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"BIO-ANALYTICAL METHOD DEVELOPEMENT AND VALIDATION OF OLMESARTAN IN HUMAN EDTA PLASMA USING LC-MS/MS DETECTION METHOD"

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ABSTRACT

A simple rapid, sensitive, accurate, precise and reproducible high performance liquid chromatographic tandem Mass spectrometer method has been developed to assay Olmesartan medoxomil in tablet dosage form. The LC-MS/MS analysis used a reversed phase X-terra, RP 8,4.6*50mm,5µ column and a mobile phase constituted of methanol, acetonitrile and buffer (60:30:10). The buffer is composed of 20mM ammonium acetate. The Parent ion and daughter ion for Olmesartan are 447.35 and 207.30 respectively. The Parent ion and daughter ion for Candesartan acid which is used as internal standard to estimate Olmesartan are 441.17 and 263.33 respectively. The validation data showed that the method is sensitive, specific and reproducible for the determination of Olmesartan in the dosage form. The method is linear from 5.000 ng/mL to 1500.590 ng/mL. . The intraday precision for LLOQ QC ranged from 2.80 to 6.10% and LQC, MQC and HQC ranged from 1.86 to 6.98%. The inter day precision for LLOQ QC is 5.03% and LQC, MQC and HQC ranged from 4.91 to 7.32%. The intraday accuracy for LLOQ QC ranged from 103.85% to 110.46% and LQC, MQC and HQC ranged from 90.90 to 108.61%. The inter day accuracy for LLOQ QC is 107.57% and LQC, MQC and HQC ranged from 95.28 to 102.66%. The proposed method provided an accurate and precise analysis of olmesartan in its pharmaceutical dosage form.

INTRODUCTION:

Chromatography is usually introduced as a technique for separating and/or identifying the components in a mixture. The basic principle is that components in a mixture have different tendencies to adsorb onto a surface or dissolve in

a solvent. It is a powerful method in industry, where it is used on a large scale to separate and purify the intermediates and products in various syntheses.

There are several different types of chromatography currently in use – i.e. paper chromatography; thin layer chromatography (TLC); gas chromatography (GC); liquid chromatography (LC); high performance liquid chromatography (HPLC); ion exchange chromatography; and gel permeation or gel filtration chromatography.

Mass spectrometry is a very powerful method to analyze the structure of organic compounds, but suffers from three major limitations:

- Compounds cannot be characterized without clean samples
- This technique has not the ability to provide sensitive and selective analysis of complex mixture
- For big molecules like peptides spectra are very complex and very difficult to interpret

The development of MS/MS allowed solving these problems .

Selective and sensitive analytical methods for the quantitative evaluation of drugs and their metabolites (analytes) are critical for the successful conduct of preclinical and/or biopharmaceutics and clinical pharmacology studies. Bioanalytical method validation includes all of the procedures that demonstrate that a particular method used for quantitative measurement of analytes in a given biological matrix, suchas blood, plasma, serum, or urine, is reliable and reproducible for the intended use. The fundamental parameters for this validation include (1) accuracy, (2) precision, (3) selectivity, (4) sensitivity, (5) reproducibility, and (6) stability. Validation involves documenting, through the use of specific laboratory investigations, that the performance characteristics of the method are suitable and reliable for the intended analytical applications. The acceptability of analytical data corresponds directly to the criteria used to validate the method.

AIM AND OBJECTIVE

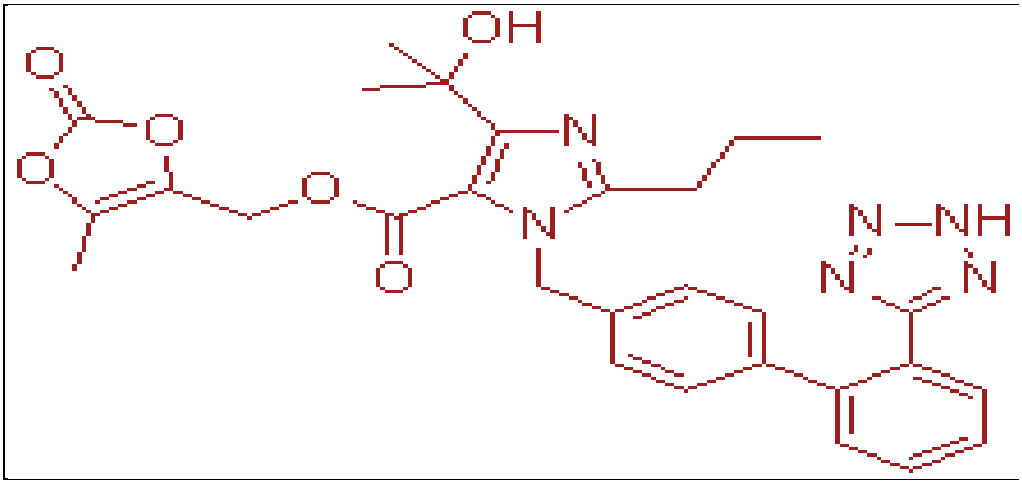
Bioanalytical methods are widely used to quantitative drugs and their metabolites in physiological matrices, and the methods could be applied to studies in areas of human clinical pharmacology and nonhuman pharmacology/toxicology. Bioanalytical method employed for the quantitative determination of drugs and their metabolites in biological fluids plays a significant role in the evaluation and interpretation of bioequivalence, pharmacokinetic (PK), and toxic kinetic studies. The major bioanalytical services are method development, method validation and sample analysis (method application).

The aim will be to achieve more selectivity, sensitivity and more rapid assay methods than have been previously described. The developed method could then be applied to clinical trials to obtain accurate pharmacokinetic parameters in human plasma.

The main objective of this work is to develop rapid, selective and sensitive LC-MS / MS method that have short and simple extraction procedures, consume small amounts of solvent and biological fluid for extraction and a short turn-around time

Drug Profile of Olmesartan Medoxomil

Chemical Structure:



IUPAC Name: (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl 5-(2-hydroxypropan-2-yl)-2-propyl-3-[[4-[2-(2H-tetrazol-5-yl) phenyl] phenyl]methyl]imidazole-4-carboxylate

Molecular weight: 558.59
Molecular Formula: $C_{29}H_{30}N_6O_6$
Pharmacologic class: Angiotensin II type 1-receptor antagonist
Therapeutic class: Antihypertensive

PHYSICAL AND CHEMICAL PROPERTIES

Physical State: white to off-white crystalline powder
Melting Point: 175 - 180 C
Solubility: Water Insoluble (sparingly soluble in strong acid, soluble in strong base, pH 3 to 9).
pKa Value: 4.96
logP value: 2.98
Decomposition: at180 ° C
CAS Registry Number: 144689-63-4
Trade Names: 1)**Benicar** in the USA

METHODOLOGY

Development for Chromatographic conditions:

Trail-1:

Column :Inertsil ODS, C18, 4.6*50mm, 5µ
Mobile Phase :70:30(Methanol: 2 mM Ammonium formate buffer)

Results:

a) The Observed Peak Shape was not good and it has tailing (asymmetric position)
b) Retention time of Analyte and Internal Standard not constant from injection to injection.
c) Observed response was low.
d) When replicate injections were given it was observed that there was no reproducibility.

Trail-2:

Column :Thermo, C18, 4.6*50mm, 5µ, Thermo, C8, 4.6*50mm, 5µ and Ace C18, 4.6*50mm, 5µ
Mobile Phase :70:30(Methanol: 2 mM Ammonium formate buffer)

Results:

a) The Observed Peak Shape was not good and it has tailing (asymmetric position)
b) Retention time of Analyte and Internal Standard not constant from injection to injection.
c) Observed response was low.
d) When replicate injections were given it was observed that there was no reproducibility.

Conclusion: It was observed that above mentioned results may be not due to the column and need to change the mobile phase.

Trail-3:

Column :X-terra, RP 8, 4.6*50mm,5µ
Mobile Phase :90:10(Methanol: 2 mM Ammonium acetate buffer)

Results:

a) The Observed Peak Shape was not good and it has tailing (asymmetric position)
b) Retention time of Analyte and Internal Standard not constant from injection to injection.
c) Observed response was good.

d) When replicate injections were given it was observed that there was no reproducibility.

Conclusion: It was observed that the response of analyte and internal standard was high. This is may be due to the changing the mobile phase composition and column.

Trail-4:

Column	:X-terra, RP 8, 4.6*50mm,5µ
Mobile Phase	:60:30:10 (Methanol: Acetonitrile: 2 mM Ammonium acetate buffer)

Results:

a) The Observed Peak Shape wasgood and it has no tailing (symmetric peak)
b) Retention time of Analyte and Internal Standard not constant from injection to injection.
c) Observed response was good.
d) When replicate injections were given good reproducibility was observed.

Conclusion: It was observed that the response of analyte and internal standard was high and reproducible. This is may be due to the changing the mobile phase composition.

Trail-5:

Column	:X-terra, RP 8, 4.6*50mm,5µ
Mobile Phase	:60:30:10 (Methanol: Acetonitrile: 20 mM Ammonium acetate buffer)

Results:

a) The Observed Peak Shape was good and it has no tailing (symmetric peak)
b) Retention time of Analyte and Internal Standard was constant from injection to injection.
c) Observed response was good.
d) When replicate injections were given good reproducibility was observed.

Conclusion:

1) It was observed that retention time was constant may be due to the increasing the buffer strength.
2) By using above mentioned chromatographic conditions (Trail-5) Aqueous linearity was injected and the results were linear and reproducible.

Extraction method trials:

Trail-7: (Liquid-Liquid extraction trail)

Extraction buffer:2mM Ammonium formate P^H 3.0
Extraction Solvent:Methyl tertiary butyl ether (MTBE)

Results

a) It was observed that recovery of Analyte and Internal Standard was low.
b) When replicate injections were given reproducibility was not observed.
c) Extracted sample has more turbidity
d) Poor chromatography was observed.

Conclusion: This may be due to extraction solvent, extraction buffer and matrix effect.

Trail-8: (Liquid-Liquid extraction trail)

Extraction buffer:1% Ammonia in water
Extraction Solvent:Methyl tertiary butyl ether (MTBE)

Results

a) It was observed that recovery of Analyte and Internal Standard was good.
b) When replicate injections were given reproducibility was not observed.
c) Extracted sample has more turbidity
d) Poor chromatography was observed.

Conclusion: This may be due to extraction solvent, extraction buffer and matrix effect.

Trail-9: (Solid-Phase extraction)

Extraction buffer:1% Ammonia in water
SPE Cartridge: HLB 1cc (30mg)

Results

a) It was observed that recovery of Analyte and Internal Standard was comparatively good.
b) When replicate injections were given reproducibility was observed.
c) Extracted sample has no turbidity
d) Good chromatography was observed.

Conclusion: This may be due to HLB1cc (30mg) cartridge, extraction buffer and no matrix effect was observed.

Trail-10: (Solid-Phase extraction)

Extraction buffer:1% Ammonia in water & SPE Cartridge: HLB 3cc (60mg)

Results

a) It was observed that recovery of Analyte and Internal Standard was very good.
b) When replicate injections were given reproducibility was observed.
c) Extracted sample has no turbidity& Good chromatography was observed.

Conclusion: This may be due to HLB3cc (60mg), extraction buffer and no matrix effect was observed.

1) Chromatographic conditions:

The following parameters for the analysis of Olmesartan in human plasma.

Column	:X-terra, RP 8,4.6*50mm,5µ
Mobile Phase	:60:30:10 (Methanol: Acetonitrile: 20mM Ammonium Acetate buffer)
Flow Rate	:0.400 mL/min
Column Temperature	: 35.0 ± 2.0 ^{o}C
Auto sampler Temperature	: 10 ± 2.0°C
Injection volume	: 20µL
Run time	: 2.5 Minutes
Retention time	: Olmesartan acid: 1.05±0.32 min Candesartan acid: 1.07±0.32 min

2) Mass spectrometer conditions for multiple Reaction Monitoring:

Scan type	:	MRM	
Acquisition duration	:	2.50min.	
Ionization Mode	:	ESI	
Polarity	:	Positive	
Inter Channel delay (sec)	:	0.050	
Inter Scan delay/time (sec)	:	0.050	

Channel	Parent (Da)	Daughter (Da)	Dwell (s)	Cone (V)	Collision (eV)
Olmesartan Acid	447.35	207.30	0.500	23.00	25.00
Candesartan Acid	441.17	263.33	0.500	21.00	11.00

3) MS tune Parameters:

Source (ES^+)	
Parameter	**Setting**
Capillary (kV)	3.50
Cone (V)	23.00
Extractor (V)	1.00
RF Lens (V)	0.4
Source Temperature (^{0}C)	100
Desolvation Temperature (^{0}C)	400
Cone Gas Flow (L/Hr)	80
Desolvation Gas Flow (L/Hr)	800
Analyser	
LM 1 Resolution	15.0
HM 1 Resolution	15.0
Ion Energy 1	0.5
Entrance	-1
Collision	25
Exit	1
LM 2 Resolution	15.0
HM 2 Resolution	15.0
Ion Energy 2	1.5
Multiplier (V)	650

4) Preparations of solutions:

4.1)20mM Ammonium acetate buffer (w/v):

Weigh accurately about 1.5416 gm of Ammonium acetate and transfer it into a 1000mL beaker, dissolve in 900mL of Milli-Q Water and make up to 1000mL with the same. Sonicate for 5 minutes.

4.2) Mobile phase (v/v):

Prepare Mobile phase by mixing 60 parts of Methanol, 30 parts of Acetonitrile and 10 parts of 20mM Ammonium acetate buffer solution and Sonicate for 5 minutes.

4.3) 80% Methanol solution (v/v):

Mix 800mL of methanol and 200mL of Milli-Q water. Sonicate for 5 minutes.

4.4) 1% Ammonia in Water (v/v):

Mix one parts of Ammonia solution and 99 Parts of Water. Sonicate for 5 Minutes.

4.5)Standard-8 spiking solution: (75.000µg/mL)

Transfer 75µL of Drug stock and make up to 1.000mL with 80% methanol solution and mix.

4.6) Internal standard (ISTD) Dilution :(20.000µg/mL)

Transfer 500µL of Candesartan Acid stock solution (1mg/mL) into a 25mL volumetric flask and make up to volume with Methanol and mix. Provide a batch number. Prepare as and when required.

4.7) Reference Solution:

Mix 20µL of Standard-8 Spiking solution and 100µL of internal standard dilution and make upto 2.000mL with Mobile Phase.

4.8) Standard-1 spiking solution :(0.250µg/mL)

Transfer 34.00µL of Standard-8 Spiking solution and make up to 10.000mL with 80% methanol solution and mix.

4.10) LLOQ Reference solution:

Mix 20µL of aqueous Standard-1 spiking solutionand 100µL of internal standard dilution and make up to 2.000mL with Mobile Phase. Provide a batch number. Prepare as and when required.

4.11) Preparation of drug stock solution:

Olmesartan Acid stock solution (w/v):

Weigh accurately about 10.000mg Olmesartan Acid Working standard and transfer it into 10.000mL volumetric flask, dissolve in about 5.000mL of methanol and make up to 10.000mL with the same to get 1.000mg/mL. Correct the final concentration of Olmesartan Acid accounting for its potency and the actual amount weighed. Store the stock solution in refrigerator below 10°C.

