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A novel method for the simultaneous determination of Azelnidipine and Olmesartan in Human plasma by using liquid chromatography-electro spray Ionization tandem mass spectrometry and application to a pharmacokinetic study

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ABSTRACT

A novel rapid, specific and sensitive liquid chromatography tandem mass spectrometry (LCMS/MS) method was developed for the simultaneous determination of Azelnidipine and Olmesartan in K_2EDTA human plasma. The method involves simple, solid phase extraction procedure and separation with an C18 column (5 µm, 100 x 4.6 mm) with Acetonitrile/5mM Ammonium Formate pH-3.00 [80/20, V/V] isocratic elution at a flow-rate of 1.0 mL/min with a total run time of 3.0 minutes. Labeled isotopes were used as the internal standard. The protonate of analyte's were quantitated in positive ionization by multiple reaction monitoring with a mass spectrometer. The mass transitions m/z 583.300 →167.200 and m/z 447.400 →207.000 were used to measure Azelnidipine and Olmesartan respectively. The method was developed and validated using 200 µL of plasma, over a concentration range of 0.100 – 40.070 ng/mL for Azelnidipine and 3.001 – 1200.340 ng/mL for Olmesartan. The intra and inter day Precision and Accuracy values were found to be within the assay variability limits as per regulatory guidelines. The method was successfully applied to a pharmacokinetic study involving a single oral administration of a combination tablet to human male volunteers.

Keywords: Azelnidipine, Olmesartan, tandem mass spectrometry, HPLC, LC-MS/MS, Human plasma.

1. INTRODUCTION

Azelnidipine is chemically 3-[1-(diphenylmethyl) azetidin-3-yl] 5-propan-2-yl 2-amino-6-methyl-4-(3-nitrophenyl) 1, 4-dihydropyridine-3, 5-dicarboxylate. Azelnidipine, a novel dihydropyridine derivative, is a L-type calcium channel blocker and antihypertensive. Unlike other L-type calcium channel blockers, Azelnidipine causes minimal stimulation of the sympathetic nervous system despite its significant depressor effect. Azelnidipine may have a protective role in inflammation in atherosclerosis. Hypertension is the most common chronic disease and the calcium channel antagonist is the most popularly used antihypertensive drug in Chinese patients. Azelnidipine is a third generation and long-acting dihydropyridine calcium channel antagonist.(1)

Olmesartan is chemically 4-(2-hydroxypropan-2-yl)-2-propyl-1-({4-[2-(1H-1, 2, 3, 4-tetrazol -5-yl) phenyl] phenyl} methyl)-1H-imidazole-5-carboxylic acid. Olmesartan is an ARB that selectively inhibits the binding of angiotensin II to AT1, which is found in many tissues such as vascular smooth muscle and the adrenal glands. This effectively inhibits the AT1-mediated vasoconstrictive and aldosterone-secreting

effects of angiotensin II and results in a decrease in vascular resistance and blood pressure. Olmesartan is selective for AT1 and has a 12,500 times greater affinity for AT1 than the AT2 receptor [(1)].

Very few analytical methods have been reported for the determination of single Azelnidipine includes HPLC [2, 3], LC-MS method [4, 5], LC-ESI -MS [6, 7], HPLC-MS-MS [8] and few analytical methods have been reported for the determination of Olmesartan in biological fluids includes HPLC [9, 10], LC-MS-MS [11-13], Simultaneous analysis with HPLC-UV [14]. The UV and HPLC methods have the issue of sensitivities. Remaining LC-MS/MS methods were developed individually. But no LCMS/MS method has so far been reported for the simultaneous determination of these drugs in pharmaceutical preparations as well as biological fluids. So this is only the method simultaneous estimation of both the analyte's in single run by using LC-ESI-MS/MS with major regulatory guidelines [15-20].

2. EXPERIMENTAL

2.1. Material and methods:

Azelnidipine {chemical purity 99.77 %, $C_{33}H_{34}N_4O_6$, MW= 582.657} and its Internal standard Azelnidipine D7 {chemical purity 98.80%, $C_{33}H_{27}D_7N_4O_6$, MW= 589.29} (Fig. 1 A & B) were obtained from Clearsynth (Mumbai, India). Olmesartan {chemical purity 96.71 %, $C_{24}H_{26}N_6O_3$, MW= 446.502} and its Internal standard Olmesartan D4 {chemical purity 98.71%, $C_{24}H_{22}N_6O_3D_4$, MW= 450.50} (Fig. 1 C & D) were obtained from Clearsynth (Mumbai, India). HPLC grade solvents acetonitrile, methanol, formic acid, water and ammonium formate were Merck products (Merck, India). Human plasma with K_2EDTA as anticoagulant was obtained from in-house clinical facility of Aizant Drug Research Solutions Pvt Ltd.

2.2. Instrumentation:

Liquid chromatography with tandem mass spectrometry detection was performed on a API Sciex 4000 (MS/MS) quadruple mass spectrometer equipped with an electro spray ionization (ESI) probe interfaced to a separation module Nexera XR UFLC system from Shimadzu.

2.3. Chromatographic condition:

A shimadzu liquid chromatography Nexera XR system, consisting of an auto sampler, a multichannel mobile phase degasser, a column heater, two pumps and a Hypurity 5µ, C18, 100 × 4.6 mm (Thermo, India), was used for the chromatographic separation of Azelnidipine, Olmesartan and its internal standard's. The mobile phase used was Acetonitrile/ 5mM Ammonium Formate (Adjusted pH-3.00 with formic acid) (80/20, v/v). Flow rate was set to 1.00 mL/min with 30 % flow splitting to the mass spectrometer. Column oven temperature was 40 ± 5 ºC and auto sampler temperature was 5 ± 3ºC. Volume of injection was 15 µL and runtime was 3.0 minutes.

