J 65:691–695. https://doi.org/10.1016/j.ihj.2013.11.001
2. Yoshimoto R, Dohmoto H, Yamala K, Goto A (1991) Prolonged inhibition of vascular contraction and calcium influx by the novel 1, 4 dihydropyridine calcium antagonist cilnidipine (FRC-8653). Japan J Pharmacol I56:225–229
3. Neil O (2006) The Merck index an encyclopedia of chemical and biological, vol 48, 14th edn. Merck & Co. Inc, p 379
4. Nadeem S, Asif H, Lakshita C, Shamsher A, Moloy M, Parminder SB (2011) Pharmacological and pharmaceutical profile of valsartan: a review. J Appl Pharm Sci 01(04):12–19
5. Goodman and Gilman's (2001) The pharmacology basis of therapeutics. Medical publishing Division, New York, pp 249–260
6. Shinji T, Denan J, Shizuka A, Kazumi N, Mizuo M (2013) Powerful vascular protection by combining cilnidipine with valsartan in stroke-prone, spontaneously hypertensive rats. Hypert Rese 36:342–348
7. Jieon L, Howard L, Kyungho J, Kyoung SL, Dongseong S, Kyung S-Y (2014) Evaluation of the pharmacokinetic and pharmacodynamic drug interactions between cilnidipine and valsartan, in healthy volunteers. Drug Design Devel Ther 8:1781–1788
8. Heon WL, Ji-Hyung S, Hyun-Su L, Seo-Young J, Young-WC, Kyung-Tae L (2008) Development of a liquid chromatography/negative-ion electrospray tandem mass spectrometry assay for the determination of cilnidipine in human plasma and its application to a bioequivalence study. J Chromatogr B 862:246–251
9. Xianhua Z, Suodi Z, Rongsheng Z, Jin O, Li X, Willy RG (2007) Determination of cilnidipine, a new calcium antagonist, in human plasma using high-performance liquid chromatography with tandem mass spectrometric. Anal Chim Acta 600:142–146
10. Alagar R, Ashwini David B, Rao KN, Selva K (2015) A new analytical method development and validation for the estimation of olmesartan medoxomil and cilnidipine in its pharmaceutical dosage form by UPLC as per ICH guidelines D2. Asian J Rese Bio Pharm Sci 3(2):78–86
11. Amit SM, Manjusha ND, Sanjay DS (2014) Development and validation of an analytical method for simultaneous estimation of cilnidipine and olmesartan medoxomil in bulk and tablet dosage form by RP-HPLC. Int J Pharm Pharm Sci 7(6):508–511
12. Krishnaiah CH, Reddy RA, Ramesh K, Mukkanti K (2015) Stability-indicating UPLC method for determination of valsartan and their degradation products in active pharmaceutical ingredient and pharmaceutical dosage forms. J Pharm Biomed Anal 53:483–489
13. Jirí V (2015) A new, rapid, stability-indicating UPLC method for separation and determination of impurities in amlodipine besylate, valsartan, and hydrochlorothiazide in their combined tablet dosage form. J Pharm Biomed Anal 109:36–44
14. Vishal G, Rajeshwari R, Ranjeet P, Dash MN (2013) Simultaneous quantification of aliskiren, valsartan and sitagliptin by LC with fluorescence detection: evidence of pharmacokinetic interaction in rats. Chromatographia 76(9-10):515–521
15. Rasha A, Shaalan TS, Belal FA, El Y, Sohila M (2013) Validated stability-indicating HPLC-DAD method of analysis for the antihypertensive triple mixture of amlodipine besylate, valsartan and hydrochlorothiazide in their tablets. Arab J Chem 10(1):S1381–S1394
16. Hiten JS, Naresh B, Kataria GS, Chagan NP (2009) Simultaneous LC-MS-MS analysis of valsartan and hydrochlorothiazide in human plasma. Chromatographia 69(9-10):1055–1060
17. Nozomu K, Hiroto K (2007) Development and validation of a method for the quantitative determination of valsartan in human plasma by liquid chromatography-tandem mass spectrometry. J Pharm Biomed Anal 43:1769–1774