Candesartan Acid stock solution (w/v): (As Internal standard)

Weigh Candesartan Acid working standard equivalent to 10.000mg of Candesartan Acid and transfer it into a 10.000mL volumetric flask, dissolve in about 5.000mL of Methanol and make up to 10.000mL with the same to get 1.000mg/mL. Correct the final concentration of Candesartan Acid accounting for its potency and the actual amount weighed. Store the stock solution in the refrigerator at below 10^0C

5) Preparation of Calibration Curve Standards

5.1) Preparation of spiking solutions:

Prepare spiking solutions from Olmesartan Acid stock solution (CC), as per the table given below in the concentration ranging from 0.250 to 75.000µg/mL. Use 80% Methanol solution as diluent.

ID	SS Conc. (µg/mL)	Volume (mL)			Final Conc. (µg/mL)	SS ID
		SS	Diluent	Final		
DS	1000.000	0.750	9.250	10.000	75.000	**SS8**
SS8	75.000	8.000	2.000	10.000	60.000	**SS7**
SS7	60.000	6.670	3.330	10.000	40.020	**SS6**
SS6	40.020	5.000	5.000	10.000	20.010	**SS5**
SS5	20.010	4.998	5.002	10.000	10.001	**SS4**
SS4	10.001	1.250	8.750	10.000	1.250	**SS3**

SS3	1.250	4.000	6.000	10.000	0.500	**SS2**
SS2	0.500	5.000	5.000	10.000	0.250	**SS1**

5.2) Preparation of Spiked Plasma CC Standards:

Spike the screened blank human plasma (K_2EDTA as anticoagulant) with the above-prepared spiking solutions to prepare the plasma standards ranging from 5.000 to 1500.000 ng/mL as per the table given below.

ID	SS Conc. (µg/mL)	Volume (mL)			Plasma Conc. (ng/mL)	CC Standard ID
		SS	Plasma	Final		
SS8	75.000	0.200	9.800	10.000	1500.000	**STD8**
SS7	60.000	0.200	9.800	10.000	1200.000	**STD7**
SS6	40.020	0.200	9.800	10.000	800.400	**STD6**
SS5	20.010	0.200	9.800	10.000	400.200	**STD5**
SS4	10.001	0.200	9.800	10.000	200.020	**STD4**
SS3	1.250	0.200	9.800	10.000	25.002	**STD3**
SS2	0.500	0.200	9.800	10.000	10.001	**STD2**
SS1	0.250	0.200	9.800	10.000	5.000	**STD1**

6) Preparation of QC Sample

6.1) **Preparation of QC spiking solutions:**

Prepare spiking solutions from Olmesartan Acid stock solution (QC) as per the given table below in the concentration ranging from 0.250 to 57.100µg/mL. Use 80% Methanol as diluent.

ID	SS Conc. (µg/mL)	Volume (mL)			Final Conc. (µg/mL)	SS ID
		SS	Diluent	Final		
DS-XX	1000.000	0.5710	9.4290	10.0000	57.100	HQCSS
HQCSS	57.100	5.5190	4.4810	10.0000	31.513	MQCSS
MQCSS	31.513	0.2380	9.7620	10.0000	0.750	LQCSS
LQCSS	0.750	3.330	6.670	10.000	0.250	LLOQ QC SS

6.2) Preparation of Spiked Plasma QC samples:

Spike the screened blank human plasma with the above-prepared QC spiking solutions to prepare the plasma quality control samples ranging from 5.000 to 1142.000ng/mL as per the table given below.

ID	SS Conc. (µg/mL)	Volume (mL)			Plasma Conc. (ng/mL)	QC ID
		SS	Plasma	Final		
HQCSS	57.100	0.200	9.800	10.000	1142.000	HQC
MQCSS	31.513	0.200	9.800	10.000	630.270	MQC
LQCSS	0.750	0.200	9.800	10.000	15.000	LQC
LLOQ QC SS	0.250	0.200	9.800	10.000	5.000	LLOQ QC

7) Spiked sample storage:

Aliquot 0.550mL of each spiked plasma into pre-labeled polypropylene vials, cap them tight, Store in deep freezer below –70°C and record them in the respective deep freezer.

8) Sample Processing:

Step-1: Aliquot 0.500mL sample into pre labelled ria vials and add 50.0µL of internal standard (ISTD) dilution (20.000µg/mL) to all except standard blank & vortex. Then add 0.500mL of 1% Ammoniated Water to all & vortex.

Step- 2: Condition HLB 3cc (60mg) Cartridges with 2mL of methanol, 2mL ofMilli-Q Water.

Step-3: Load the Prepared Sample in to Cartridges.

Step-4: Elute the Sample slowly and completely.

Step-5: Wash the Cartridges with 2mL of Milli-QWater, followed by 2 mL ofn- Hexane and allowed to dry for two minutes

Step- 6: Elute the Samples with 1.000mL of Methanol in to Pre Labelled ria vials
Step- 7: Evaporate the eluent under dry nitrogen gas at about 50°C & 15 psi for about 25 min or till the samples are dried.
Step- 8: Reconstitute the evaporated tubes with 1.000mL of Mobile phase and vortex.
Step- 9: Transfer the Reconstituted samples into pre labelled auto sampler vials.

FLOW CHART FOR SAMPLE PREPARATION OF OLMESARTAN ACID

Aliquot 0.500mL of plasma
↓
Add 50.000µL of internal standard (ISTD) dilution (20.000 µg/mL) & vortex.
(Do not add ISTD dilution to the blank samples). Add 0.500mL of 1% Ammonia in Water Solution to all &Vortex.
↓
Condition HLB 3cc (60mg) Cartridges with 2mL of methanol, 2mL of Milli-Q Water
↓
Load the Prepared Sample in to Cartridges.
↓
Elute the Samples slowly and completely.
↓
Wash the Cartridges with 2 mL of Milli-Q Water followed by 2 mL of n-Hexane and allowed to dry for two minutes.
↓
Elute the Samples with 1.000mL of Methanol in to Pre labelled ria vials
↓
Evaporate the eluent at 50°C and 15 psi of dry N2 gas pressure,
For 25 minutes or till the tubes is dried.
↓
Reconstitute the dried samples with 1.000 mL of Mobile Phase and vortex.
↓
Transfer the Reconstituted samples into pre-labeled auto sampler vials.

VALIDATION RESULTS &DISCUSSION

1) System Suitability:

Procedure: The System suitability was measured by injecting six replicates of reference solution (equalant to ULOQ Standard) and evaluated the RT and area ratio.

Acceptance criteria: The obtained %CV of retention time should not be more than 5%, the %CV of area or area ratio should not be more than 2% in case of HPLC and 4% in case of LC-MS/MS.

Result: The obtained %CV is 0.00% for RT of Olmesartan and 0.80% for RT of Candesartan (ISTD).The obtained %CV of area ratio is 0.69%. Results are presented in table – 1(A).

2) Auto sampler carryover test:

Procedure: The Auto sampler carry over test was measured by injecting one reference solution, two mobile phase solution and one extracted ULOQ standard, two extracted blank matrix in sequential order and evaluated the area.
Acceptance criteria: The obtained % carryover is not exceeded more than 0.5 % of the areas of blank samples when comparing with high standard sample at Retention time of analyte or ISTD.

Result: The obtained % carryover is 0.00 % at RT of Olmesartan and 0.00% at RT of Candesartan (ISTD).Results are presented in table – 1(B).

3) Selectivity:

Procedure: The selectivity of the present method was evaluated by checking the blank K_2EDTA (Ethylene Diamine Tetra acetate) plasma (without spiking Olmesartan and Candesartan (ISTD)) obtained from different blood donors. Eight different batches of K_2EDTA plasma samples were screened and all the batches were found to have no significant endogenous interferences at the retention times of analyte and internal standard (ISTD).6 LOQ samples are prepared in interference free blank for evaluation. Candesartan is used as internal standard.

Acceptance criteria: Response of interfering peaks at the retention time of Analyte (s) must be ≤ 20% of the mean response of extracted LOQ standard. Response of interfering peaks at the retention time of IS must be ≤ 5% of the mean response of Internal standard. At least 80% of 6 different volunteer matrix lots should meet above criteria.

Result: All the batches found to have no significant endogenous interferences at the retention time of analyte and internal standard. Human K_2EDTA plasma batches, free of significant interferences, were used to prepare calibration standards and QC samples for the validation. Results are presented in table – 2.

4) Linearity:

Procedure: The linearity of the calibration curve was determined by injecting the samples of standard-1 to standard-8 in the range of 5.000 ng/mLto 1500.590 ng/mL.

Acceptance criteria: A minimum 6 of 8 standards, including ULOQ should fall within ±15% except LLOQ for which it should be within ±20% when back calculated. Regression should be simple using appropriate weighting factor for goodness of fit.

Result: The linearity of the method was determined by a weighted ($1/x^2$) least square regression analysis of standard plots associated with eight-point standard curve for Olmesartan. The calibration line was linear for the standards ranging from 5.000 ng/mLto 1500.590 ng/mL for Olmesartan as shown in figure 2.

A straight-line fit is made through the data points by least square regression analysis and a constant proportionality is observed with minimal data scattering. The correlation coefficients (r^2) ranged from 0.9932 to 0.9974 for Olmesartan during the course of validation. Results are presented in the Table 3.

5) Chromatography (Figures No. 3 -17):

Representative chromatograms of aqueous standards (Olmesartan andCandesartan), blank plasma, and blank plasma spiked with ISTD, Calibration curve standards (STD-1 to STD-8), LLOQ QC, low, middle and high QC samples are shown in figures 3 to 17 respectively.

6) Precision and Accuracy:

Procedure: The precision and accuracy was measured by injecting one mobile phase sample, one reference solution, one calibration curve (includes one blank sample, one blank+ISTD and STD-1 to STD-8) and quality control samples six at each level of LLOQ QC, LQC, MQC and HQC.

Calculation: The precision of the assay was measured by the percent coefficient of variation for QC samples of Olmesartan and the accuracy of the assay was measured by calculating the ratio of the calculated mean values of the QC samples to their respective nominal values, expressed as percentage. Results are presented in the Table 4(A) & 4(B).

Acceptance criteria: A minimum of 4 P & A batches should be evaluated. The precision should not exceed 15% of coefficient of variation for all the QC samples except for the LLOQ QC where it should not exceed 20% of CV.

Result:

Within-batch Precision for Olmesartan
Within-batch precision for LLOQ QC was ranged from 2.80 to 6.10%.
Within-batch precision for LQC, MQC and HQC ranged from 1.86 to 6.98%.

Within-batch Accuracy for Olmesartan
Within-batch accuracy for LLOQ QC was ranged from 103.85 to 110.46%.
Within-batch accuracy for LQC, MQC and HQC ranged from 90.90 to 108.61%.

Between-batch Precision for Olmesartan
Between-batch precision for LLOQ QC was 5.03%.
Between-batch precision for LQC, MQC and HQC ranged from 4.91 to 7.32%.

Between-batch Accuracy for Olmesartan
The between batch accuracy for LLOQ QC was 107.57%.
The between batch accuracy for LQC, MQC and HQC ranged from 95.28 to 102.66%.

6) Stability: Stability of Drugs in Stock solution

6.1) Short-term stability of drugs in Stock solution:

Procedure: Stock solutions of about 1 mg/mL Olmesartan and Candesartan (Internal standard) were prepared freshly and aliquots of freshly prepared stock solutions were left at room temperature of ≤25°C for 6.25 hours for Olmesartan and Candesartan. The freshly prepared stock solutions stored in refrigerator below 10°C, were used as comparison samples. The short-term stock solution stability of Olmesartan in Methanol and Candesartan (Internal standard) in Methanol was successfully assessed by comparing mean responses of six replicates of stability samples Vs six replicates of comparison samples. The mean responses of both stability and comparison solutions were subsequently compared.

Acceptance criteria: Comparable stability against comparative samples for analyte and internal standard should be within the range of 90-110%.

Result: After storage for 6.25 hours at room temperature of ≤ 25°C, the percent stabilities were 97.53% and 99.01% for Olmesartan and Candesartan respectively. Results are presented in the Table-5.

6.2) Long-term stability of drugs in Stock solution:

Procedure: Stock solutions of about 1 mg/mL Olmesartan and Candesartan (Internal standard) were prepared and aliquots of these stock solutions were stored in refrigerator below 10°C (Stability samples) for 7.14 days for Olmesartan and Candesartan (ISTD). On the day of analysis, freshly prepared stock solutions stored in refrigerator ≤ 10°C, were used as comparison samples. The long-term stock solution stability of Olmesartan in Methanol and Candesartan (Internal standard) in Methanol was successfully assessed by comparing mean responses of six replicates of stability samples Vs six replicates of comparison samples. The responses of both stability and comparison solutions were subsequently compared.

Acceptance criteria: Comparable stability against comparative samples for analyte and internal standard should be within the range of 90-110%.

Result: After storage for 7.14 days for Olmesartan and Candesartan (ISTD) in refrigerator below 10°C, the percent stabilities were 100.37% and 95.51% for Olmesartan and Candesartan respectively. Results are presented in the Table 6.

6.3) Stability of analyte in Biological Matrix:

Zero day Long-term stability of analyte in human K_2EDTA plasma (First day of LT Stabilitytesting)

Procedure: The Zero day long-term stability of Olmesartan in human K_2EDTA plasma was successfully assessed by analyzing fresh QC samples at low and high concentrations. The concentrations of QC samples were determined with the freshly prepared calibration curve standards. These back calculated concentrations will be used as nominal concentrations for calculating long term stability in matrix.

The mean back calculated concentrations of fresh QC samples (before storage for stability evaluation at-70°C & -20°C) are observed to be 14.407 ng/mL (LQC level) and 1166.259 ng/mL (HQC level).

Acceptance criteria: The mean concentration obtained for LQC & HQC samples should be within ± 15% of nominal concentration. %CV should not exceed 15%.
Result: The %CV and %nominal values for LQC are 2.35% and 96.09% where as for HQC are 1.78% and 101.29%. Results are presented in the Table 7.

7) Freeze-thaw stability:

Procedure: The freeze-thaw (FT) stability of Olmesartan in human plasma was successfully assessed by analyzing six replicates of quality control samples at low and high levels, previously frozen and thawed (at room temperature of ~ 25°C) over 5 cycles along with freshly prepared calibration curve standards and by calculating the percent nominal of mean concentrations of stability samples at low and high QC levels.

Acceptance criteria: The mean concentration obtained for LQC & HQC samples should be within ± 15% of nominal concentration. %CV should not exceed 15%.

Result: The freeze-thaw stability of Olmesartan after five FT cycles was 97.79% and 102.38% at low and high concentrations respectively. Results are presented in the Table 8.

8) Bench top stability:

Date and retrieval time of samples from deep freezer: 09-04-12 at 11:05 AM
Date and time of processing: 09-04-12 at 04:22 PM

Procedure: The Bench top stability of Olmesartan in human plasma was successfully assessed by unprocessed six replicates of stability samples, maintained at a temperature of ~ 25°C for 5.28 hours, at low and high QC concentrations and analyzing the stability samples along with freshly prepared calibration curve standards. The percentage stability was determined by calculating the percent nominal of mean concentrations of stability QC samples at high and low QC levels.

Acceptance criteria: The mean concentration obtained for LQC & HQC samples should be within ± 15% of nominal concentration. %CV should not exceed 15%.

Result: The bench top stability of Olmesartan in human plasma at a temperature of ~25°C for 5.28 hours was 98.80% and 99.92% at low and high concentrations respectively. Results are presented in the Table 9.

9) In-injector stability:

Date and time of loading of samples into auto injector 07-04-12 at 03:20 PM
Date and time of injection: 09-04-12 at 05:07 PM

Procedure: In-Injector stability of analyte was successfully assessed by analyzing processed QC samples at high and low concentrations after storage for 49.78 hours at 10°C in auto sampler and the concentrations of QC samples were determined with the freshly prepared calibration curve standards. The percentage stability was determined by calculating the percentage nominal of mean concentrations of QC samples stored at 10°C for 49.78 hours in auto sampler.