2.4. Mass spectrometric conditions:

Analyte's were detected by tandem mass spectrometry using multiple reaction monitoring (MRM) of precursor-product ion transitions with 200 ms dwell time, at m/z 583.300/167.200 for Azelnidipine, m/z 590.400/167.100 for Azelnidipine D7 and at m/z 447.400/207.000 for Olmesartan, m/z 451.400/211.000 for Olmesartan D4 . The instrument dependent parameters were optimized and maintained as follows: curtain gas gas, 30; Gas 1 and Gas 2, 45; Ion spray voltage, 5.5 kV; source temperature, 500ºC. Compound dependent parameters like Declustering Potential 90 for Azelnidipine and 115 for Olmesartan, collision energy 40 for Azelnidipine and 33 for Olmesartan, entrance potential 10 for Azelnidipine and Olmesartan. Data acquisition and processing were performed using Analyst software, Version 1.6.2.

2.5. Preparation of stock solution, standard and quality control samples:

Stock solutions of Azelnidipine, Azelnidipine D7 and Olmesartan, Olmesartan D4 were prepared by dissolving accurately weighed standard compounds in methanol to yield a concentration of 1 mg/mL. All subsequent dilutions were made with methanol/water 50/50 v/v. Standard working solutions at concentrations of 0.100, 0.200, 0.401, 0.801, 2.003, 4.007, 10.017, 20.035, 32.056 and 40.070 ng/mL for Azelnidipine and 3.001, 6.002, 12.003, 24.007, 60.017, 120.034, 300.085, 600.170, 960.272 and 1200.340 for Olmesartan were prepared by serial dilutions. QC working solutions at concentrations of 0.100, 0.300, 1.467, 3.667, 18.333 and 30.556 ng/mL for Azelnidipine and 3.001, 8.987, 43.966, 109.914, 549.571 and 915.952 for Olmesartan were also prepared by successively diluting the 1mg/mL QC stock solution.

The internal standard stock solution was diluted to a working concentration of 20 ng/ml for Azelnidipine and 250 ng/mL for Olmesartan. These working solutions were stored at 2-8 ºC. The linearity curve was built by 2% spiking of drug into the screened human plasma.

2.6. Solid-Phase Extraction (SPE) procedure:

Extracted Sample Preparation:

Plasma samples frozen at below -25ºC were thawed at room temperature followed by vortexing to ensure homogeneity. For the determination of Azelnidipine and Olmesartan, 50 µL of ISTD working solution was transferred to polypropylene tubes followed by 200 µL of spiked plasma and vortexed for 5 seconds. To this

200 µL of extraction buffer was added and vortexed for about 10 seconds, then loaded the sample into pre-labeled Strata-X polymeric reversed phase extraction cartridges (30 mg/1mL). Washed the cattridges with 0.1% formic acid in water followed by 5% methanol in water, dry the cattridge with high pressure for 1 minute, eluted the cartridge with 0.600 mL of methanol and evaporated to dryness under nitrogen gas at 40 ± 5°C using TurboVap (Caliper life sciences, United States). Finally 0.300 mL of reconstitution solution was added to all the tubes and vortexed for about 1 minute. An appropriate volume of the reconstituted solution was transferred into pre-labeled autosampler vials and injected 15µL into LC-MS/MS.

***Aqueous Sample Preparation*:**
About 250 µL of mixed ISTD working solution were added into pre-labeled tubes. To this 20µL of respective spiking solution was added and vortexed, followed by 1230 µL of reconstitution solution and vortexed. An appropriate volume of the reconstituted solution was transferred into pre-labeled autosampler vials and 15µL was injected into LC-MS/MS.

2.7. Method validation
A full Method validation was performed according to guidelines set by US FDA (33). The validation of this procedure was performed in order to evaluate the method in terms of selectivity, sensitivity, linearity of response, accuracy, precision, recovery, matrix effect, and matrix factor, ruggedness, reinjection reproducibility, effect of potential interfering drugs, stability of analytes during both short-term sample processing and long-term storage.

3. RESULTS AND DISCUSSION

3.1. LC-MS/MS condition optimization:
The product ion spectra of Azelnidipine, Olmesartan and it's internal standards were obtained (Fig. 2 A to D). Several fragment ions were observed in the product ion spectra for both Azelnidipine and Olmesartan. The fragment ion at m/z 167.200 was chosen as product ion for Azelnidipine, m/z 167.100 for Azelnidipine D7 and fragment ion at m/z 207.000 was chosen as product ion for Olmesartan, m/z 211.000 for Olmesartan D4. As these ions presented a higher abundance and stability with no cross-talk effect.
The composition of mobile phase includes acetonitrile/ 5mM ammonium formate adjusted pH3.0 with formic acid. Lesser mM of ammonium formate and low pH was chosen in the mobile phase to increase the sensitivity and for better peak shape. The retention time of Azelnidipine was 2.26 and Olmesartan was 1.19 minutes. A representative chromatogram of double blank (A & B), standard Zero (C & D), and lower limit of quantitation (LLOQ) (E & F) and upper limit of quantitation (ULOQ) (G & H) samples were summarized in (Fig. 3).

3.2. Sample preparation optimization
Solid phase extraction and liquid-liquid extraction are often used in preparation of biological samples due to their ability to improve the sensitivity and robustness of assay. Due to interference peaks observed in liquid-liquid extraction, method was developed in solid-phase extraction technique. In SPE technique extraction buffer and washing solvents plays a major role for their selectivity and extraction issues. As the p^H of the buffer is important in maintaining the stability of the analyte. For extraction purpose we tried ammonium acetate p^H 2.50, 1%Formic acid, 0.1%Acetic acid, finally 1%Formic acid was chosen because of better recovery. For eluting the interference peaks different washing solutions were tried and finally chosen acidic solution and lesser organic solutions. The Optimization of these parameters in the SPE technique made the method more sensitive, rugged, no matrix interferences and good recoveries.

3.3. Method validation parameters
3.3.1. Carryover Effect
The carryover effect due to the auto sampler was investigated by injecting a sequence of unextracted samples consisting of RS, AQ ULOQ, RS, RS, AQ LLOQ and extracted samples containing STD Blk, ULOQ, STD Blk,STD Blk and LLOQ. No significant carry over observed during this experiment.

