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Development and Validation of RP-HPLC Method for the Estimation of Olmesartan Medoxomil and Cilnidipine in Tablet Dossage Form

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A B S T R A C T

A simple, Accurate, precise method was developed for the simultaneous estimation of the Olmesartan medoxomil and Cilnidipine in Tablet dosage form. Chromatogram was run through kromosil (150mm 4.6mm, 5µ). Mobile phase containing Buffer and Acetonitrie in the ratio of 40:60 was pumped through column at a flow rate of 1 ml/min. Temperature was maintained at 30°C. Optimized wavelength for Olmesartan medoxomil and Cilnidipine was 294nm. Retention time of Olmesartan medoxomil and Cilnidipine were found to be 2.55 min and 3.92 min. %RSD of the Olmesartan medoxomil and Cilnidipine were and found to be 1.02 and 1.30respectively. %Recover was Obtained as 100.15% and 100.04% for Olmesartan medoxomil and Cilnidipine. LOD, LOQ values were obtained from regression equations of Olmesartan medoxomil and Cilnidipine were 0.14ppm, 0.10ppm and 0.41ppm, 0.30ppm respectively. Regression equation of Olmesartan medoxomil is y = 1843x + 261.1, and of Cilnidipine is y = 3611x + 213.0.

Keywords: Olmesartan medoxomil, Cilnidipine, RP-HPLC.

A R T I C L E I N F O

CONTENTS

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1. Introduction

In general terms pharmaceutical analysis comprises those procedures necessary to determine the identity, quality, strength, and purity of the drug and drug products [1]. Pharmaceutical analysis can be defined as an analysis of a pharmaceutical compound and drug [2]. These analyses range from an assay of the New Chemical Entity (NCE) in the presence of related compounds including optical isomers to complex determination of trace or ultra-trace level of various related substance and transformed/degraded products.

High Performance Liquid Chromatography

High Performance Liquid Chromatography (HPLC) is one of the most used analytical techniques due to the presence of numerous principle of separation with different types of stationary phases and availability of many type of detection technique with many types of detectors. Chromatographic process can be defined as separation technique involving mass-transfer between stationary and mobile phase [4]. To separate the components of a mixture, HPLC uses a liquid mobile phase. Liquid or a solid phase can be the stationary phase [3].

Olmesartan Medoxomil: Olmesartan Medoxomil is an antihypertensive agent, which belongs to the class of medications called angiotensin II receptor blockers [5].

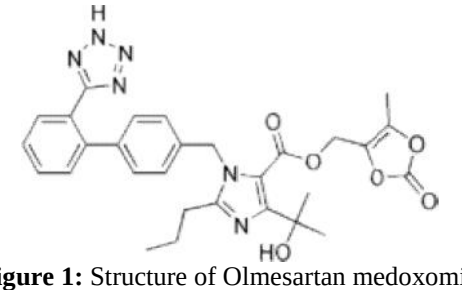

Figure 1: Structure of Olmesartan medoxomil

Cilnidipine: Cilnidipine is a calcium channel blocker. Clinidipine is the novel calcium antagonist accompanied with L-type and N-type calcium channel blocking function [7].

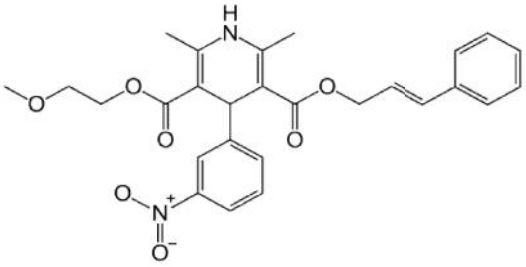

Figure 2: Structure of Cilnidipine

2. Materials and Methods

Materials:

Olmesartan Medoxomil and Cilnidipine, Combination of Olmesartan Medoxomil and Cilnidipine tablet dosage forms, distilled water, acetonitrile, phosphate buffer, ammonium acetate buffer, glacial acitic acid, methanol, potassium dihydrogen phosphate buffer, tetra hydrofuran, tri ethyl amine, ortho-phosphoric acid etc[9].