Acceptance criteria: The mean concentration obtained for LQC & HQC samples should be within ± 15% of nominal concentration. %CV should not exceed 15%.

Result: The In-Injector stability of Olmesartan for 49.78 hours at 10°C was 101.50% for LQC and 97.61% for HQC. Results are presented in the Table 10.

10) Wet Extract stability:

Date and storage time of processed samples: 07-04-12 at 03:28 PM
Date and retrieval time of stored samples: 09-04-12 at 12:50 PM

Procedure: The stability of Olmesartan in Wet extract form, was successfully assessed by analyzing six replicates of Wet Extract stability samples stored at a temperature below 10°C for 45.37 hours at low and high concentrations and the concentrations of stability QC samples were determined with the freshly prepared calibration curve standards. The percentage stability was determined by calculating the percentage nominal of mean concentrations of QC samples stored below 10°C for 45.37 hours.

Acceptance criteria: The mean concentration obtained for LQC & HQC samples should be within ± 15% of nominal concentration. %CV should not exceed 15%.

Result: The Wet extract stability of Olmesartan at a temperature of below 10°C for 45.37 hours was 96.62% and 101.97% at low and high concentrations respectively. Results are presented in Table 11.

11) Dry Extract stability:

Date and storage time of processed samples: 07-04-12 at 03:28 PM
Date and retrieval time of stored samples: 09-04-12 at 12:50 PM

Procedure: The stability of Olmesartan in Dry extract form was successfully assessed by analyzing six replicates of Dry Extract stability samples stored at a temperature below 10°C for 45.37 hours at low and high concentrations. And the concentrations of stability QC samples were determined with the freshly prepared calibration curve standards. The percentage stability was determined by calculating the percentage nominal of mean concentrations of QC samples stored below 10°C for 45.37 hours.

Acceptance criteria: The mean concentration obtained for LQC & HQC samples should be within ± 15% of nominal concentration. %CV should not exceed 15%.

Result: The Dry Extract stability of Olmesartan at a temperature of below 10°C for 45.37 hours was 96.93% and 99.54% at low and high concentrations respectively. Results are presented in Table 12.

12) Long-term stability of analyte in biological matrix:

Date and storage time of fresh QC samples for Stability (at –70°C): 04-04-12 at 03:15 PM
Date and retrieval time of stored stability samples for analysis (from –70°C) 13-04-12 at 10:30 AM

Procedure: The long-term stability of Olmesartan in human plasma was successfully assessed by analyzing QC samples at low and high concentrations after storage for 8.80 days at -70°C. The concentrations of stability QC samples were determined with the freshly prepared calibration curve standards prepared in human K_2EDTA plasma. The percentage stability was determined by comparing the mean concentrations of stability QC samples at low and high QC levels with mean back calculated concentrations of fresh QC samples analysed on day zero (i.e. First day of LT Stability Testing).

Acceptance criteria: The mean concentration obtained for stability LQC & HQC samples should be within ± 15% of zero day mean back calculated concentrations of fresh QC samples. The %CV of stability samples should not exceed 15%.

Result: The long-term stability of Olmesartan in human plasma after storage for 8.80 days at –70°C was 114.05% and 99.13% at low and high concentrations respectively. Results are presented in the Table 13.

13) Dilution Integrity:

Procedure: A dilution QC pool (3001.180 ng/mL) was prepared in human K_2EDTA plasma (containing K_2EDTA as anticoagulant) at a concentration approximately twice the high CC standard (ULOQ-upper limit of quantitation) for Olmesartan to assess dilution integrity. Blank human K_2EDTA plasma was used to dilute the dilution quality control (DQC) samples (prepared in human K_2EDTA plasma). The precision and accuracy for

dilution integrity after ½nd dilution and ¼th dilution were determined against processed calibration curve standards.

Acceptance criteria: The accuracy of the dilution integrity QC samples should be within ±15%. %CV should not exceed 15%

Result: The precision for dilution quality control sample of Olmesartan in human K_2EDTA plasma was 2.95% at ½nd dilution and 6.98% at ¼th dilution. The accuracy for dilution quality control sample of Olmesartan in human K_2EDTA plasma was 104.02% for ½nd dilution and 92.27% for ¼th dilution.

Dilution integrity for Olmesartan in human K_2EDTA plasma was observed after ½nd and ¼th dilution. Results are presented in the Table 14.

14) Ruggedness (P&A-5)

Ruggedness was evaluated using a single precision and accuracy batch performed by a same instrument, different column lot, and different sample processing analyst.

Result: The % CV and nominal values for LLOQ QC was 5.35% and 110.66% and for LQC, MQC & HQC ranged from 4.42 to 6.77% and 100.27 to 104.05% respectively. Results are presented in the Table 15.

15) Simultaneous evaluation of Matrix effect, Recovery and Process efficiency:

Matrix effect, Recovery and Process efficiency for Olmesartan are evaluated with Aqueous (neat) samples, Post-extracted and extracted samples from 6 different K_2EDTA blank plasma lots. The results are calculated using peak area responses.

Matrix Effect:

Procedure: Matrix Effect was performed by analyzing six replicates of post extracted samples at five different CC standard i.e. STD-1,STD-3,STD-5,STD-6 and STD-8 concentrations (prepared by spiking aqueous solutions into extracted blank plasma samples from 6 different blank K_2EDTA plasma lots) along with 6 replicates of equivalent, similarly prepared aqueous (neat) samples. The percentage matrix effect was determined by comparing the mean peak area responses of post-extracted samples with mean peak area responses of aqueous (neat) samples at five different CC standard i.e. STD-1,STD-3,STD-5,STD-6 and STD-8 concentration levels.

Acceptance criteria: The mean response obtained for post extracted samples should be within ±15% of the mean response of aqueous (neat) samples at five different CC standard levels.%CV should not exceed 15% except std1 where it is 20%.

Result: The %CV and % matrix effect on Olmesartan in K_2EDTA plasma ranged from 1.54 to 3.22% and 97.93 to 101.96 respectively. Results are presented in the Table 16.

The %CV and % matrix effect on Candesartan in K_2EDTA plasma ranged from 1.50 to 2.64% and 98.64 to 101.51% respectively. Results are presented in the Table 16.

Recovery:

Procedure: Recovery was performed by analyzing six replicates of extracted CC standard samples (spiked in 6 different blank K_2EDTA plasma lots) along with post extracted CC standard samples (prepared by spiking aqueous solutions into extracted blank plasma samples from 6 different blank K_2EDTA plasma lots) at five different CC standard concentration levels i.e. STD-1,STD-3,STD-5,STD-6 and STD-8. The percentage recovery is determined by comparing the areas of the extracted QC samples against equivalent post-extracted QC samples at five different CC standards i.e. STD-1, STD-3, STD-5, STD-6 and STD-8 concentration levels.

Acceptance criteria: Percent recovery for analyte and internal standard should be ≤ 115%. The %CV of the mean recoveries at five different CC standard levels for analyte is ≤ 15% except STD-1 where it is 20%.

Result: Recovery of Olmesartanranged from 84.76 to 92.29%. Results are presented in Table 16.

Recovery of Candesartan ranged from 66.39 to 72.92%. Results are presented in Table 16.

Process Efficiency

Procedure: Process efficiency was performed by analyzing six replicates of extracted CC standard samples (spiked in 6 different blank K_2EDTA plasma lots) along with 6 replicates of equivalent, similarly prepared aqueous (neat) samples. The percentage process efficiency is determined by comparing the mean peak areas of the extracted samples against equivalent aqueous samples (neat samples) at five different CC standard i.e. STD-1, STD-3, STD-5, STD-6 and STD-8 concentration levels.

Acceptance criteria: The mean response obtained for extracted samples should be less than 115% of the mean response of aqueous (neat) samples at five different CC standard levels. %CV should not exceed 15% except std1 where it is 20%.

Result: Process efficiency for Olmesartan ranged from 83.01 to 93.25%. Results are presented in Table 16.

Process efficiency for Candesartan ranged from 65.49 to 73.55%. Results are presented in Table 16.

TABLE 1(A)

System Suitability

Name of the experiment :System suitability				Instrument Name :LC-MS/MS	
Sr.No	**Name of the Analyte**	**Mean Retention Time**	**% CV of Retention Time**	**% CV of Area Area-ratio (Analyte/IS)**	**System suitable Yes/No**
1	Olmesartan	1.02	0.00	0.69	Yes
	Candesartan(ISTD)	1.05	0.80	-	

TABLE 1(B)

Auto Sampler Carry over Test

Name of the experiment : Auto Sampler Carry Over Test				Instrument Name :LC-MS/MS				
Sr No	**Analyte ID**	**Area of Reference Solution**	**Area of R.S-1***	**Area of R.S-2***	**Area of Ext High Std**	**Area of STD Blank-1**	**Area of STD Blank-2***	**%Carry-Over of extracted** High STD
1	Olmesartan	177772	0	0	201636	0	0	0.00
2	Candesartan (ISTD)	94032	2	3	98660	1	0	0.00

RS - Reconstitution solution/Mobile Phase

*-Qualitative evaluation only

TABLE 2

SELECTIVITY OF OLMESARTAN

Instrument Name: LC-MS/MS							
	Selectivity	**Olmesartan**			**Candesartan (ISTD)**		
S.No	**K_2EDTA Plasma Batch No.**	**LLOQ area**	**Interference**		**ISTD Area**	**Interference**	
			Area	**%Area**		**Area**	**%Area**
1	2705	975	3	0.41	109655	0	0.00
2	2706	716	0	0.00	88137	2	0.00
3	2707	707	0	0.00	89575	4	0.00
4	2708	692	0	0.00	87386	3	0.00
5	2709	654	0	0.00	78956	1	0.00
6	2710	677	0	0.00	85274	2	0.00
7	2711	-	0	0.00	-	0	0.00
8	2712	-	0	0.00	-	3	0.00
	Mean	**737**			**89831**		

TABLE 3

Back Calculated Standard Concentrations (ng/mL)of Olmesartan and Calibration curve parameters

CC ID	STD-1	STD-2	STD-3	STD-4	STD-5	STD-6	STD-7	STD-8	slope	intercept	r^2
Nominal Concentrations (ng/mL)											
Instrument	**5.000**	**10.001**	**25.002**	**200.019**	**400.037**	**800.715**	**1200.472**	**1500.590**			
Calculated Concentrations (ng/mL)											
LC-MS/MS	5.214	9.080	25.402	194.300	421.123	800.107	1237.047	1469.726	0.0012	-0.0005	0.9972
	5.148	9.260	25.837	200.081	428.630	797.626	1186.120	1433.141	0.0014	-0.0005	0.9974
	5.246	8.910	25.658	194.919	420.690	804.049	1220.329	1480.733	0.0014	-0.0004	0.9967
	5.174	9.605	23.408	173.866	408.640	836.212	1326.912	1542.915	0.0011	-0.0001	0.9932
Mean	**5.196**	**9.214**	**25.076**	**190.792**	**419.771**	**809.499**	**1242.602**	**1481.629**			
±SD	**0.0432**	**0.2974**	**1.1264**	**11.5775**	**8.2675**	**18.0043**	**60.0703**	**45.6419**			
%CV	**0.83**	**3.23**	**4.49**	**6.07**	**1.97**	**2.22**	**4.83**	**3.08**			
%Nominal	**103.91**	**92.13**	**100.30**	**95.39**	**104.93**	**101.10**	**103.51**	**98.74**			

r^2 – Regression coefficient

TABLE 4(A)

Back-Calculated Concentration of QC Samples (ng/mL) for Olmesartan (Within-Batch) (Contd…)

Batch ID	Instrument ID	LLOQ QC			LQC			MQC			HQC		
		Nominal Concentrations (ng/mL)											
		QC ID	5.000	%Nominal	QC ID	14.994	%Nominal	QC ID	635.326	%Nominal	QC ID	1151.370	%Nominal
P & A –I & (–70°C)	LC-MS/MS	QC-01	5.612	**112.24**	QC-01	14.691	**97.98**	QC-01	529.640	**83.37**	QC-01	1164.211	**101.12**
		QC-02	5.312	**106.24**	QC-02	15.270	**101.84**	QC-02	613.132	**96.51**	QC-02	1200.962	**104.31**
		QC-03	5.434	**108.68**	QC-03	15.822	**105.52**	QC-03	631.469	**99.39**	QC-03	1190.864	**103.43**
		QC-04	5.624	**112.48**	QC-04	15.651	**104.38**	QC-04	610.341	**96.07**	QC-04	1221.935	**106.13**
		QC-05	5.432	**108.64**	QC-05	15.736	**104.95**	QC-05	604.815	**95.20**	QC-05	1212.109	**105.28**
		QC-06	5.725	**114.50**	QC-06	15.828	**105.56**	QC-06	614.078	**96.66**	QC-06	1223.430	**106.26**
		Mean	**5.523**		**Mean**	**15.500**		**Mean**	**600.579**		**Mean**	**1202.252**	
		±SD	**0.1547**		**±SD**	**0.4466**		**±SD**	**35.8875**		**±SD**	**22.4123**	
		%CV	**2.80**		**%CV**	**2.88**		**%CV**	**5.98**		**%CV**	**1.86**	
		%Nominal	**110.46**		**%Nominal**	**103.37**		**%Nominal**	**94.53**		**%Nominal**	**104.42**	

P & A – II & (– 70°C)	LC-MS/MS	QC-07	5.743	**114.86**	QC-07	14.182	**94.58**	QC-07	545.137	**85.80**	QC-07	1103.085	**95.81**
		QC-08	5.342	**106.84**	QC-08	14.158	**94.42**	QC-08	568.698	**89.51**	QC-08	1079.494	**93.76**
		QC-09	4.835	**96.70**	QC-09	13.792	**91.98**	QC-09	574.852	**90.48**	QC-09	1144.268	**99.38**
		QC-10	5.541	**110.82**	QC-10	15.474	**103.20**	QC-10	603.340	**94.97**	QC-10	1184.782	**102.90**
		QC-11	5.405	**108.10**	QC-11	15.499	**103.37**	QC-11	591.803	**93.15**	QC-11	1169.361	**101.56**
		QC-12	5.706	**114.12**	QC-12	15.242	**101.65**	QC-12	633.055	**99.64**	QC-12	1194.754	**103.77**
		Mean	**5.429**		**Mean**	**14.725**		**Mean**	**586.148**		**Mean**	**1145.957**	
		±SD	**0.3313**		**±SD**	**0.7634**		**±SD**	**30.4690**		**±SD**	**46.2482**	
		%CV	**6.10**		**%CV**	**5.18**		**%CV**	**5.20**		**%CV**	**4.04**	
		%Nominal	**108.57**		**%Nominal**	**98.20**		**%Nominal**	**92.26**		**%Nominal**	**99.53**	
P & A – III & (– 70°C)	LC-MS/MS	QC-13	5.576	**111.52**	QC-13	13.828	**92.22**	QC-13	583.220	**91.80**	QC-13	1115.765	**96.91**
		QC-14	4.924	**98.48**	QC-14	14.379	**95.90**	QC-14	563.842	**88.75**	QC-14	1122.742	**97.51**
		QC-15	5.181	**103.62**	QC-15	14.538	**96.96**	QC-15	573.449	**90.26**	QC-15	1099.178	**95.47**
		QC-16	5.036	**100.72**	QC-16	14.146	**94.34**	QC-16	565.899	**89.07**	QC-16	1124.764	**97.69**
		QC-17	5.064	**101.28**	QC-17	14.352	**95.72**	QC-17	587.288	**92.44**	QC-17	1153.253	**100.16**
		QC-18	5.373	**107.46**	QC-18	15.280	**101.91**	QC-18	591.474	**93.10**	QC-18	1159.528	**100.71**
		Mean	**5.192**		**Mean**	**14.421**		**Mean**	**577.529**		**Mean**	**1129.205**	
		±SD	**0.2420**		**±SD**	**0.4869**		**±SD**	**11.5007**		**±SD**	**22.9849**	
		%CV	**4.66**		**%CV**	**3.38**		**%CV**	**1.99**		**%CV**	**2.04**	
		%Nominal	**103.85**		**%Nominal**	**96.18**		**%Nominal**	**90.90**		**%Nominal**	**98.07**	