3.3.2. Linearity and Sensitivity:
The linearity of the method was determined (in K_2EDTA) by using a $1/x^2$ weighted least square regression analysis of standard plots associated with an Ten-point standard curve. All the three calibration curves analyzed during the course of validation were found to be linear. The correlation coefficient (r) was observed to be $\geq$ 0.9984 during the course of validation.
The Sensitivity of the method was evaluated by analyzing six LLOQ samples. The % CV and % mean accuracy at LLOQ level were found to be 1.53 and 100.30 for Azelnidipine and 1.36 and 98.52 for Olmesartan.
The S/N ratio Calculated for sensitivity experiment was found more than 356 for Azelnidipine and 370 for Olmesartan.

3.3.4. Precision and accuracy:
The precision (% CV) of the LC-MS/MS method was evaluated in K_2EDTA by analyzing 6 replicates at different concentration levels corresponding to HQC, MQC1, MQC2, MQC3, LQC, DQC and LLOQ during the course of validation.

Within Batch Precision and accuracy

The % CV of back calculated concentrations for all quality control samples were ranged from 0.84 to 2.78. The % CV of back calculated concentration for all the samples of LLOQ was found to be 5.66. The % mean accuracy of back calculated concentrations for all quality control samples at all QC concentration levels were ranged from 93.90 to 99.05. The % mean accuracy of back calculated concentration for all the samples of LLOQ was found to be 85.20 for Azelnidipine.

The % CV of back calculated concentrations for all quality control samples concentration levels were ranged from 0.47 to 2.18. The % CV of back calculated concentration for all the samples of LLOQ was found to be 2.78. The % mean accuracy of back calculated concentrations for all quality control samples at concentration levels were ranged from 95.39 to 101.67. The % mean accuracy of back calculated concentration for all the samples of LLOQ was found to be 102.135 for Olmesartan.

Between Batch Precision and accuracy

The % CV of back calculated concentrations for all quality control samples of concentration levels were ranged from 0.71 to 1.99. The % CV of back calculated concentration for all the samples of LLOQ was found to be 4.15. The % mean accuracy of back calculated concentrations for all quality control samples concentration levels were ranged from 96.67 to 100.26. The % mean accuracy of back calculated concentration for all the samples of LLOQ was found to be 97.20 for Azelnidipine.

The % CV of back calculated concentrations for all quality control samples of concentration levels were ranged from 1.46 to 4.24. The % CV of back calculated concentration for all the samples of LLOQ was found to be 11.35. The % mean accuracy of back calculated concentrations for all quality control samples concentration levels were ranged from 96.81 to 102.64. The % mean accuracy of back calculated concentration for all the samples of LLOQ was found to be 102.75 for Olmesartan.

The results were summarized in table 1.

3.3.5. Recovery

Recovery for Analyte

The % mean recoveries were determined by measuring the responses of the extracted plasma quality control samples against unextracted quality control samples at HQC, MQC1 and LQC levels. The % mean recovery at HQC, MQC1 and LQC levels was found to be 69.08, 62.41 and 61.95 respectively. Over all % mean recovery and % CV at all QC levels was found to be 64.48 and 6.19 respectively for Azelnidipine.

The % mean recovery at HQC, MQC1 and LQC levels was found to be 61.89, 57.41 and 49.12 respectively. Over all % mean recovery and % CV at all QC levels was found to be 56.14 and 11.54 respectively for Olmesartan. This is within the acceptance limit of 15.00 %.

Recovery for Internal Standard

The % mean recoveries were determined by measuring the responses of internal standard in the extracted samples against unextracted samples at HQC, MQC1, MQC2 and LQC levels respectively. The % mean recovery at HQC, MQC1 and LQC levels was found to be 70.32, 68.35 and 67.33 respectively. Over all % mean recovery and % CV at all QC levels was found to be 68.67 and 2.21 respectively for Azlendipine D7.

The % mean recovery at HQC, MQC1 and LQC levels was found to be 64.67, 63.56 and 55.41 respectively. Over all % mean recovery and % CV at all QC levels was found to be 61.21 and 8.26 respectively for Olmesartan D4. The results were summarized in table 2.

3.3.6. Matrix Effect

Matrix effect was assessed by using 8 different lots (4 normal plasma, 2 haemolytic plasma and 2 lipemic plasma) of previously screened plasma lots. Blank samples in duplicate for each lot in each level were processed as per the respective method SOP, samples were spiked to achieve the concentration equivalent to HQC and LQC were injected. Un-extracted samples concentration equivalent to HQC and LQC were also prepared and injected. The % CV of ISTD normalized matrix factor at HQC and LQC samples were found to be 0.46 and 1.29 respectively for Azelnidipine. The % CV of ISTD normalized matrix factor at HQC and LQC samples were found to be 0.74 and 1.00 respectively for Olmesartan. The results were summarized in table 3.

3.3.7. Selectivity of Concomitant Drugs

Concomitant drugs was performed using 1 STD Blk by spiking concomitant spiking solution separately for each concomitant drug (Paracetamol, Caffeine, Diclofenac, Nicotine, Ondansetron, Pantoprazole and Hyoscine) and 3 samples equivalent to LLOQ using screened blank plasma and analyzed.

There was no effect observed by all the above drugs on Azelnidipine and Olmesartan.

3.3.8. Stability of Analytes:

The stabilities of Azelnidipine were investigated at two concentrations of QC samples {Low (LQC) and High (HQC) concentrations} to cover expected conditions during analysis, storage and processing of all samples. Stability was assessed by comparing the stability samples against the comparison samples with freshly prepared calibration curve. Which include the stability data from various stability exercise like auto sampler,

dry extract, wet extract, bench-top, freeze thaw, blood, short term and long-term stability tests. These data were summarized in table 4.