Instrument: HPLC instrument used was of WATERS HPLC 2965 SYSTEM with Auto Injector and PDA Detector [10]. Software used is Empower2. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz was be used for measuring absorbance for Olmesartan Medoxomil and Cilnidipine solutions.

Methods:

(Preparation of buffer)

Buffer: (0.1%OPA)

1ML of Ortho phosphoric acid solution in a 1000ml of volumetric flask add about 100ml of milli-Q water and final volume make up to 1000 ml with milli-Q water.

Standard Preparation:

Accurately Weighed and transferred 10mg of olmesartan Medoxomil and 5 mg of cilnidipine working Standards into a 25ml clean dry volumetric flask, add 3/4th volume of diluent, sonicated for 5 minutes and make up to the final volume with diluents. 1ml from the above two stock solutions was taken into a 10ml volumetric flask and made up to 10ml.

Sample Preparation: 1tablet was weighed, powdered and then was transferred into a 25mL volumetric flask, 10mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 0.5ml was pipeted out into a 10 ml volumetric flask and made upto 10ml with diluent.

Linearity:

Linearity solutions are prepared such that 0.25, 0.5, 0.75, 1, 1.25, 1.5ml from the Stock solutions of Olmesartan Medoxomil and Cilnidipine are taken in to 6 different volumetric flasks and diluted to 10ml with diluents to get 10ppm,20ppm, 30ppm, 40ppm, 50ppm, 60ppm of Olmesartan and 5ppm, 10ppm, 15ppm 20ppm, 25ppm, 30ppm of Cilnidipine[11].

Accuracy:

accuracy solutions are prepared such that 0.25ml ,0.5ml and 0.75ml of sample stock solution in 10ml volumetric flasks and 1ml of standard stock solution added to each flask and make up the volume with diluent to produce final concentration[12,13].

Method Development: There are many trials were done by changing columns and Mobile phases and were reported below.[14]

Trial 1: This trial was run through kromosil 250 column with mobile phase composition of 40:60 ammonium acetate Buffer and Acetonitrile, Flow rate set at 1ml/min.

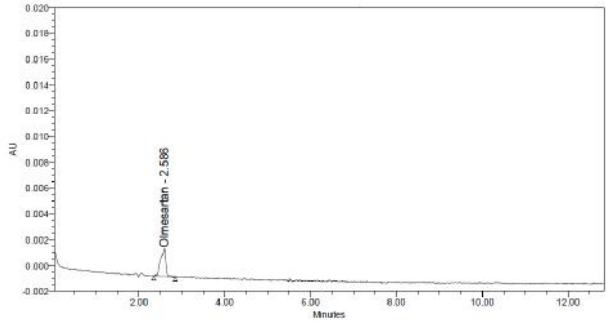

Figure 3: Trial chromatogram1

Observation: only olmesartan eluated and also peak shape was not good.

Trial 2: This trial was run through ODS 250mm column with mobile phase composition of 35:60:5 OPA Buffer and Acetonitrile, Flow rate set at 1ml/min.

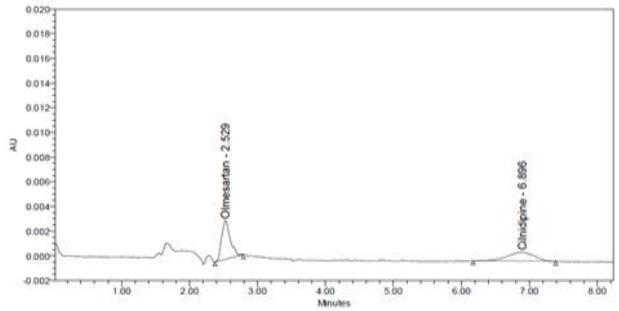

Figure 4: Trial chromatogram 2

Observation: Plate count is low for cilnidipine.