TABLE 4 (B)

Back-Calculated Concentration of QC Samples (ng/mL) for Olmesartan (Between-Batch)

Batch ID	Instrument	LLOQ QC			LQC			MQC			HQC		
		Nominal Concentrations (ng/mL)											
		QC ID	**5.000**	**%Nominal**	**QC ID**	**14.994**	**%Nominal**	**QC ID**	**635.326**	**%Nominal**	**QC ID**	**1151.370**	**%Nominal**
P & A – IV & (–70°C)	LC-MS/MS	QC-19	5.226	**104.52**	QC-19	14.917	**99.49**	QC-19	717.906	**113.00**	QC-19	1296.015	**112.56**
		QC-20	5.250	**105.00**	QC-20	13.199	**88.03**	QC-20	695.863	**109.53**	QC-20	1245.635	**108.19**
		QC-21	5.627	**112.54**	QC-21	15.618	**104.16**	QC-21	642.888	**101.19**	QC-21	1244.872	**108.12**
		QC-22	5.797	**115.94**	QC-22	14.974	**99.87**	QC-22	586.463	**92.31**	QC-22	1256.482	**109.13**
		QC-23	5.132	**102.64**	QC-23	15.876	**105.88**	QC-23	649.092	**102.17**	QC-23	1187.573	**103.14**
		QC-24	5.185	**103.70**	QC-24	16.101	**107.38**	QC-24	648.865	**102.13**	QC-24	1272.105	**110.49**
		Mean	**5.370**		**Mean**	**15.114**		**Mean**	**656.846**		**Mean**	**1250.447**	
		±SD	**0.2736**		**±SD**	**1.0521**		**±SD**	**45.8761**		**±SD**	**36.3024**	
		%CV	**5.10**		**%CV**	**6.96**		**%CV**	**6.98**		**%CV**	**2.90**	
		%Nominal	**107.39**		**%Nominal**	**100.80**		**%Nominal**	**103.39**		**%Nominal**	**108.61**	

Back-Calculated Concentration of QC Samples for Olmesartan (Between-batch)

QC ID	LLOQ QC	LQC	MQC	HQC
Actual Conc. (ng/mL)	**5.000**	**14.994**	**635.236**	**1151.370**
Mean	**5.378**	**14.940**	**605.275**	**1181.965**
±SD	**0.2707**	**0.7965**	**44.3160**	**58.0577**
%CV	**5.03**	**5.33**	**7.32**	**4.91**
%Nominal	**107.57**	**99.64**	**95.28**	**102.66**

TABLE 5

SHORT TERM STOCK SOLUTION STABILITY FOR OLMESARTAN

Short-term stability of Olmesartan and Candesartan (ISTD) in stock solution

Date of Experiment: 03-04-12			Instrument :LC-MS/MS	
Sr. No.	Olmesartan		Candesartan (ISTD)	
	Comparison	Stability (6.25 Hours)	Comparison	Stability (6.25 Hours)
1	78465	74734	87456	87757
2	78087	73820	85459	85590
3	74180	72704	85656	85150
4	72829	71413	84885	83318
5	71737	71310	83263	81527
6	69827	70156	82982	81300
Mean	**74188**	**72356**	**84950**	**84107**
±SD	**3474.7**	**1717.1**	**1659.0**	**2520.6**
%CV	**4.68**	**2.37**	**1.95**	**3.00**
%Stability	**97.53**		**99.01**	

TABLE 6

LONG TERM STOCK SOLUTION STABILITY FOR OLMESARTAN

Long-term stability of Olmesartan & Candesartan (ISTD) in stock solution

Date of Experiment: 10-04-12			Instrument :LC-MS/MS	
Sr. No.	Olmesartan		Candesartan(ISTD)	
	Comparison	Stability (7.14 days)	Comparison	Stability (7.14 days)
Conc. (ng/mL)	**1000.593**	**1001.191**	**1001.742**	**1000.583**
Correction factor	**N/AP**	**0.9994**	**N/AP**	**1.0012**
1	116956	116554	138503	134838
2	111897	113998	134596	125956
3	106393	107130	130341	123304
4	101936	101986	127711	122859
5	97868	100448	124562	119781
6	94897	92512	123753	116891
Mean	**104991**	**105438**	**129911**	**123938**
± SD	**8423.5**	**8984.4**	**5785.0**	**6186.9**
%CV	**8.02**	**8.52**	**4.45**	**4.99**
Corrected Mean	**-**	**105375**	**-**	**124082**
%Stability	**100.37**		**95.51**	

TABLE 7

Zero-day Long-term stability of analyte in human K_2EDTA plasma

Back Calculated Concentrations for CC Standards of Olmesartan

CC ID	STD 1	STD 2	STD 3	STD 4	STD 5	STD 6	STD 7	STD 8	slope	Intercept	r^2
Actual Conc. (ng/mL)	**5.000**	**10.001**	**25.002**	**200.019**	**400.037**	**800.715**	**1200.472**	**1500.590**			
LT Fresh CC Curve	5.114	9.153	27.769	174.351	416.030	824.351	1227.137	1482.581	0.0018	-0.0006	0.9930
% Nominal	**102.28**	**91.52**	**111.07**	**87.17**	**104.00**	**102.95**	**102.22**	**98.80**			

r^2= regression coefficient

Zero-day Long-term stability of Olmesartan in human K_2EDTA plasma

Date of Experiment: 04-04-12		**Instrument :LC-MS/MS**
Olmesartan in human K_2EDTA plasma	**Long-Term stability in human K_2EDTA plasma for zero day**	
	LQC	**HQC**
	Nominal Concentration (ng/mL)	
QC ID	**14.994**	**1151.370**
1 (LT)	14.548	1132.057
2 (LT)	14.245	1156.096
3 (LT)	14.060	1193.039
4 (LT)	14.785	1172.541
5 (LT)	14.039	1177.878
6 (LT)	14.767	1165.942
Mean	**14.407**	**1166.259**
±SD	**0.3390**	**20.8077**
%CV	**2.35**	**1.78**
%Nominal	**96.09**	**101.29**

TABLE 8

FREEZE THAW STABILITY OF OLMESARTAN

Back Calculated Concentrations for CC Standards of Olmesartan

CC ID	STD 1	STD 2	STD 3	STD 4	STD 5	STD 6	STD 7	STD 8	slope	Intercept	r^2
Actual Conc. (ng/mL)	**5.001**	**10.001**	**25.004**	**200.029**	**400.057**	**800.115**	**1200.472**	**1500.590**			
CC Curve	5.245	9.369	22.838	197.535	403.221	798.917	1232.643	1621.073	0.0009	-0.0008	0.9962
% Nominal	**104.88**	**93.68**	**91.34**	**98.75**	**100.79**	**99.85**	**102.68**	**108.03**			

Freeze thaw stability of Olmesartan

Date of Experiment: 09-04-12		**Instrument :LC-MS/MS**
Olmesartan	**Freeze thaw stability (5 Cycles)**	
	LQC	**HQC**
	Nominal Concentration (ng/mL)	
QC ID	**14.994**	**1151.370**
QC-49	15.430	1216.475
QC-50	14.271	1202.897
QC-51	14.307	1208.741
QC-52	14.646	1152.221
QC-53	14.651	1145.581
QC-54	14.673	1147.034
Mean	**14.663**	**1178.825**
±SD	**0.4169**	**33.8099**
%CV	**2.84**	**2.87**
%Nominal	**97.79**	**102.38**

Details of Freeze-thaw Cycles:

FT Cycle ID	Freezing Time & Date (at-70°C) Storage Location: Deep Freezer	Thawing Time & Date (at room temperature of ~ 25°C) Storage Location: Sample processing area	Time difference between Freezing & Thawing
FT Cycle-1	03:15 PM & 04-04-2012	03:55 PM & 05-04-2012	24.67 hrs
FT Cycle-2	05:56 PM & 05-04-2012	09:30 AM &06-04-2012	15.57 hrs
FT Cycle-3	11:15 AM & 06-04-2012	09:32 AM & 07-04-2012	22.28 hrs
FT Cycle-4	11:31 AM & 07-04-2012	10:53AM & 08-04-2012	23.37 hrs
FT Cycle-5	11:59AM & 08-04-2012	01:35PM & 09-04-2012	25.60 hrs

Note: The storage time of samples in deep freezer is considered as freezing time & retrieval time of samples from Deep freezer is considered as thawing time.

TABLE 9

BENCH TOP STABILITY OF OLMESARTAN

Back Calculated Concentrations for CC Standards of Olmesartan

CC ID	STD 1	STD 2	STD 3	STD 4	STD 5	STD 6	STD 7	STD 8	slope	Intercept	r^2
Actual Conc. (ng/mL)	**5.001**	**10.001**	**25.004**	**200.029**	**400.057**	**800.115**	**1200.472**	**1500.590**			
CC Curve	5.264	9.291	23.004	182.346	415.574	831.343	1233.357	1623.053	0.0009	-0.0006	0.9943
% Nominal	**105.26**	**92.90**	**92.00**	**91.16**	**103.88**	**103.90**	**102.74**	**108.16**			

r^2= regression coefficient

Bench Top stability of Olmesartan

Date of Experiment: 09-04-12		**Instrument :LC-MS/MS**
Olmesartan	**Bench top stability (5.28 hrs)**	
	LQC	**HQC**
	Nominal Concentration (ng/mL)	
QC ID	**14.994**	**1151.370**
QC-61	16.151	1133.205
QC-62	14.614	1168.817
QC-63	14.832	1126.146
QC-64	14.661	1166.108
QC-65	13.208	1143.448
QC-66	15.414	1165.271
Mean	**14.813**	**1150.499**
±SD	**0.9788**	**18.6508**
%CV	**6.61**	**1.62**
%Nominal	**98.80**	**99.92**

TABLE 10
IN-INJECTOR STABILITY OF OLMESARTAN
Back Calculated Concentrations for CC Standards of Olmesartan

CC ID	STD 1	STD 2	STD 3	STD 4	STD 5	STD 6	STD 7	STD 8	slope	Intercept	r^2
Actual Conc. (ng/mL)	**5.001**	**10.001**	**25.004**	**200.029**	**400.057**	**800.115**	**1200.472**	**1500.590**			
FT-IIJ FCC	5.245	9.369	22.838	197.535	403.221	798.917	1232.643	1621.073	0.0009	-0.0008	0.9962
% Nominal	**104.88**	**93.68**	**91.34**	**98.75**	**100.79**	**99.85**	**102.68**	**108.03**			

In-Injector stability of Olmesartan

Date of Experiment: 09-04-12		**Instrument ID: LC-MS/MS**
Olmesartan	**In-Injector stability at 10°C (49.78 hr)**	
	LQC	**HQC**
	Nominal Concentration (ng/mL)	
QC ID	**14.994**	**1151.370**
QC-43	15.041	1195.365
QC-44	14.551	1182.548
QC-45	15.074	1187.689
QC-46	14.619	1099.108
QC-47	16.036	1041.903
QC-48	15.991	1036.181
Mean	**15.219**	**1123.799**
±SD	**0.6515**	**74.3621**
%CV	**4.28**	**6.62**
%Nominal	**101.50**	**97.61**

Table 11

WET EXTRACT STABILITY OF OLMESARTAN

Back Calculated Concentrations for CC Standards of Olmesartan

CC ID	STD 1	STD 2	STD 3	STD 4	STD 5	STD 6	STD 7	STD 8	slope	Intercept	r^2
Actual Conc. (ng/mL)	**5.001**	**10.001**	**25.004**	**200.029**	**400.057**	**800.115**	**1200.472**	**1500.590**			
CC Curve	5.284	9.238	22.786	187.911	405.980	819.700	1261.261	1619.215	0.0009	-0.0008	0.9947
% Nominal	**105.66**	**92.37**	**91.13**	**93.94**	**101.48**	**102.45**	**105.06**	**107.91**			

r^2= regression coefficient

Wet extract stability of Olmesartan

Date of Experiment: 09-04-12		**Instrument :LC-MS/MS**
Olmesartan	**Wet extract stability below 10°C (45.37 hr)**	
	LQC	**HQC**
	Nominal Concentration (ng/mL)	
QC ID	**14.994**	**1151.370**
QC-37	14.363	1153.318
QC-38	14.576	1178.598
QC-39	14.454	1148.521
QC-40	14.482	1172.006
QC-41	14.350	1204.025
QC-42	14.699	1188.125
Mean	**14.487**	**1174.099**
±SD	**0.1328**	**20.9905**
%CV	**0.92**	**1.79**
%Nominal	**96.62**	**101.97**

Table 12

DRY EXTRACT STABILITY OF OLMESARTAN

Back Calculated Concentrations for CC Standards of Olmesartan

CC ID	STD 1	STD 2	STD 3	STD 4	STD 5	STD 6	STD 7	STD 8	slope	Intercept	r^2
Actual Conc. (ng/mL)	**5.001**	**10.001**	**25.004**	**200.029**	**400.057**	**800.115**	**1200.472**	**1500.590**			
CC Curve	5.284	9.238	22.786	187.911	405.980	819.700	1261.261	1619.215	0.0009	-0.0008	0.9947
% Nominal	**105.66**	**92.37**	**91.13**	**93.94**	**101.48**	**102.45**	**105.06**	**107.91**			

r^2= regression coefficient

Dry Extract stability of Olmesartan

Date of Experiment: 09-04-12		Instrument :LC-MS/MS
Olmesartan	Dry Extract stability below 10°C (45.37 hr)	
	LQC	HQC
	Nominal Concentration (ng/mL)	
QC ID	14.994	1151.370
QC-31	14.515	1152.244
QC-32	14.400	1158.221
QC-33	14.519	1126.134
QC-34	14.704	1156.765
QC-35	14.398	1139.143
QC-36	14.666	1144.008
Mean	14.534	1146.086
±SD	0.1291	12.2460
%CV	0.89	1.07
%Nominal	96.93	99.54

TABLE 13

Long-term stability of Analyte in human K_2EDTA plasma (after storage for 8.80 days at-70°C)

Back Calculated Concentrations for CC Standards of Olmesartan

CC ID	STD 1	STD 2	STD 3	STD 4	STD 5	STD 6	STD 7	STD 8	slope	Intercept	r^2
Actual Conc. (ng/mL)	5.001	10.003	25.007	200.059	400.118	800.236	1199.754*	1499.692			
CC Curve	5.238	9.300	23.527	193.163	392.770	883.205	1446.703	1546.639	0.0050	0.0035	0.9949
% Nominal	104.74	92.97	94.08	96.55	98.16	110.37	120.58	103.13			

r^2= regression coefficient * - Out of acceptance limits, not included in CC curve