3.3.9. Drug-Drug Reactivity (DDR):

DDR experiment was performed to evaluate the effect of Azelnidipine in presence of Olmesartan and the effect of Olmesartan in presence of Azelnidipine. Screened blank sample of Azelnidipine was spiked with Olmesartan Cmax concentration and Olmesartan was spiked with Azelnidipine Cmax concentration for interference check. There was no drug-drug reaction observed.

3.3.10. Application to a pharmacokinetic study:

An open-label, balanced, randomized, two-treatment, two-period, two-sequence, single dose, crossover, oral bioequivalence study of Azelnidipine 8mg and Olmesartan 10 mg Tablets in normal healthy adult human subjects under fed conditions. Mean plasma concentration of Azelnidipine and Olmesartan with corre-sponding pharmacokinetic parameters listed in Table 5.

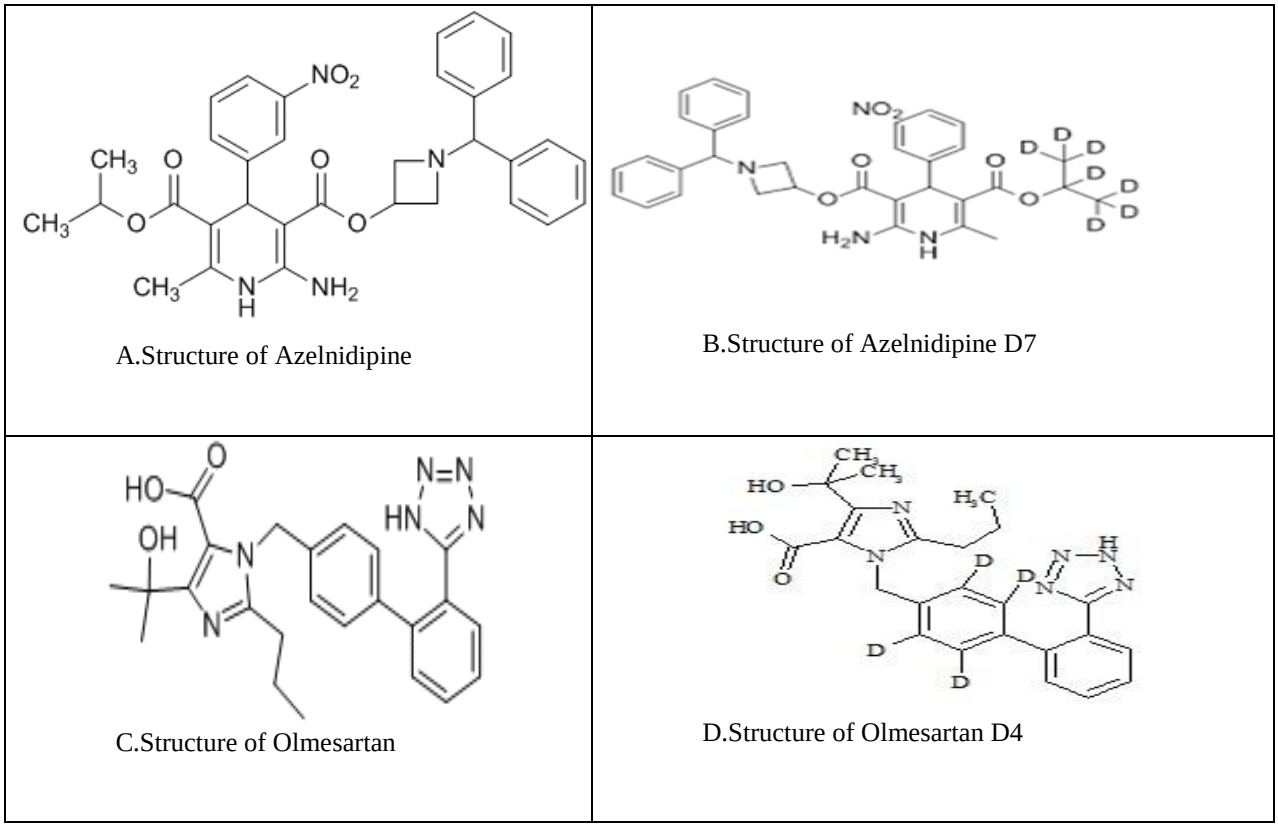
4. CONCLUSION

A novel, simple, sensitive, accurate and reproducible LC-MS/MS method has been developed and validated for the simultaneous estimation of Azelnidipine and Olmesartan in human plasma. This method was developed and validated using 200 µL of plasma, over a concentration range of 0.100 – 40.070 ng/mL for Azelnidipine and 3.001 – 1200.340 ng/mL for Olmesartan. This is a novel, more sensitive and less runtime than previously reported techniques with individual methods. To extract Azelnidipine and Olmesartan from plasma, simple SPE extraction was used. Total run time was 3.0 min only for each sample analysis. This method provided a very simple simultaneous procedure with much better sensitivity for the determination of Azelnidipine and Olmesartan in human plasma. The method was successfully applied to a human pharmacokinetic study and has the potential to be useful for bioequivalence studies and routine therapeutic drug monitoring.

Acknowledgments

The authors gratefully acknowledge Aizant Drug Research Solutions Pvt Ltd, Hyderabad, India. For providing the necessary facilities to carry out this work.