Trial 3: This trial was run through Xterra 150mm column with mobile phase composition of 40:60 Na_2HPO_4 Buffer and Acetonitrile, Flow rate set at 1 ml/min.

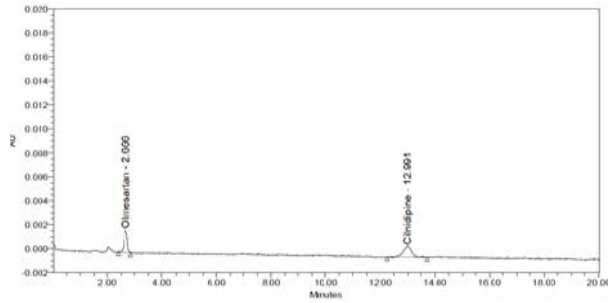

Figure 5: Trial chromatogram 3

Observation: cilindipine eluated with high retention time

Optimized Method: Drugs were eluted with good resolution, retention time all the parameters like Plate count and Tailing factor were within the limits[15].

Mobile phase:

Buffer and Acetonitrile taken in the ratio 60:40

Chromatographic conditions:

Flow rate : **1**ml/min
Column : Kromosil 150 x 4.6 mm, 5µ.
Detector wave length : 294nm
Column temperature : 30°C
Injection volume : 10µL
Run time : 7 min

Diluent: First dissolved in methanol & made up with water

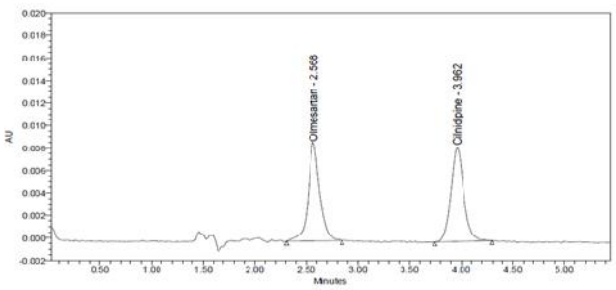

Figure 6: Optimized chromatogram of Olmesartan Medoxomil and Cilnidipine

3. Results and discussions

1. System Suitability:

All the system suitability parameters are within range and satisfactory as per ICH guidelines.

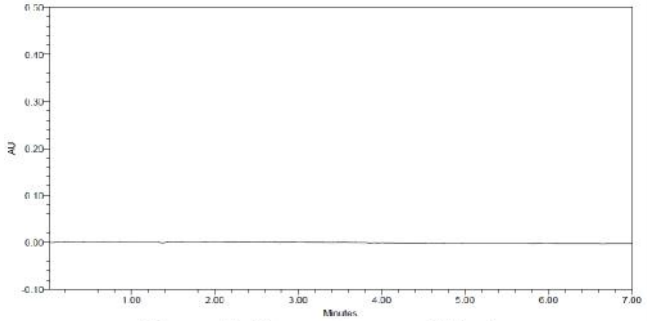

Figure 7: Chromatogram of blank

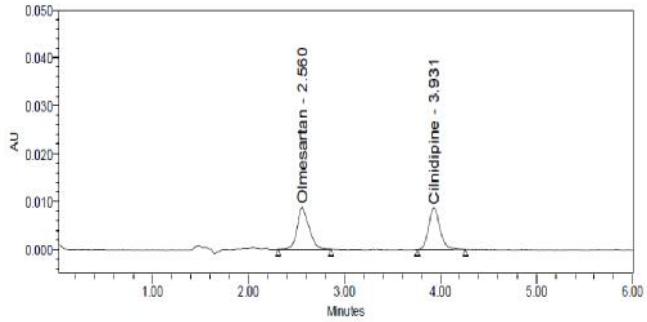

Figure 8: Typical chromatogram of Olmesartan and Cilnidipine

2. Linearity:

Six Linear concentrations of Olmesartan (50-300ppm) and Cilnidipine (25ppm to 150ppm) are prepared and Injected. Regression equation of the the Olmesartan and Cilnidipine are found to be, y = 1843x + 261.1, and y = 3611x + 213.0 and the regression co-efficient was 0.999.