Zero day stability Data of Olmesartan Long-Term Stability data of Olmesartan in human K_2EDTA plasma in human K_2EDTAplasmaafter storage of 8.80 days (-70°C)

Back calculated concentrations of Fresh QC samples

Date of Experiment: 04-04-12		Instrument ID: LC-MS/MS
Nominal Concentrations (ng/mL)		
QC ID	LQC	HQC
	14.994	1151.370
1 (LT)	14.548	1132.057
2 (LT)	14.245	1156.096
3 (LT)	14.060	1193.039
4 (LT)	14.785	1172.541
5 (LT)	14.039	1177.878
6 (LT)	14.767	1165.942
Mean	14.407	1166.259
±SD	0.3390	20.8077
%CV	2.35	1.78
%Nominal	96.09	101.29

Back calculated concentrations of Stability QC Samples

Date of Experiment: 13-04-12		Instrument ID: LC-MS/MS
Nominal concentrations (ng/mL)		
QC ID	LQC	HQC
	14.994	1151.370
13 (LT)	16.139	1195.610
14(LT)	16.645	1175.842
15(LT)	16.183	1151.440
16(LT)	16.732	1129.563
17(LT)	16.911	1131.476
18(LT)	15.978	1153.085
Mean	16.431	1156.169
±SD	0.3791	25.6598
%CV	2.31	2.22
%Nominal	109.59	100.42

LT Stability in Human K_2EDTA Plasma at -70°C	LQC	HQC
Zero day QC's Mean back Calculated Concentrations (ng/mL)	14.407	1166.259
Stability QC's Mean back Calculated Concentrations (ng/mL) after storage of 8.80 days (-70°C)	16.431	1156.169
%Stability compare to zero day concentration (-70°C)	114.05	99.13

TABLE 14

DILUTION INTEGRITY OF OLMESARTAN

Back Calculated Concentrations for CC Standards of Olmesartan

CC ID	STD 1	STD 2	STD 3	STD 4	STD 5	STD 6	STD 7	STD 8	slope	Intercept	r^2
Actual Conc. (ng/mL)	5.000	10.001	25.002	200.019	400.037	800.715	1200.472	1500.590			
CC Curve	5.108	9.547	25.257	195.454	365.008	856.749	1235.234	1538.050	0.0019	0.0006	0.9970
% Nominal	102.16	95.46	101.02	97.72	91.24	107.00	102.90	102.50			

r^2= regression coefficient

Dilution Integrity for DQC in human K_2EDTA plasma

Date of Experiment: 07-04-12		Instrument :LC-MS/MS
Olmesartan in K_2EDTA plasma	Dilution Integrity	
	1/2 Dilution (50:50)	1/4 Dilution (25:75)
	Nominal Concentration (ng/mL)	
DQC ID	**3001.180**	**3001.180**
DQC-01	3096.350	2505.585
DQC-02	3173.668	2589.020
DQC-03	3262.835	2859.735
DQC-04	3004.594	3036.626
DQC-05	3051.802	2837.769
DQC-06	3142.443	2785.827
Mean	**3121.949**	**2769.094**
±SD	**91.9694**	**193.2092**
%CV	**2.95**	**6.98**
%Nominal	**104.02**	**92.27**

TABLE 15
RUGGEDNESS (P&A-5)
Back Calculated Concentrations for CC Standards of Olmesartan

	STD 1	STD 2	STD 3	STD 4	STD 5	STD 6	STD 7	STD 8	Slope	Intercept	r^2
Actual conc. (ng/mL)	**5.000**	**10.001**	**25.002**	**200.019**	**400.037**	**800.715**	**1200.472**	**1500.590**			
CC 05	5.211	9.338	23.997	186.092	409.867	824.629	1238.825	1572.025	0.0012	-0.0003	0.9969
% Nominal	**104.22**	**93.37**	**95.98**	**93.04**	**102.46**	**102.99**	**103.19**	**104.76**			

r^2= regression coefficient

Back Calculated Concentrations for QC samples of Olmesartan

Batch ID & Date	Instrument ID	LLOQ QC			LQC			MQC			HQC		
		Nominal Concentrations (ng/mL)											
		QC ID	5.000	%Nominal	QC ID	14.994	%Nominal	QC ID	635.326	%Nominal	QC ID	1151.370	%Nominal
P & A-5	LC-MS/MS	**QC-25**	5.158	**103.16**	**QC-25**	16.247	**108.36**	**QC-25**	645.784	**101.65**	**QC-25**	1167.867	**101.43**
		QC-26	5.635	**112.70**	**QC-26**	15.543	**103.66**	**QC-26**	636.819	**100.23**	**QC-26**	1261.700	**109.58**
		QC-27	5.948	**118.96**	**QC-27**	15.158	**101.09**	**QC-27**	589.956	**92.86**	**QC-27**	1273.768	**110.63**
		QC-28	5.752	**115.04**	**QC-28**	13.237	**88.28**	**QC-28**	676.888	**106.54**	**QC-28**	1229.296	**106.77**

		QC-29	5.369	**107.38**	**QC-29**	15.661	**104.45**	**QC-29**	630.002	**99.16**	**QC-29**	1110.061	**96.41**
		QC-30	5.335	**106.70**	**QC-30**	15.153	**101.06**	**QC-30**	642.692	**101.16**	**QC-30**	1144.992	**99.45**
		Mean	**5.533**		**Mean**	**15.167**		**Mean**	**637.024**		**Mean**	**1197.947**	
		±SD	**0.2957**		**±SD**	**1.0273**		**±SD**	**28.1523**		**±SD**	**66.6769**	
		%CV	**5.35**		**%CV**	**6.77**		**%CV**	**4.42**		**%CV**	**5.57**	
		%Nominal	**110.66**		**%Nominal**	**101.15**		**%Nominal**	**100.27**		**%Nominal**	**104.05**	

Table18

Matrix effect, Recovery and Process efficiency for Olmesartan and Candesartan (IS) evaluated with Aqueous (neat) samples, Post-extracted and Extracted from 6 different K_2EDTA blank plasma lots

Standard ID	**Analyte Conc (ng/mL)**	**Number of samples**	**Analyte Peak response (Olmesartan Data)**								
			Aqueous samples (neat)			**Post-extracted samples**			**Extracted samples**		
			Mean	**S.D**	**%CV**	**Mean**	**S.D**	**%CV**	**Mean**	**S.D**	**%CV**
Standard-1	5.000	N=6	643	16.7	2.59	645	9.9	1.54	592	35.0	5.91
Standard-3	25.002	N=6	2660	68.5	2.57	2708	63.9	2.36	2379	55.5	2.33
Standard-5	400.037	N=6	44001	1611.0	3.66	43092	1338.6	3.11	36526	549.3	1.50
Standard-6	800.715	N=6	84765	2198.1	2.59	86423	1631.9	1.89	77638	2000.7	2.58
Standard-8	1500.590	N=6	155021	2480.7	1.60	156631	5046.4	3.22	144551	5363.5	3.71
Standard ID	**Analyte Conc (ng/mL)**	**Number of samples**	**ISTD Peak response (CandesartanData)**								
			Aqueous samples (neat)			**Post-extracted samples**			**Extracted samples**		
			Mean	**S.D**	**%CV**	**Mean**	**S.D**	**%CV**	**Mean**	**S.D**	**%CV**
Standard-1	5.000	N=6	99866	1569.9	1.57	101360	2254.6	2.22	68681	2672.1	3.89
Standard-3	25.002	N=6	101155	1874.4	1.85	99850	2365.0	2.37	68132	1904.8	2.80
Standard-5	400.037	N=6	101262	3771.1	3.72	99885	2100.3	2.10	66318	997.1	1.50
Standard-6	800.715	N=6	94951	1412.0	1.49	96382	1449.2	1.50	67781	1584.4	2.34
Standard-8	1500.590	N=6	93591	1676.9	1.79	94399	2496.4	2.64	68840	2412.6	3.50
Standard ID	**Analyte Conc (ng/mL)**	**Number of samples**	**%Matrix effect**			**% Recovery**			**% Process efficiency**		
			Analyte	**ISTD**		**Analyte**	**ISTD**		**Analyte**	**ISTD**	
Standard-1	5.000	N=6	100.34	101.50		91.75	67.76		92.06	68.77	
Standard-3	25.002	N=6	101.78	98.71		87.88	68.23		89.44	67.35	
Standard-5	400.037	N=6	97.93	98.64		84.76	66.39		83.01	65.49	
Standard-6	800.715	N=6	101.96	101.51		89.83	70.33		91.59	71.38	
Standard-8	1500.590	N=6	101.04	100.86		92.29	72.92		93.25	73.55	
		Mean	**100.61**	**100.24**	**Mean**	**89.30**	**69.13**	**Mean**	**89.87**	**69.31**	
		S.D	**1.627**	**1.455**	**S.D**	**3.075**	**2.549**	**S.D**	**4.074**	**3.202**	
		%CV	**1.62**	**1.45**	**%CV**	**3.44**	**3.69**	**%CV**	**4.53**	**4.62**	

%Matrix effect: Post extracted mean response/Aqueous (neat) mean response x 100, **%Recovery:** Extracted mean response / Post extracted mean response x 100, % **Process efficiency:** Extracted mean response / Aqueous mean response x 100

FIGURE NO.1
Representative Parent ions for Olmesartan and Candesartan

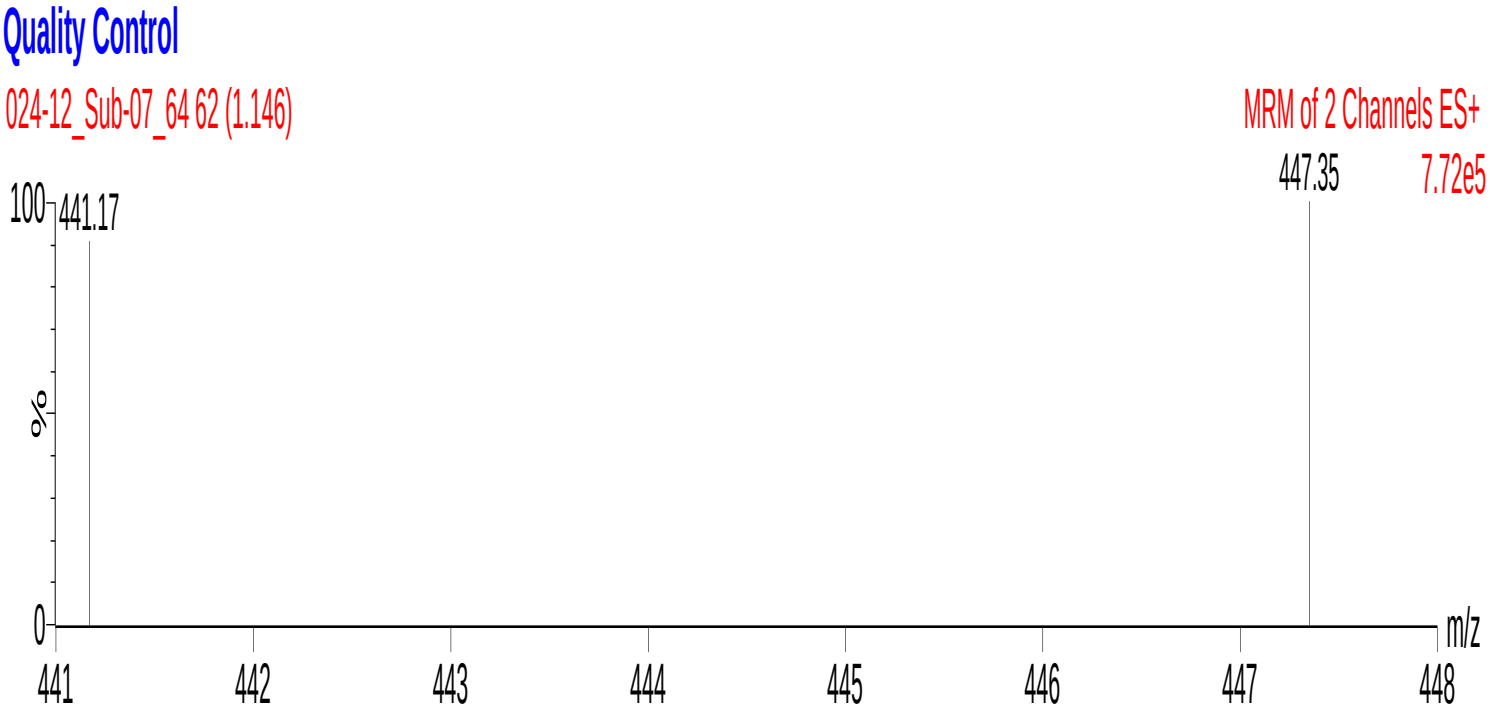


FIGURE No.2
Representative Calibration Curve of Olmesartan

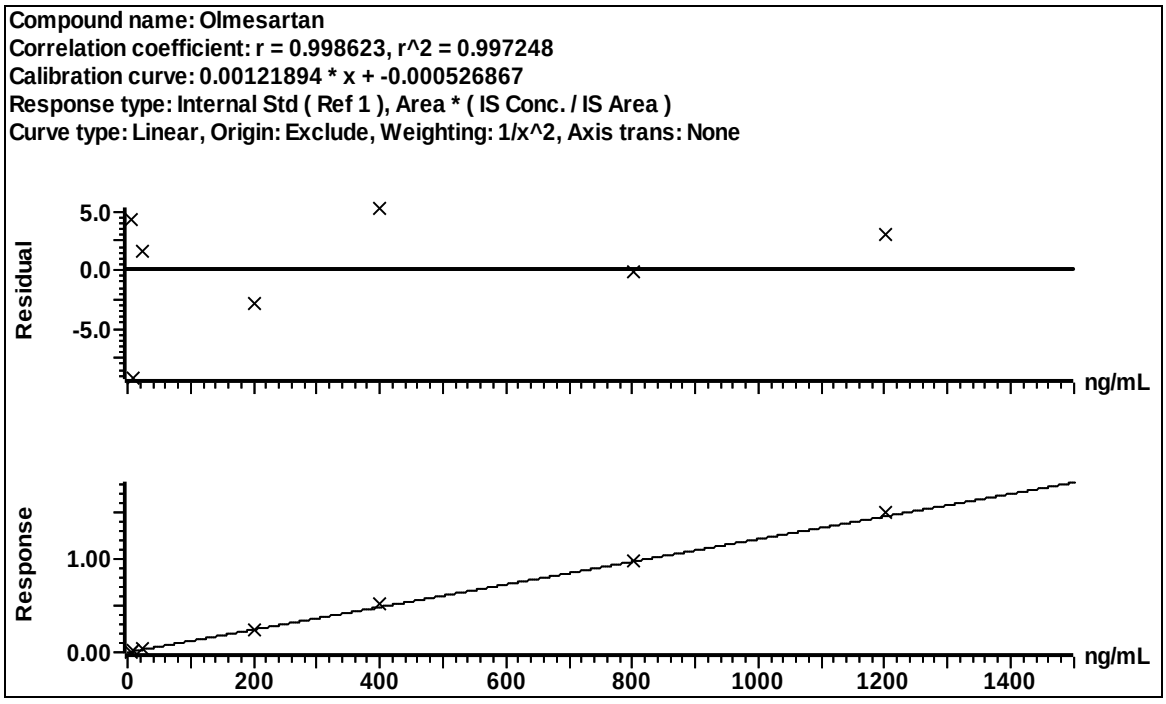
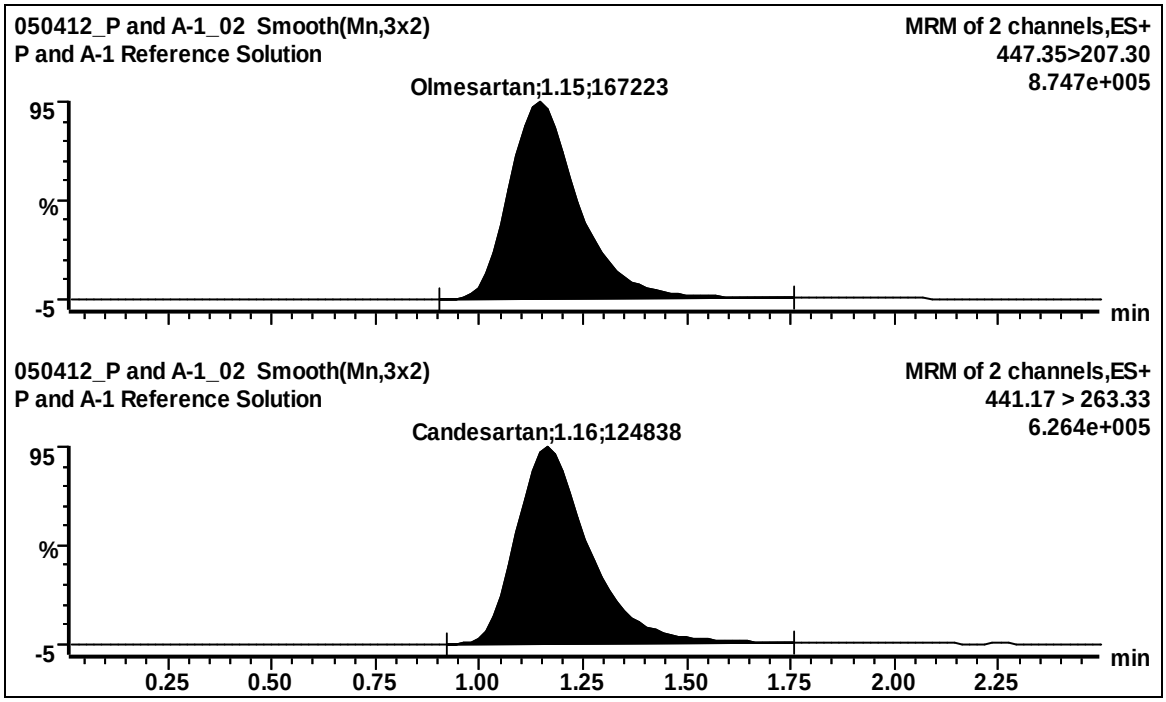