Fig. 1. Chemical Structures of Drugs and ISTD



A.Structure of Azelnidipine

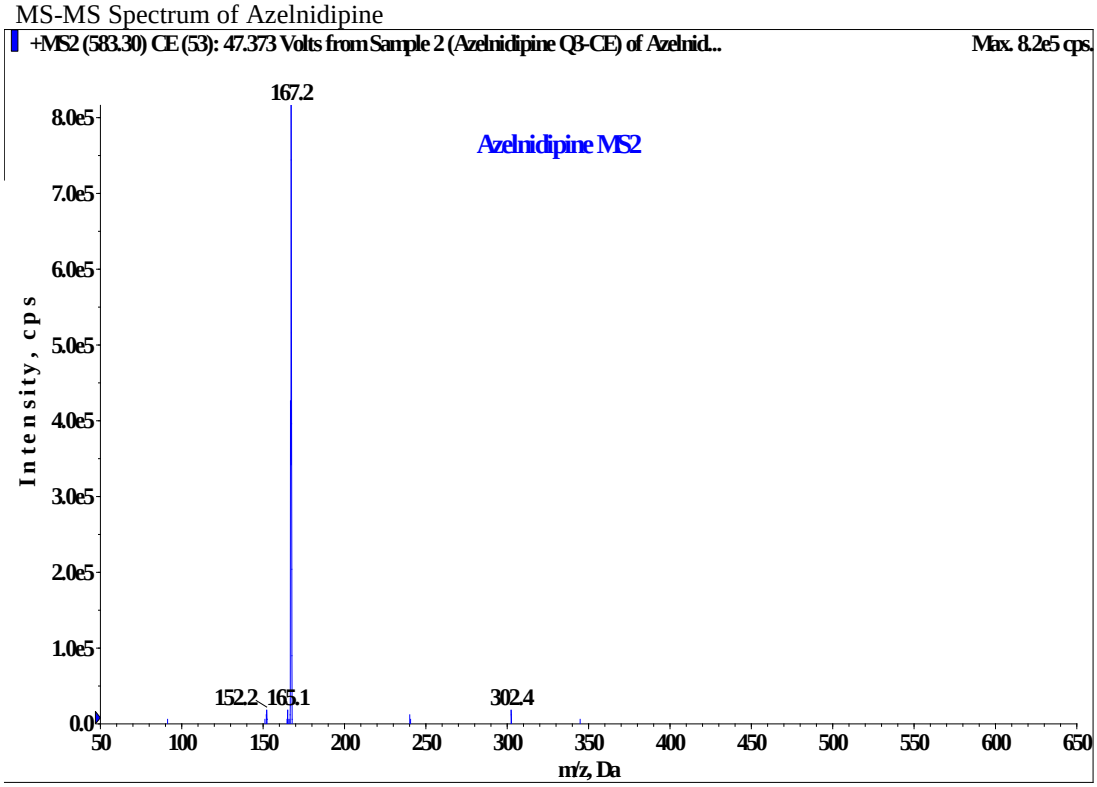
B.Structure of Azelnidipine D7

C.Structure of Olmesartan

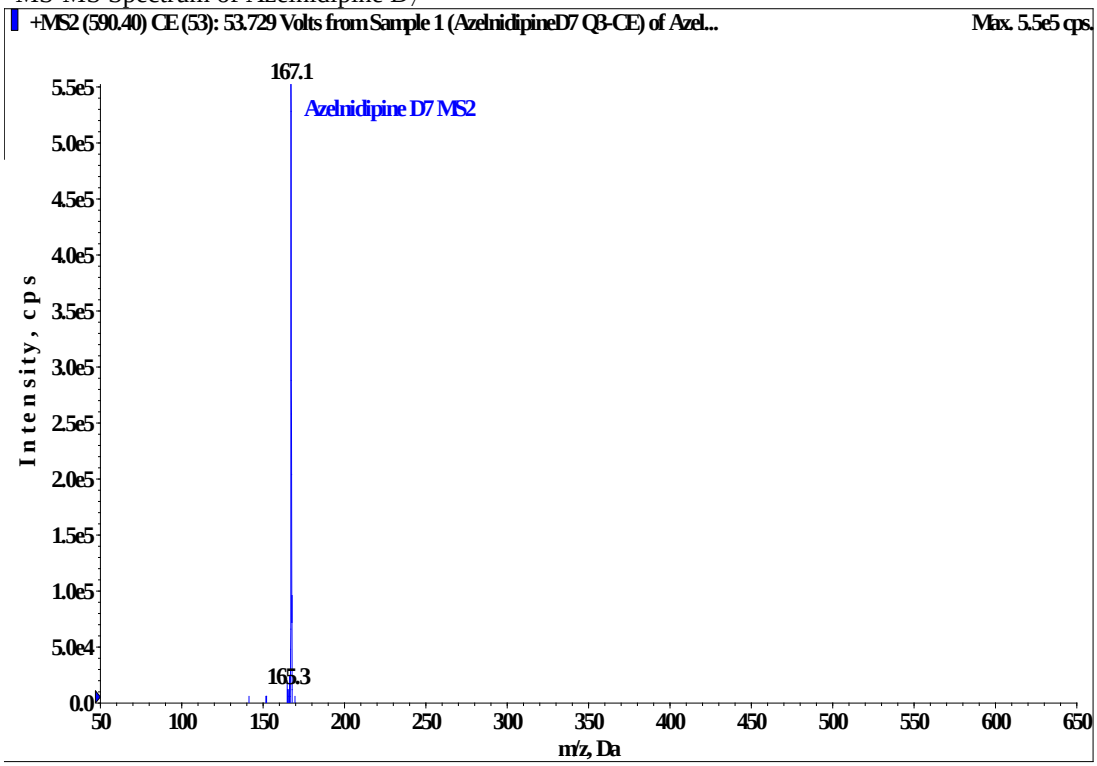
D.Structure of Olmesartan D4

Fig. 2.