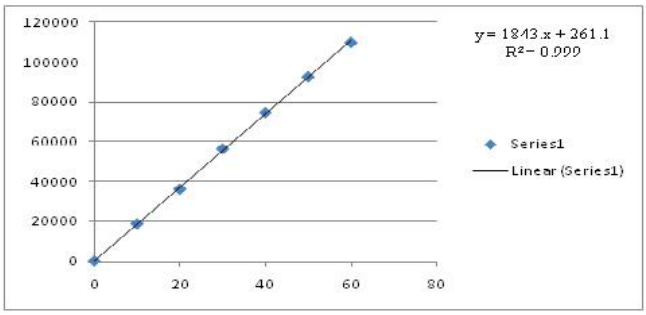

Figure 9: Calibration curve of Olmesartan

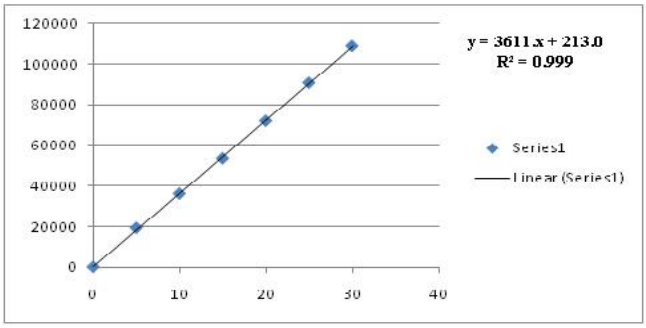

Figure 10: Calibration curve of Cilnidipine

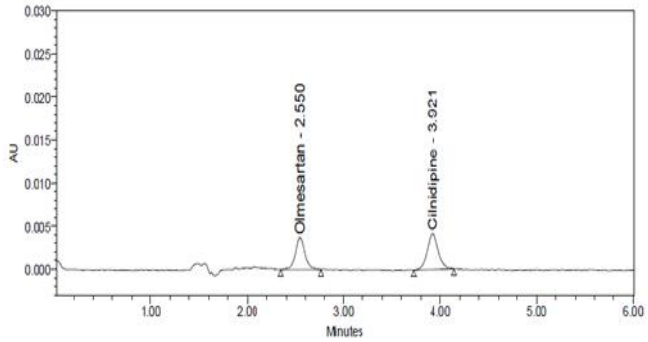

Figure 11: Linearity 50% Chromatogram of Olmesartan and Cilnidipine method

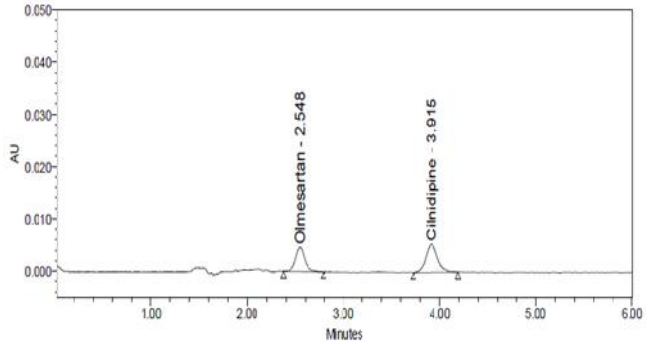

Figure 12: Linearity 100% Chromatogram of Olmesartan and Cilnidipine method

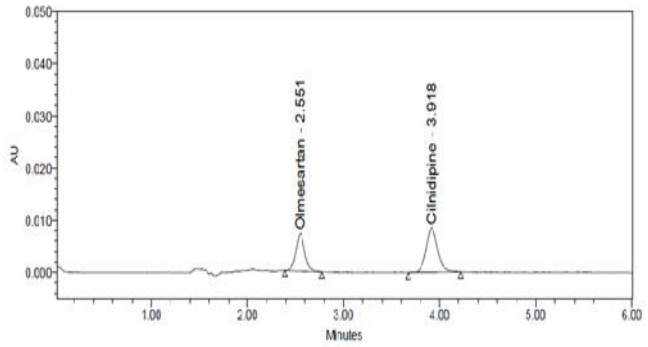

Figure 13: Linearity 150% Chromatogram of Olmesartan and Cilnidipine method

3. Precision:

Intraday precision (Repeatability): Intraday Precision was performed and % RSD for Olmesartan and Cilnidipine were found to be 1.0% and 0.8% respectively.