FIGURE No.3

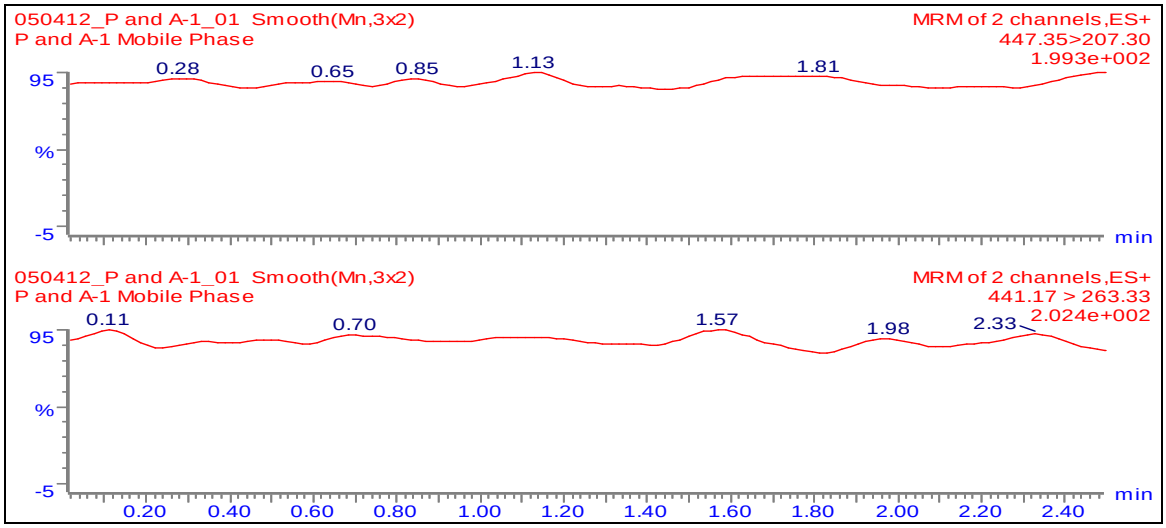
Representative Chromatogram of Aqueous Standards (Olmesartan& Candesartan)



Drug Name	Retention time	Area
Olmesartan	1.15	167223
Candesartan (ISTD)	1.16	124838

FIGURE No. 4

Representative Chromatogram of Blank Plasma

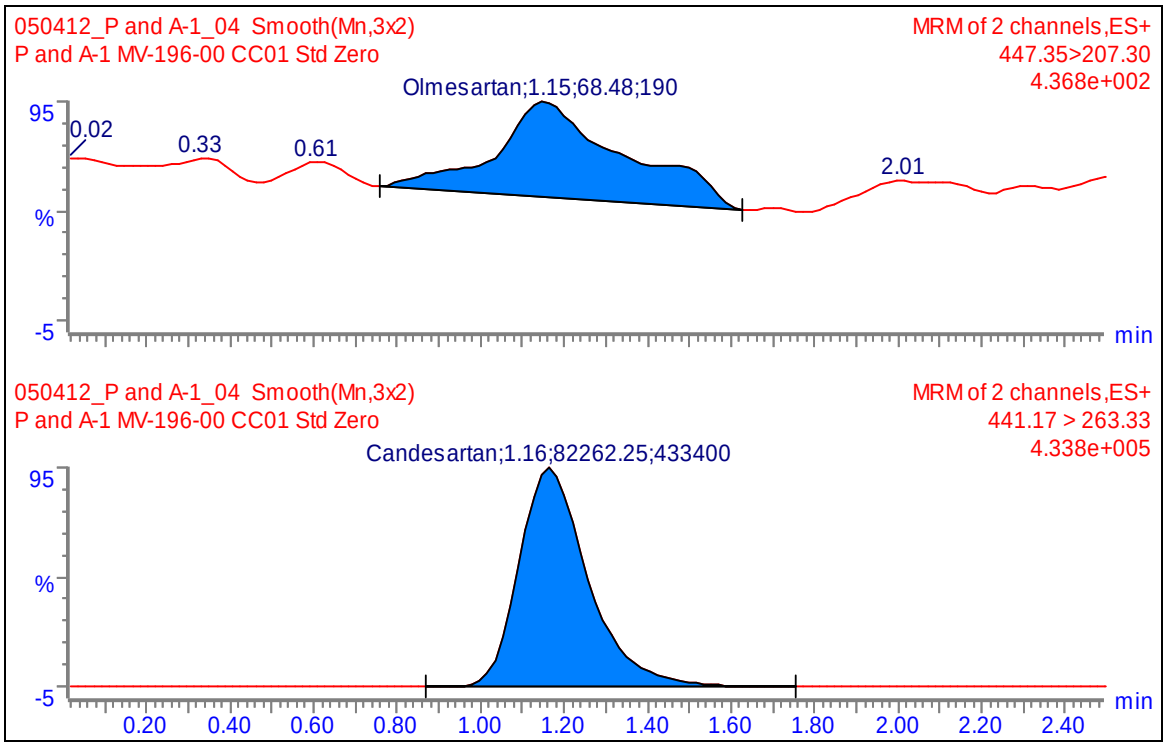


Drug Name	Retention time	Area

Olmesartan	-	0
Candesartan (ISTD)	-	0

FIGURE No. 5

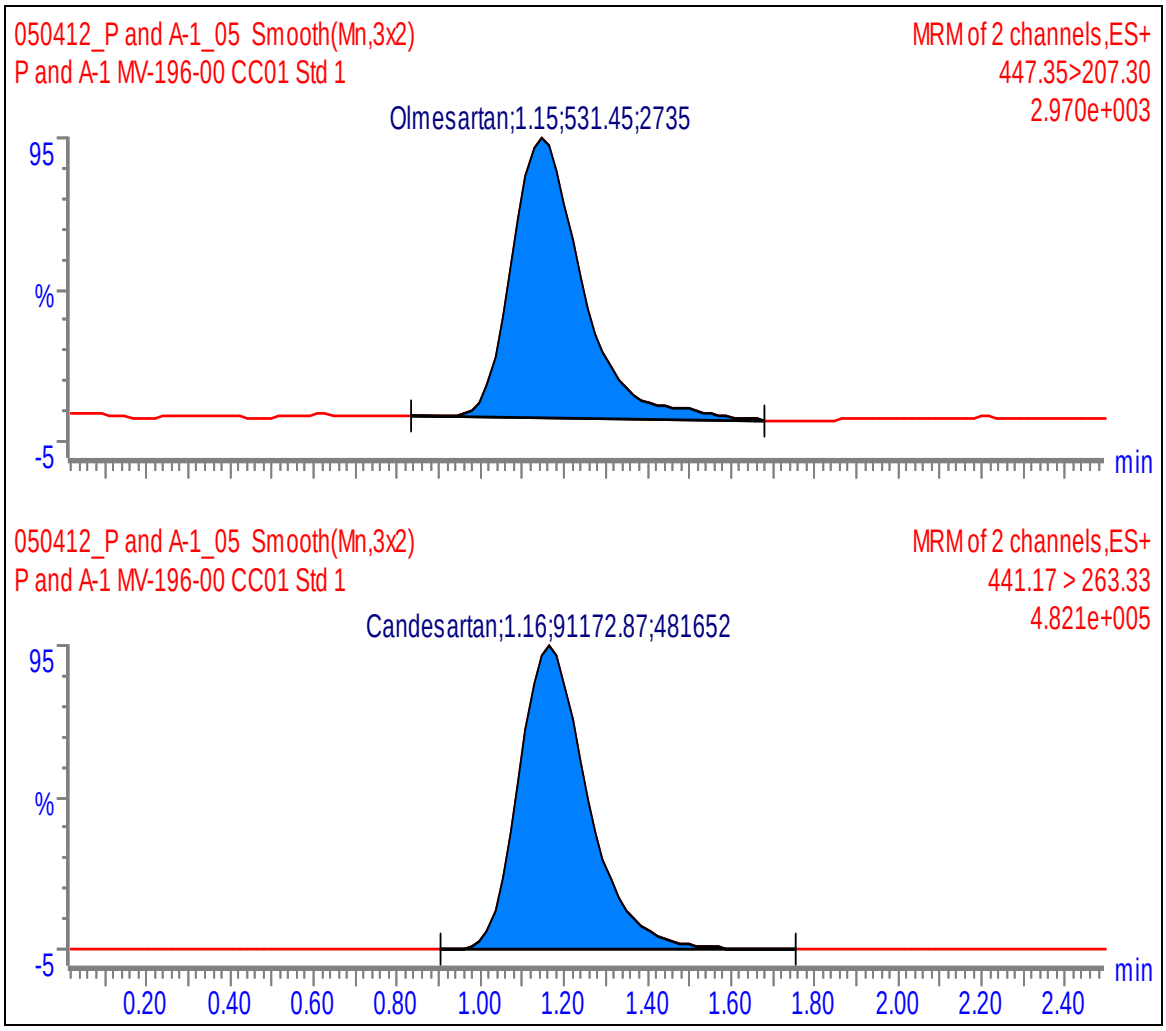
Representative Chromatogram of Blank Plasma with Internal Standard (Candesartan)



Drug Name	Retention time	Area
Olmesartan	1.15	68
Candesartan (ISTD)	1.16	82262

FIGURE No. 6

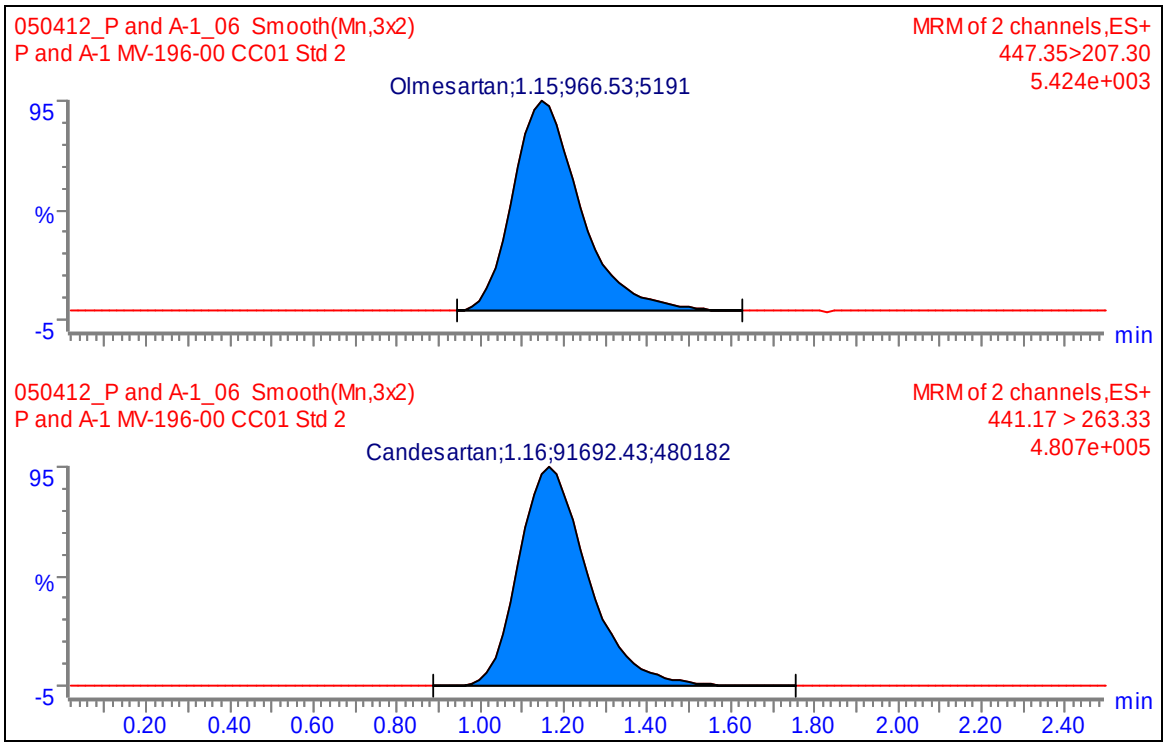
Representative Chromatogram of Calibration Curve Standard-1 Sample (Olmesartan)