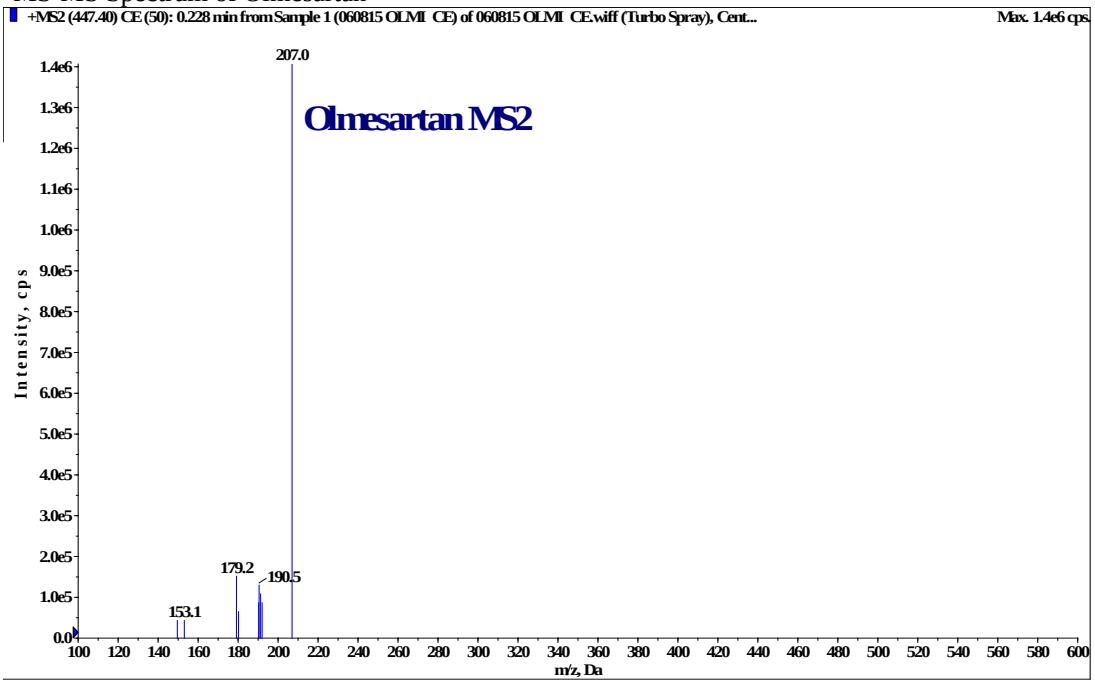
A. MS-MS Spectrum of Azelnidipine



B. MS-MS Spectrum of Azelnidipine D_7



C. MS-MS Spectrum of Olmesartan



D. MS-MS Spectrum of Olmesartan D4

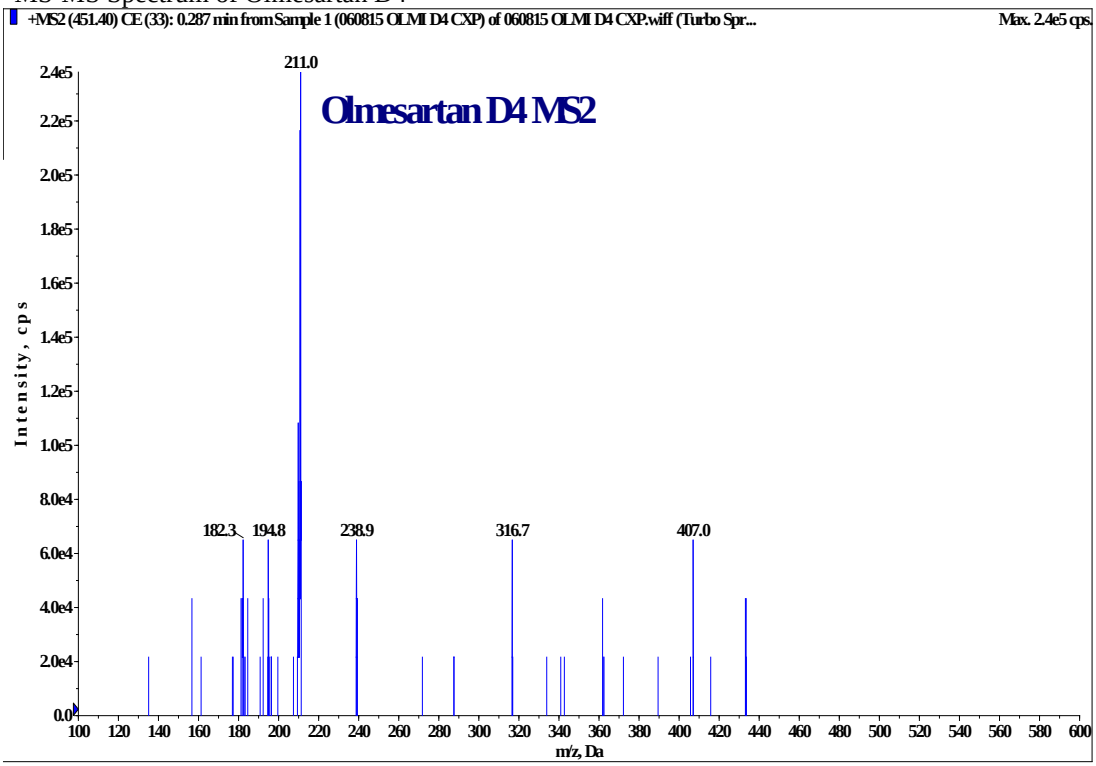
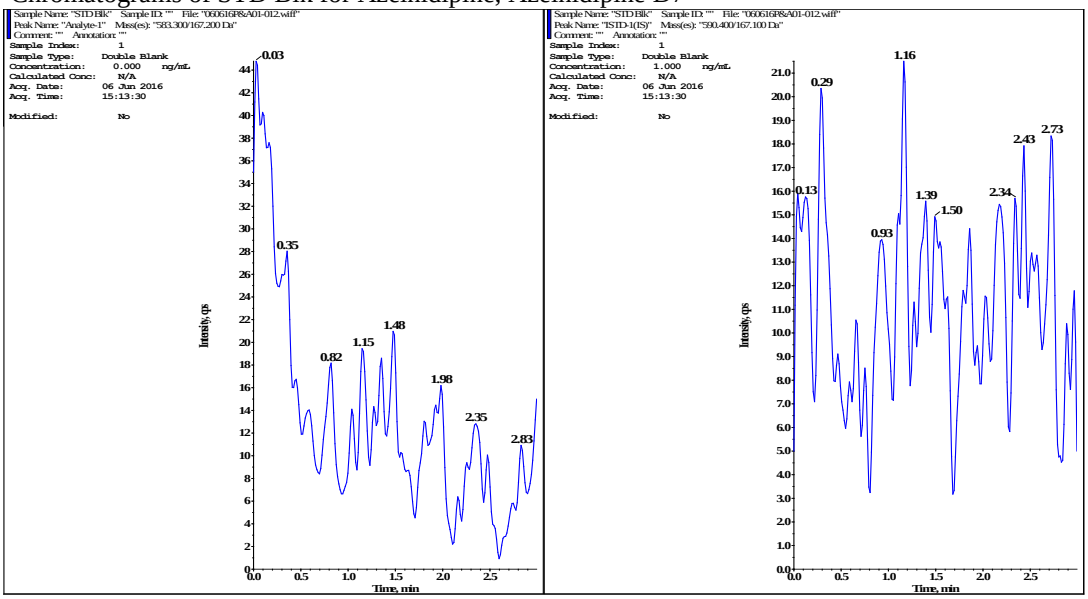
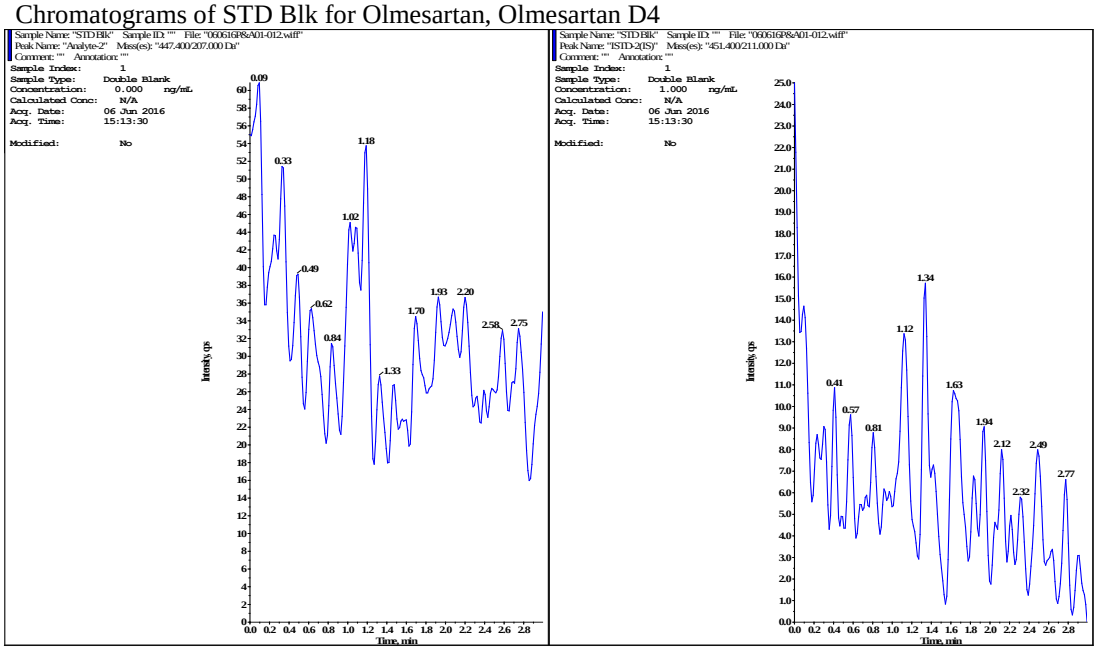