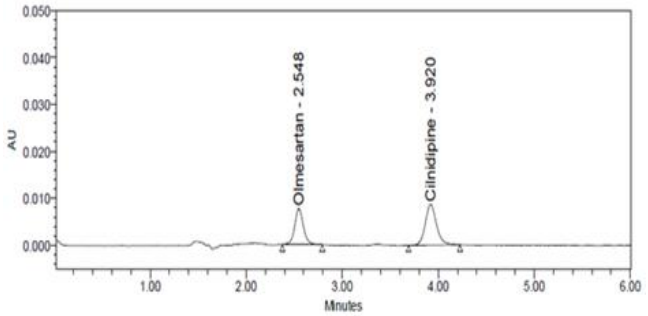

Figure 14: Repeatability Chromatogram of Olmesartan and Cilnidipine method

Intermediate precision: Inter day precision was performed with 24 hrs time lag and the %RSD Obtained for Olmesartan and Cilnidipine were 1.5% and 1.0%.

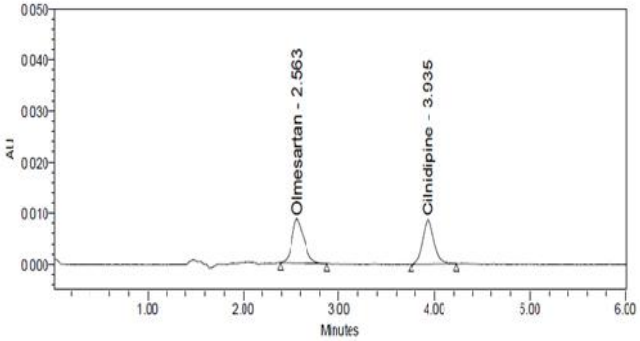

Figure 15: Inter Day precision Chromatogram of Olmesartan and Cilnidipine method

4. Accuracy:

Three concentrations 50%, 100%, 150%, were injected in a triplicate manner and amount Recovered and % Recovery were displayed in Table 6.5.