Drug Name	Retention time	Area
Olmesartan	1.15	531
Candesartan (ISTD)	1.16	91173

FIGURE No. 7

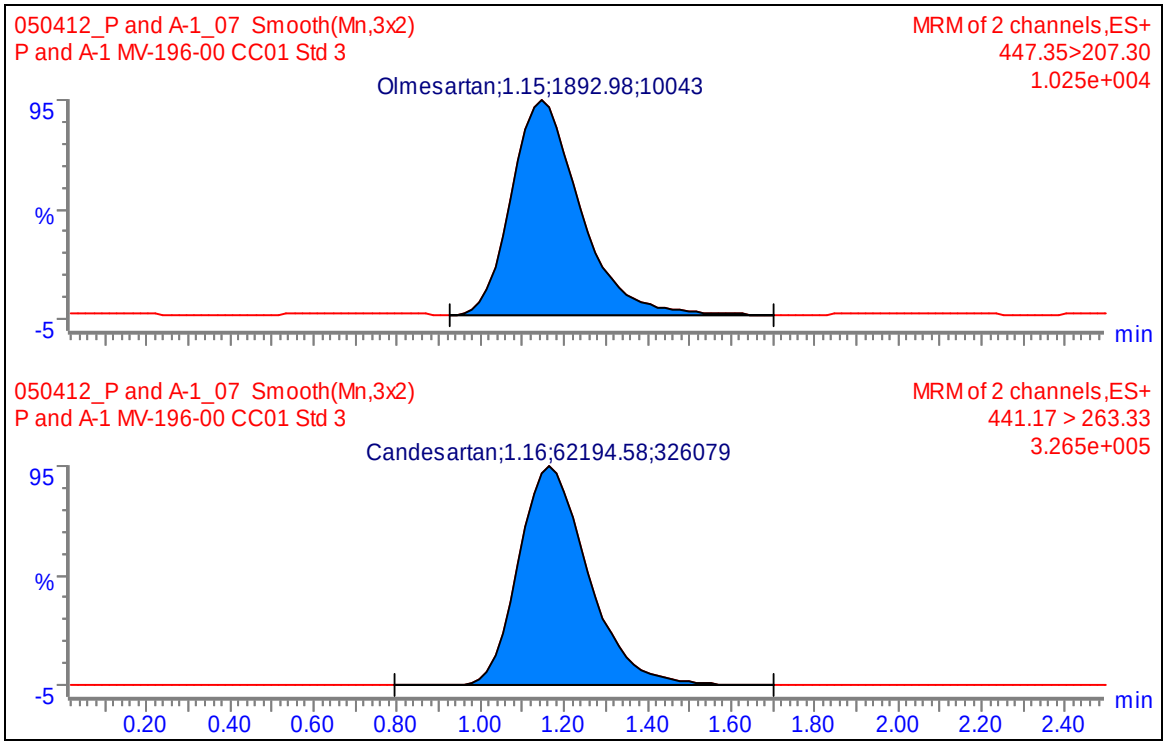
Representative Chromatogram of Calibration Curve Standard-2 Sample (Olmesartan)



Drug Name	Retention time	Area
Olmesartan	1.15	967
Candesartan (ISTD)	1.16	91692

FIGURE No. 8

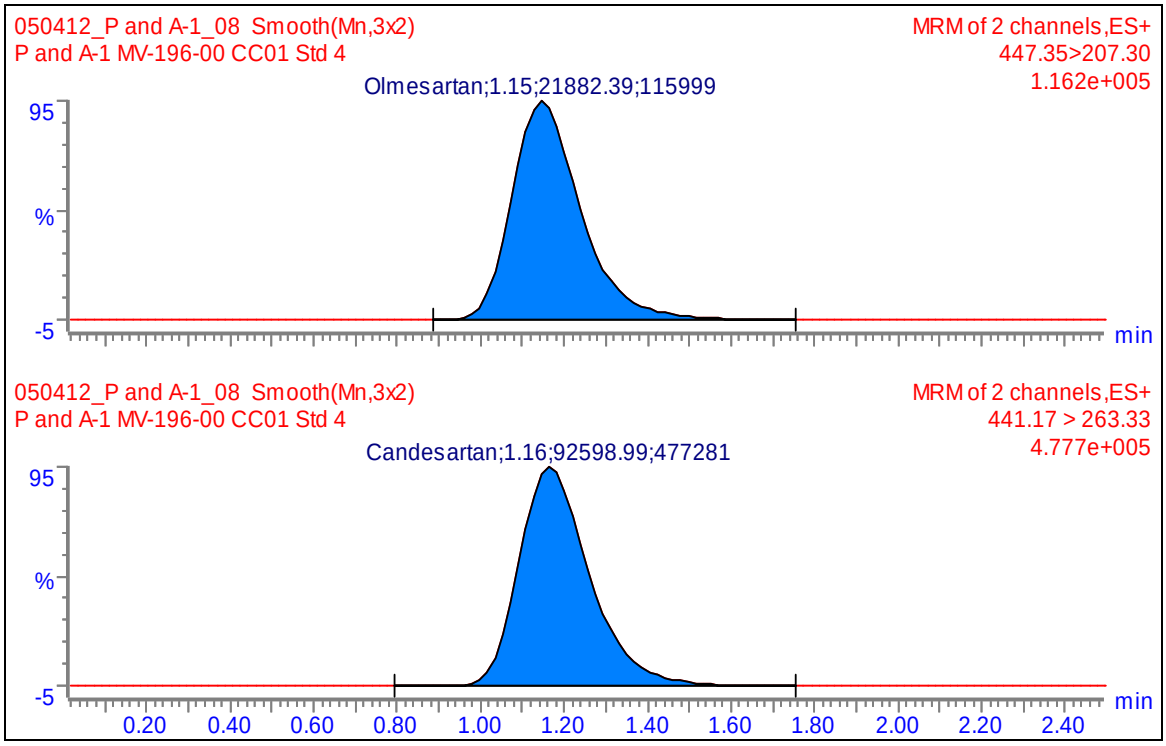
Representative Chromatogram of Calibration Curve Standard-3 Sample (Olmesartan)



Drug Name	Retention time	Area
Olmesartan	1.15	1893
Candesartan (ISTD)	1.16	62195

FIGURE No. 9

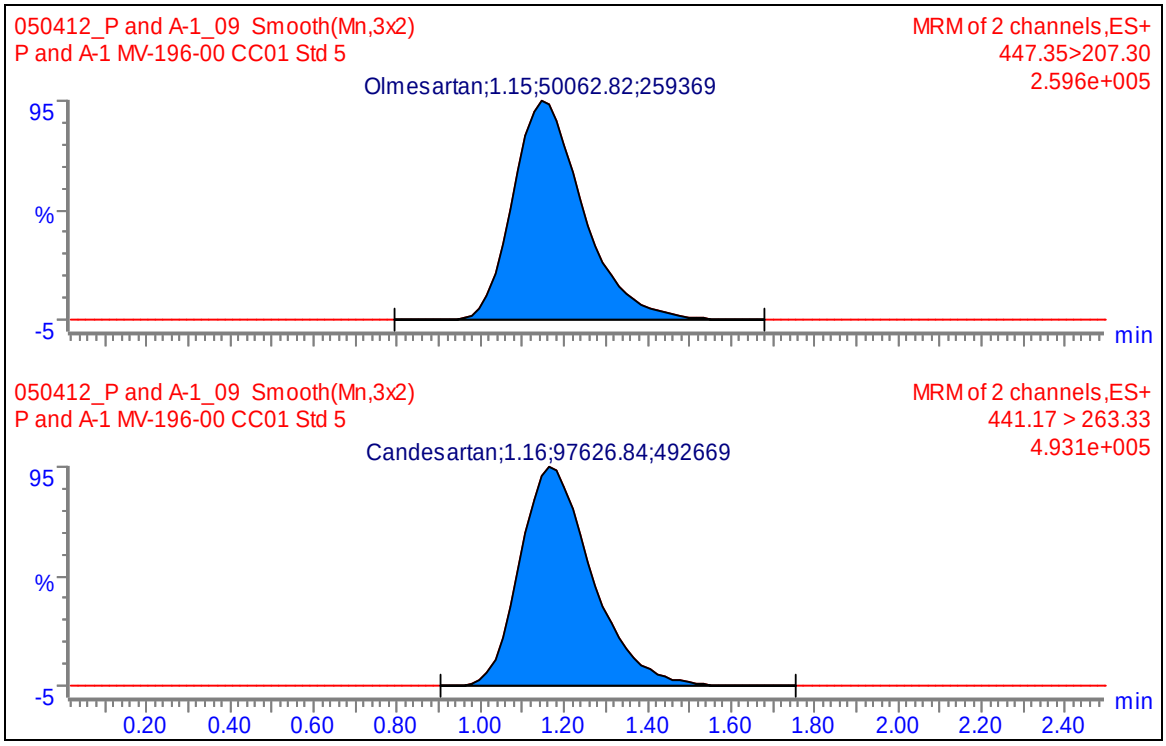
Representative Chromatogram of Calibration Curve Standard-4 Sample (Olmesartan)



Drug Name	Retention time	Area
Olmesartan	1.15	21882
Candesartan (ISTD)	1.16	92599

FIGURE No. 10

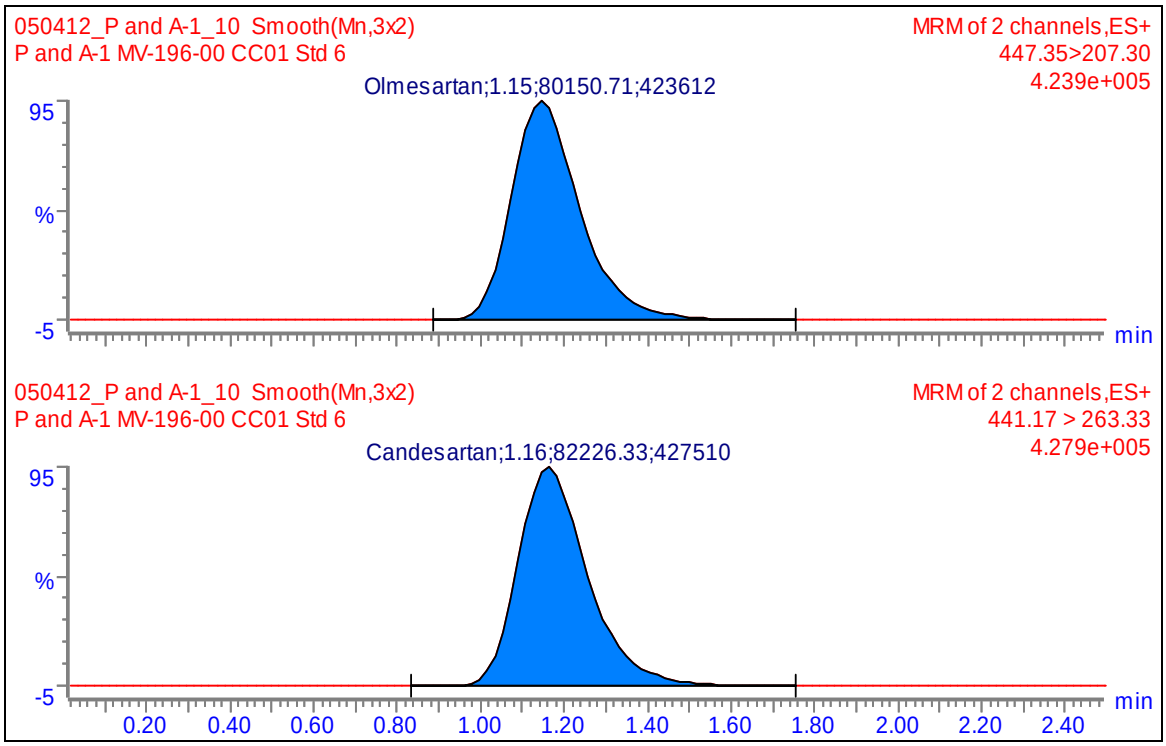
Representative Chromatogram of Calibration Curve Standard-5 Sample (Olmesartan)



Drug Name	Retention time	Area
Olmesartan	1.15	50063
Candesartan (ISTD)	1.16	97626

FIGURE No. 11

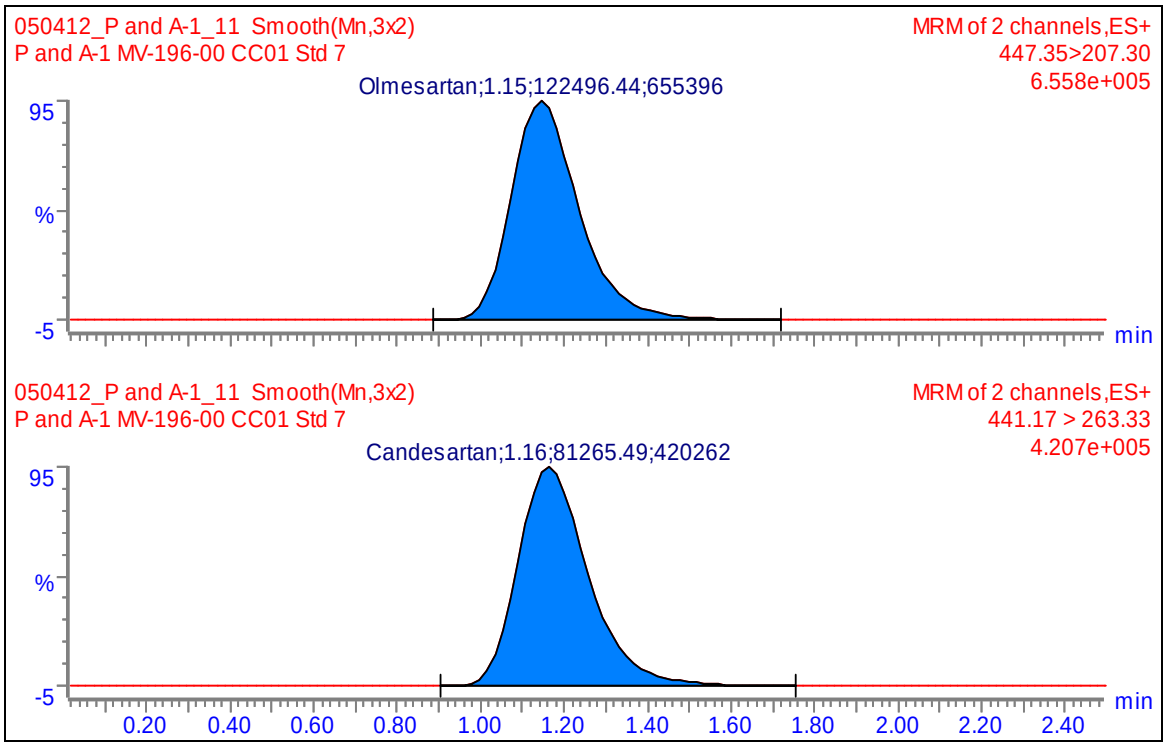
Representative Chromatogram of Calibration Curve Standard-6 Sample (Olmesartan)



Drug Name	Retention time	Area
Olmesartan	1.15	80151
Candesartan (ISTD)	1.16	82226