18. Senthamil SP, Veeran GK, Mandal U, Sam-Solomon WD, Pal TK (2007) Simultaneous determination of fixed-dose combination of nebivolol and valsartan in human plasma by liquid chromatographic-tandem mass spectrometry and its application to a pharmacokinetic study. J Chromatogr B 858(1-2):143–150
19. Shankar GG, Pragney D, Madhusudana K, Ramakrishna S (2014) Simultaneous determination of amlodipine, valsartan, and hydrochlorothiazide by LC–ESI-MS/MS and its application to pharmacokinetics in rats. J of Pharm Ana 6(4):399–406
20. del RB M (2009) Determination of losartan, Telmisartan, and valsartan by direct injection of human urine into a column-switching liquid chromatographic system with fluorescence detection. J Pharm Biomed Anal 50:194–199
21. U. S .Department of Health and Human Services (2013) Food and Drug Administration: guidance for industry, bioanalytical method validation, September
22. Eboka MB, Sabi M, Janvier EA, Run Z, Li Q, Li D (2016) Simultaneous determination of a fixed-dose combination of lercanidipine and valsartan in human plasma by LC-MS-MS: application to a pharmacokinetic study. J Chromatogr Sci 54(9):1553–1559
23. Aruna G, Bharathi K, Prasad K (2017) Development and validation of bioanalytical HPLC method for simultaneous estimation of clinidipine and nebivolol in human plasma. Int J Pharm Pharm Sci 9(10):253–259
24. Mir-Ali F, Parisa K, Azar P (2016) Simultaneous determination of atorvastatin and valsartan in human plasma by solid- based disperser liquid-liquid microextraction followed by high-performance liquid chromatography-diode array detection. J Chromatogr B 1017:62–69
25. Jiezhao Z, Meilling C, Li Y, Yu F, Xiaohui C, Hui ZXM (2017) Simultaneous determination of metolazone and valsartan in plasma by on-line SPEcoupled with liquid chromatography/tandem mass spectrometry. Eur J of Mass Spect 23(5):305–312
26. Jaldeepsinh VR, Maheshwari DG (2015) Development and validation of second order derivative spectrophotometric method for simultaneous estimation of cilnidipine and valsartan in synthetic mixture. Ame J of Pharm Tech Res 2(5):313–323
27. Bhole RP, Pawara VC, Chitlange SS (2015) Development and validation of HPLC method for simultaneous estimation of cilnidipine and valsartan in bulk and tablet dosage form. Res & Re J of Pharm Sci 6(2):28–36
28. Pooja JP, Ankit BC, Vijay PJ, Ruchi PS (2017) Stability indicating RP-HPLC method development and validation for estimation of cilnidipine and valsartan. World J Pharm Pharm Sci 5(5):1231–1245
29. Bhoomi DA, Aniket B, Chaudhary PJ, Parmar VN (2016) Development and validation of reverse-phase high-performance liquid chromatography method for simultaneous estimation of nebivolol HCl and clinidipine in combined tablet dosage form. Pharm & Biom Eva 3(2):208–214
30. Pavan KV, Lokeswara G, Haribaskar V, Gobinath M (2015) Development and validation of RP-HPLC method for simultaneous estimation of metoprolol succinate and cilnidipine in combined tablet dosage form. Int J Pharm 5(4): 1196–1202
31. Priyanka RP, Nilam P, Samir KS (2015) Analytical method development and validation for simultaneous estimation of nebivolol hydrochloride and cilnidipine in the combined dosage form. J Chem Pharm Res 7(9):951–960
32. Ritesh PB, Vinayak CP, Sohan SC, Sager BW (2015) Development and validation of HPLC method for simultaneous estimation of cilnidipine and valsartan in bulk and tablet dosage form. Int J of Pharm Chem Ana 2(1): 102–107
33. Sunitha N, Subhash CM, Sai SJ, Venu A, Narasimha BV, Appa B (2015) Method development and validation of RP-HPLC method for the simultaneous estimation of olmesartan and cilnidipine in bulk and formulations. Int J of Pharm Res & Allied Sci 4(3):127–135

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