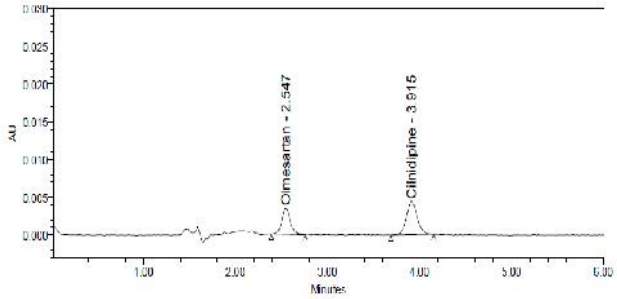

Figure 16: Accuracy 50% Chromatogram of Olmesartan and Cilnidipine method

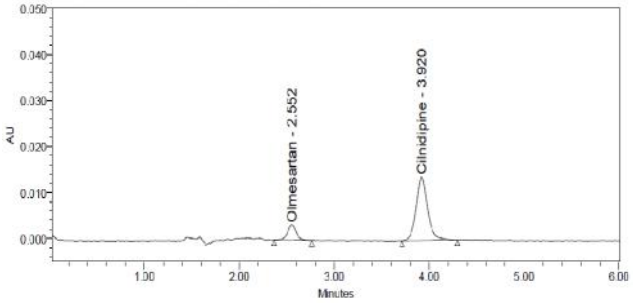

Figure 17: Accuracy 100% Chromatogram of Olmesartan and Cilnidipine method

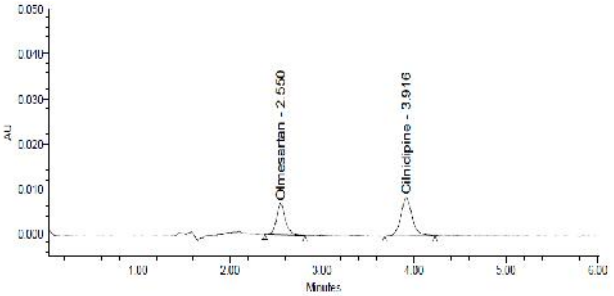

Figure 18: Accuracy 150% Chromatogram of Olmesartan and Cilnidipine method

5. LOD:

Limit of ditection was calculated by Olmesartan and Cilnidipinept method and LOD for Olmesartan was found to be 0.14 and Cilnidipine was 0.10 respectively.

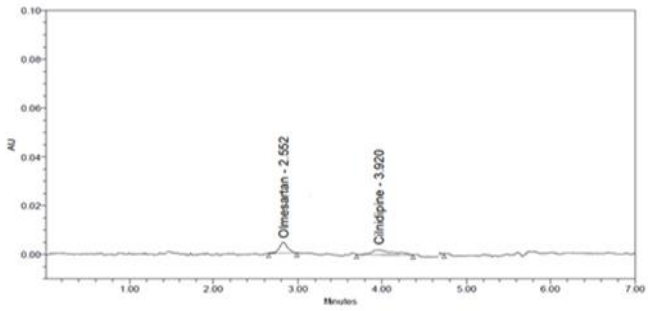

Figure 19: LOD Chromatogram of Olmesartan and Cilnidipine method

6. LOQ:

Limit of Quantification was calculated by Olmesartan and Cilnidipinept method and LOQ for Olmesartan and Cilnidipine were found to be 0.41 and 0.30 respectively.

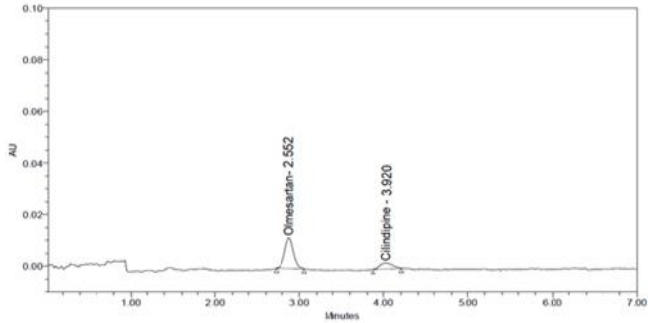

Figure 20: LOQ Chromatogram of of Olmesartan and Cilnidipine method

7. Robustness:

Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines.

Assay: Standard preparations are made from the API and Sample Preparations are from Formulation. Both sample and standards are injected six homogeneous samples. Drug in the formulation was estimated by taking the standard as the reference. The Average %Assay was calculated and found to be 100.15 % and 100.04% for Olmesartan and Cilnidipine respectively.