FIGURE No. 12

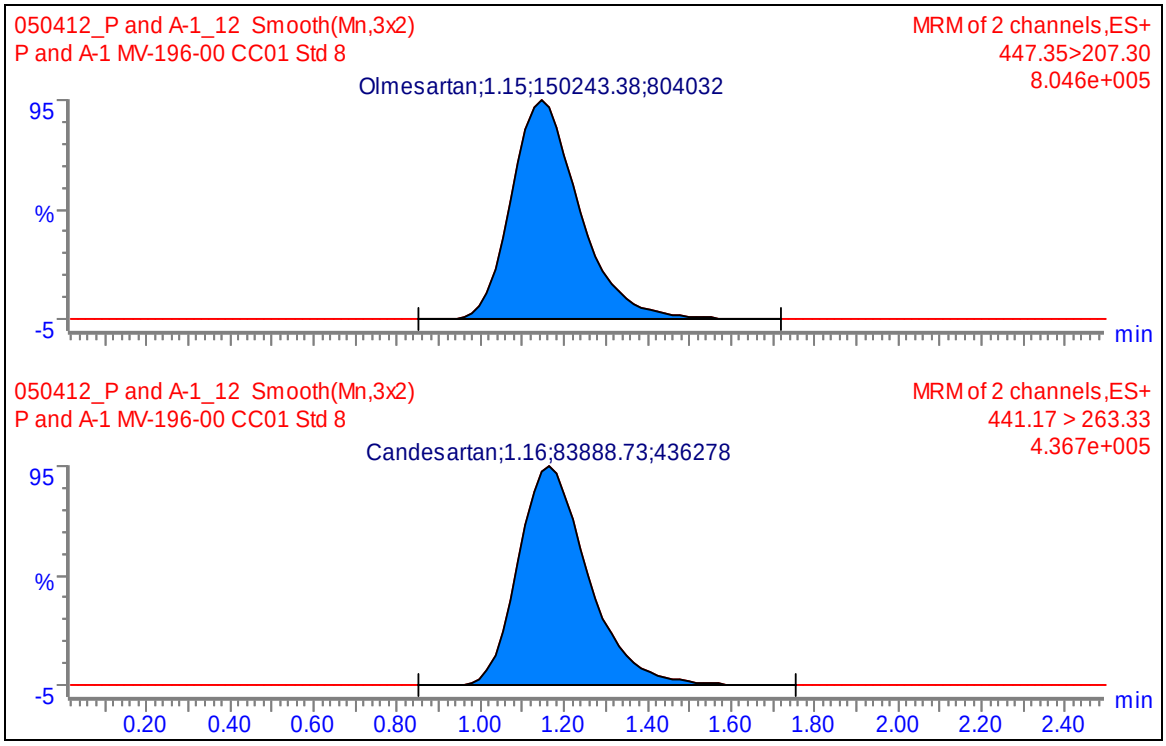
Representative Chromatogram of Calibration Curve Standard-7 Sample (Olmesartan)



Drug Name	Retention time	Area
Olmesartan	1.15	122496
Candesartan (ISTD)	1.16	81265

FIGURE No. 13

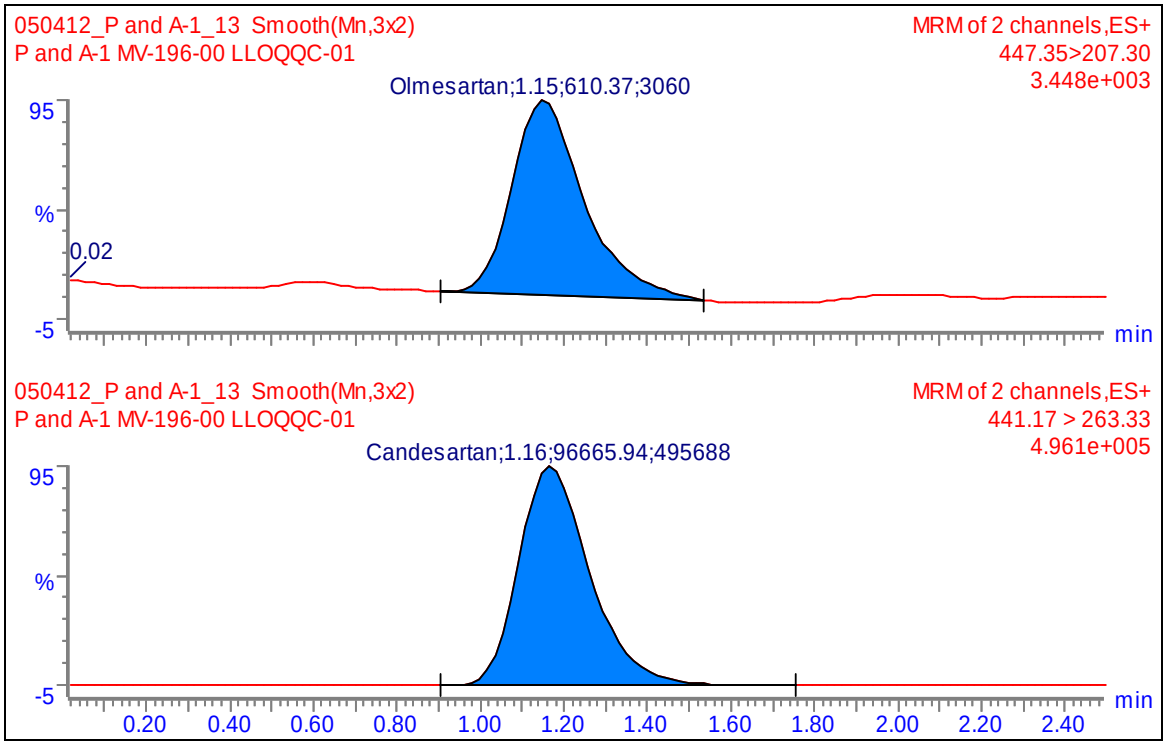
Representative Chromatogram of Calibration Curve Standard-8 Sample (Olmesartan)



Drug Name	Retention time	Area
Olmesartan	1.15	150243
Candesartan (ISTD)	1.16	83888

FIGURE No. 14

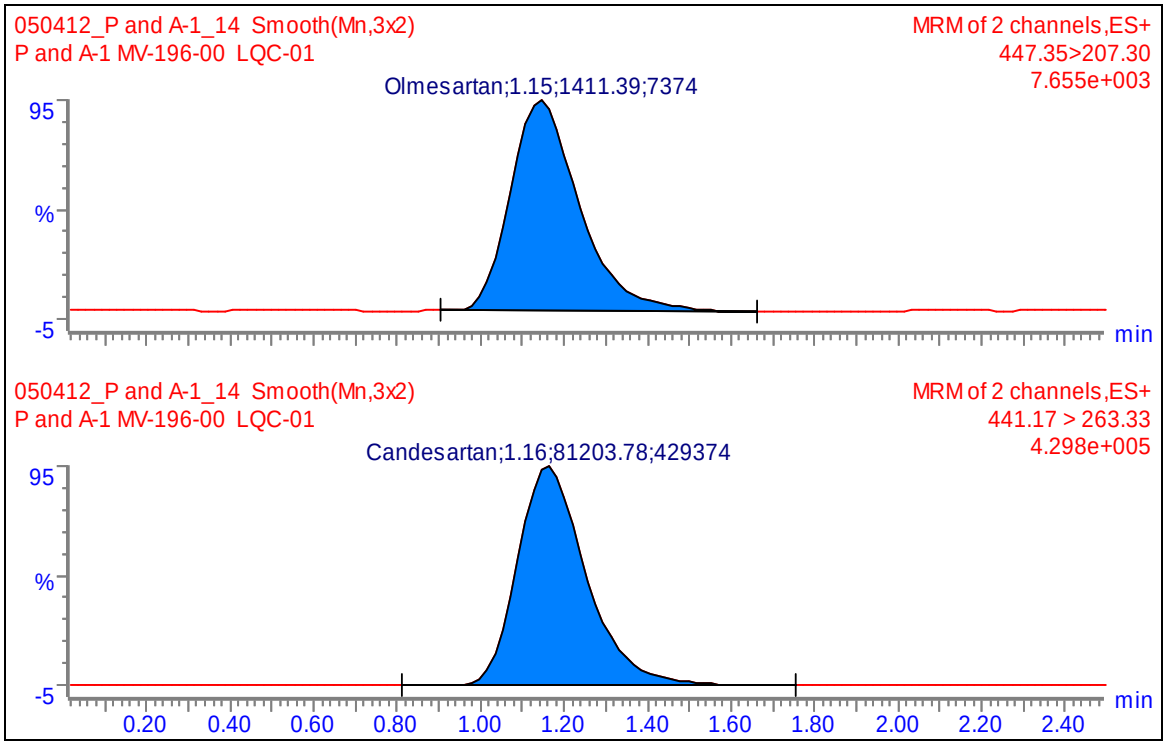
Representative Chromatogram of LLOQ QC Sample (Olmesartan)



Drug Name	Retention time	Area
Olmesartan	1.15	610
Candesartan (ISTD)	1.16	96666

FIGURE No. 15

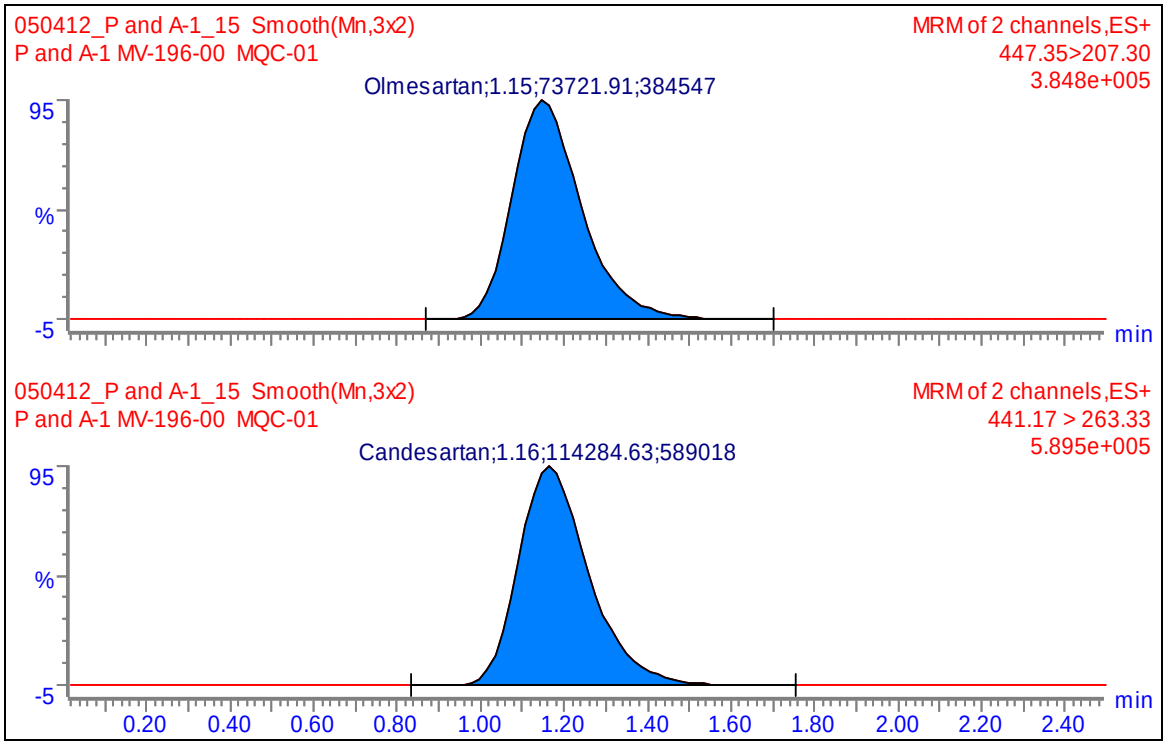
Representative Chromatogram of LQC Sample (Olmesartan)



Drug Name	Retention time	Area
Olmesartan	1.15	1411
Candesartan (ISTD)	1.16	81204

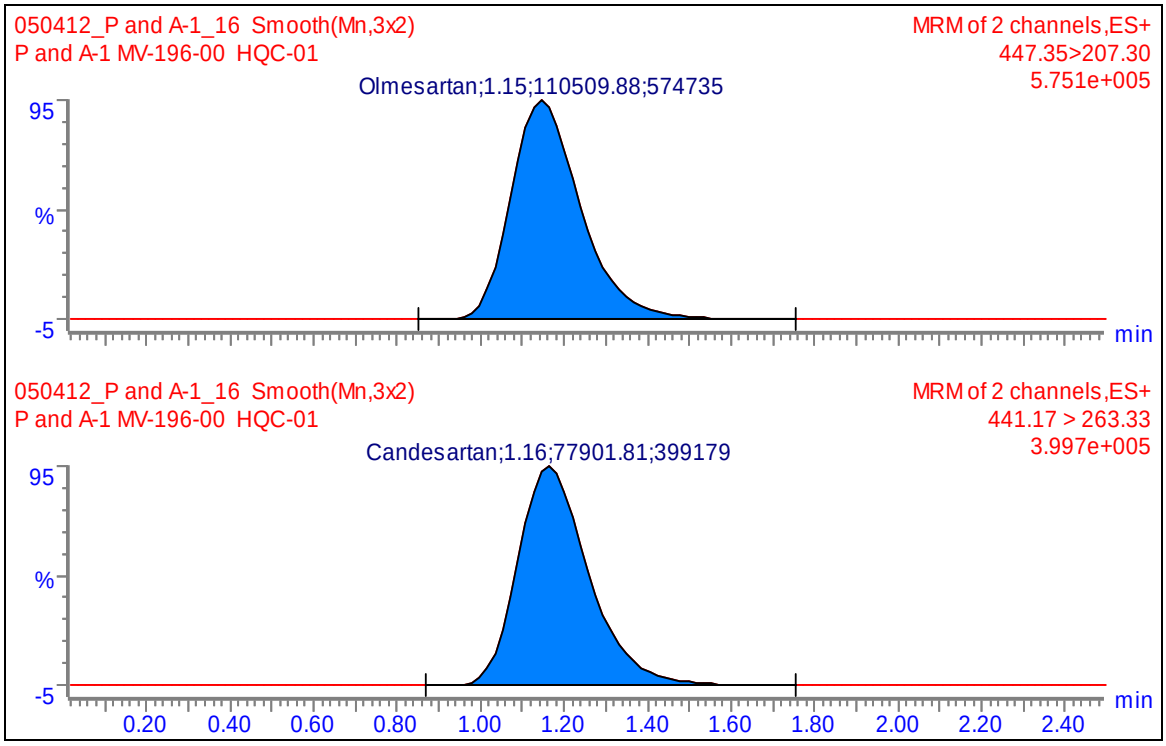
FIGURE No. 16

Representative Chromatogram of MQC Sample (Olmesartan)



Drug Name	Retention time	Area
Olmesartan	1.15	73722
Candesartan (ISTD)	1.16	114285

FIGURE No. 17
Representative Chromatogram of HQC Sample (Olmesartan)



Drug Name	Retention time	Area
Olmesartan	1.15	110510
Candesartan (ISTD)	1.16	77902

CONCLUSION

Based on the data presented in this report, it can be concluded that the present method is validated for the estimation of Olmesartan in human K_2EDTA plasma over the concentration range of 5.000 to 1500.590 ng/mL. The method for determination of Olmesartan in human plasma using HPLC with tandem mass spectrometric detection has met the acceptance criteria with respect to selectivity, precision, accuracy, linearity, recovery, re-injection reproducibility, Process efficiency, extended P&A, dilution integrity and matrix effect over a theoretical concentration range of 5.000 to 1500.590 ng/mL for Olmesartan. Stability evaluations performed in human K_2EDTA plasma have met the acceptance criteria, demonstrating insignificant degradation of Olmesartan over the specified storage durations and conditions. Stability evaluations performed in stock solutions have met the acceptance criteria, demonstrating insignificant degradation of Olmesartan & Internal standard (Candesartan) over the specified storage durations and conditions.

This method can be used for quantitation of Olmesartan in human K_2EDTA plasma for Bioequivalence studies with tandem mass spectrometric detection.

SUMMARY

The contents of the Thesis have been divided in to Six Chapters. The Aim and Objective was placed after 1st Chapter and the conclusion was placed after 5th chapter

Chapter-I: Begins with the introduction giving a brief account of Pharmaceutical Analysis, Analytical method development, High Performance liquid Chromatography, MassSpectroscopy and Bioanalytical method and its validation Procedures. After that Aim and Objective of the present thesis was explained.
Chapter-II: It explains the brief information about Drug profile.
Chapter-III: Review of Literature that gives important details collected from previously published bioanalytical methods for Olmesartan.
Chapter-IV: It presents the detailed information about the developed method parameters.
Chapter-5: This chapter gives the Validation experiments details and its Acceptance criteria and the performed experiment results along the tables and chromatogram figures. After that conclusion was written for above results.
Chapter-VI: The list of references were given

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