Fig. 3

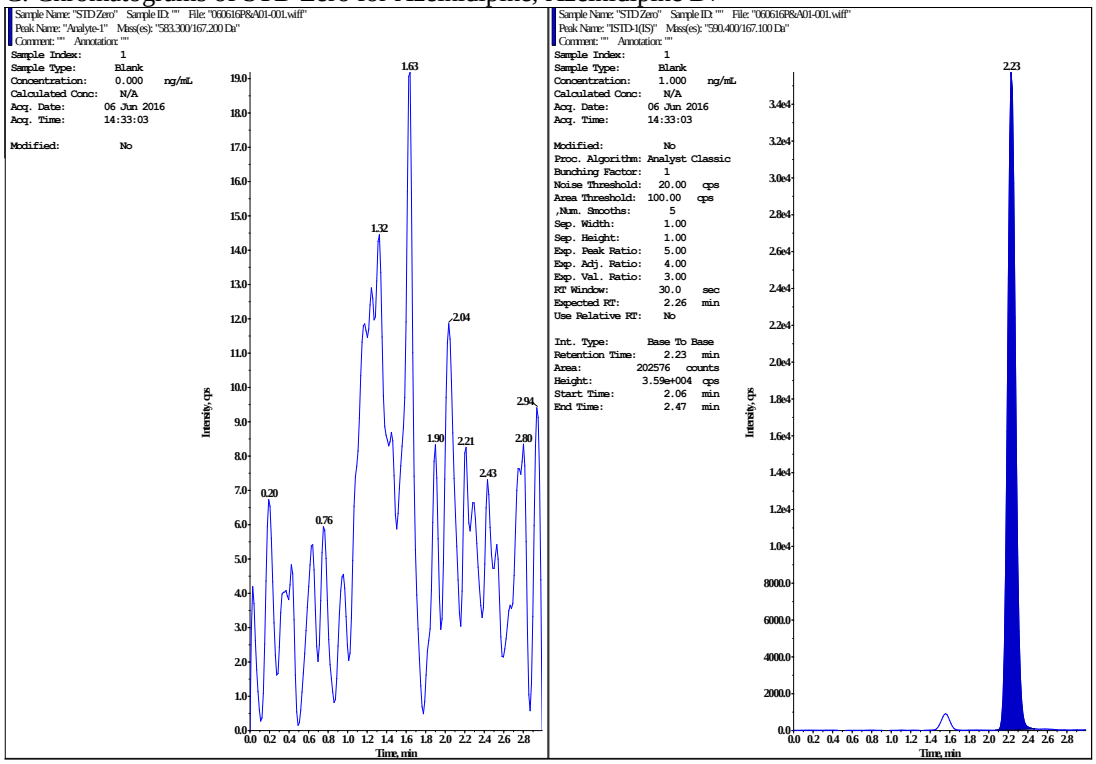
A. Chromatograms of STD Blk for Azelnidipine, Azelnidipine D7