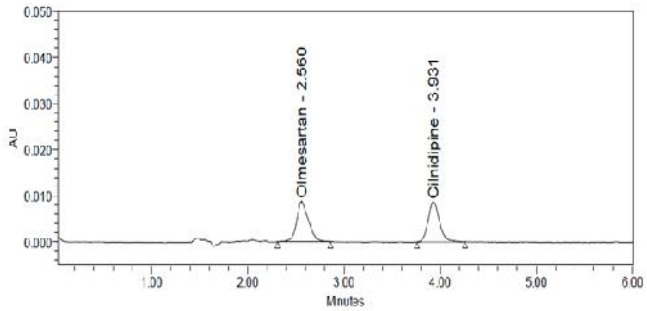

Figure 21: Assay Chromatogram

4. Conclusion

The proposed RP-HPLC (Reverse Phase High performance Liquid Chromatography) method has been evaluated for the accuracy, precision and linearity. The method was found to be precise, accurate and linear over the linear concentration range. The analytical method validation of Olmesartan medoxomil and cilnidipine by RP-HPLC was found to be satisfactory and could be used for the routine pharmaceutical analysis of Olmesartan medoxomil and cilnidipine. Method was validated as per ICH guidelines like system suitability, accuracy, precision, linearity, specificity, forced degradation studies, ruggedness, robustness and solution stability, Therefore, this HPLC method can be used as a routine analysis of these drugs in bulk, pharmaceutical formulations and also for stability studies.

5. Acknowledgement

I gives my immense pleasure to express my sincere thanks to my guide Dr. V. Hari Baskar M.Pharm., Ph.D, Ratnam Institute of Pharmacy for giving guidance at all stages of my work.

Table 1: System suitability studies of Olmesartan and Cilnidipine method

Property	Olmesartan	Cilnidipine
Retention time (t_R)	2.55± 0.3 min	3.92±0.3min
Theoretical plates (N)	1777 ± 163.48	5557±163.48
Tailing factor (T)	1.23 ± 0.117	1.15± 0.117

Table 2: Calibration data of Olmesartan and Cilnidipine method

S.NO	Conc. Olmesartan (µg/ml)	Response	Conc. Cilnidipine (µg/ml)	Response
1	0	0	0	0
2	10	18811	5	19270
3	20	36224	10	36170
4	30	56502	15	53517
5	40	74720	20	72084
6	50	92705	25	90786
7	60	109937	30	108899

Table 3: Repeatability results for Olmesartan and Cilnidipine

S.NO	Olmesartan	Cilnidipine
1	76148	73365
2	76746	73715
3	77630	72531
4	77785	72193
5	76035	73284
6	76170	72593
Mean	76752	72947
Std. Dev.	781.7	590.8
%RSD	1.0	0.8

*Average of six determinations

Table 4: Intermediate precision results for Olmesartan and Cilnidipine

S. NO.	Olmesartan	Cilnidipine
1	77453	72132
2	76236	72491
3	76591	71975
4	77841	73965
5	75317	72716
6	78545	72487
Mean	76997	72628
Std. Dev.	1173.3	708.0
%RSD	1.5	1.0

Table 5: Accuracy results of Olmesartan and Cilnidipine

Sample	Amount added (µg/ml)	Amount Recovered (µg/ml)	Recovery (%)	% RSD
Olmesartan	20	19.86	99.32	0.29
	40	40.24	100.60	0.75
	60	59.94	99.90	0.97
Cilnidipine	10	9.94	99.39	0.66
	20	19.98	99.91	1.41
	30	30.07	100.24	1.07

Table 6: Robustness data of Olmesartan and Cilnidipine method

S.NO	Robustness condition	Olmesartan % RSD	Cilnidipine % RSD
1	Flow minus	1.5	0.3
2	Flow Plus	1.3	1.0
3	Mobile phase minus	1.6	1.4
4	Mobile phase Plus	1.6	1.4
5	Temperature minus	0.9	1.0
6	Temperature Plus	1.2	0.1

Table 7: Assay of Tablet

S. No.	Olmesartan %Assay	Cilnidipine %Assay
1	99.36	101.08
2	100.14	98.57
3	101.30	98.30
4	101.50	100.08
5	99.22	101.05
6	99.39	101.15
AVG	100.15	100.04
STDEV	1.02	1.305
%RSD	1.02	1.30

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