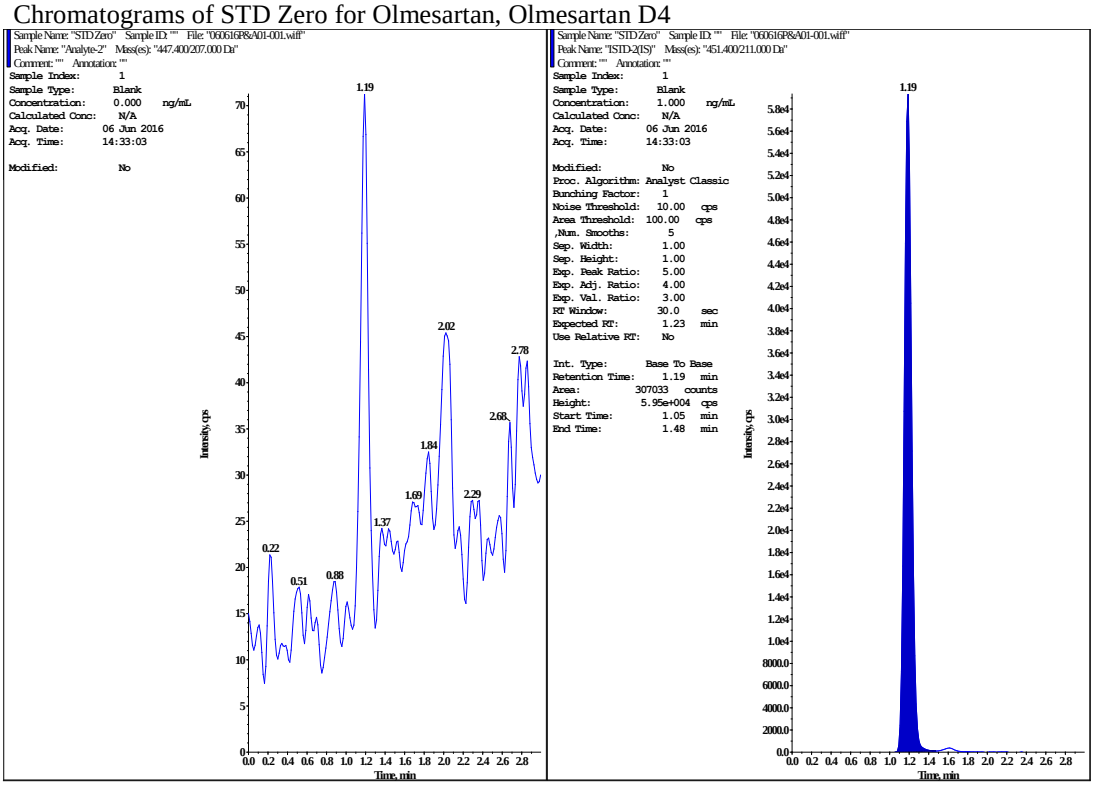
B. Chromatograms of STD Blk for Olmesartan, Olmesartan D4



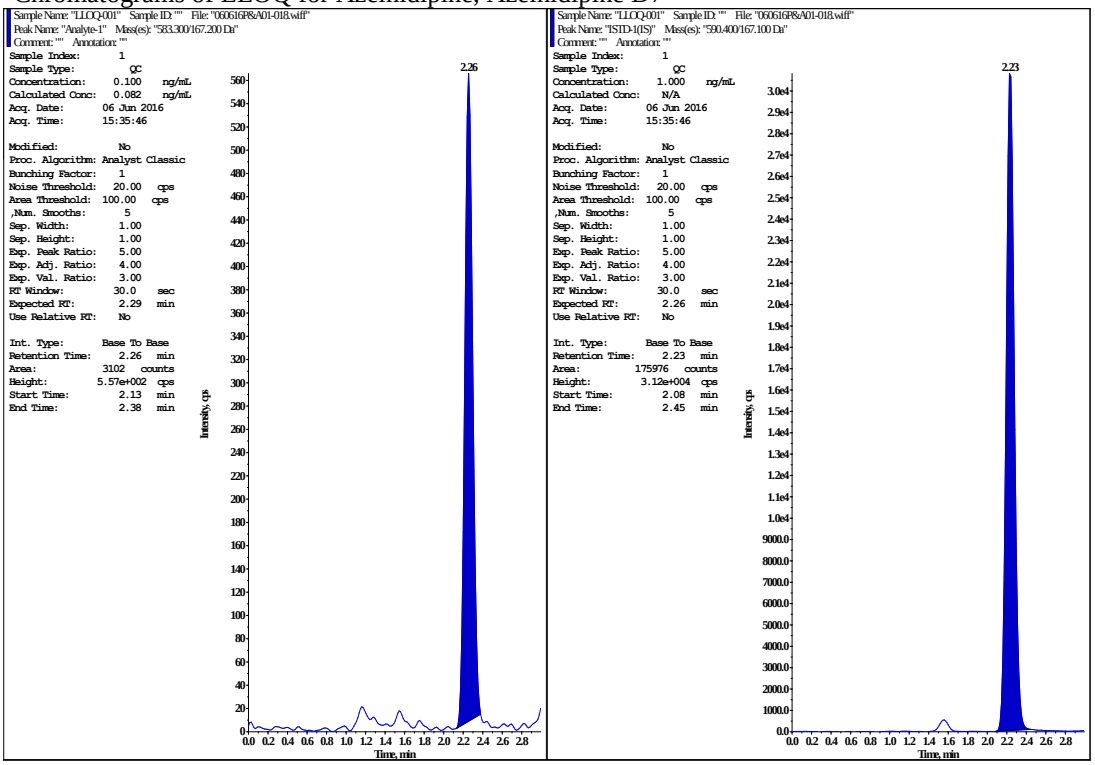
C. Chromatograms of STD Zero for Azelnidipine, Azelnidipine D7



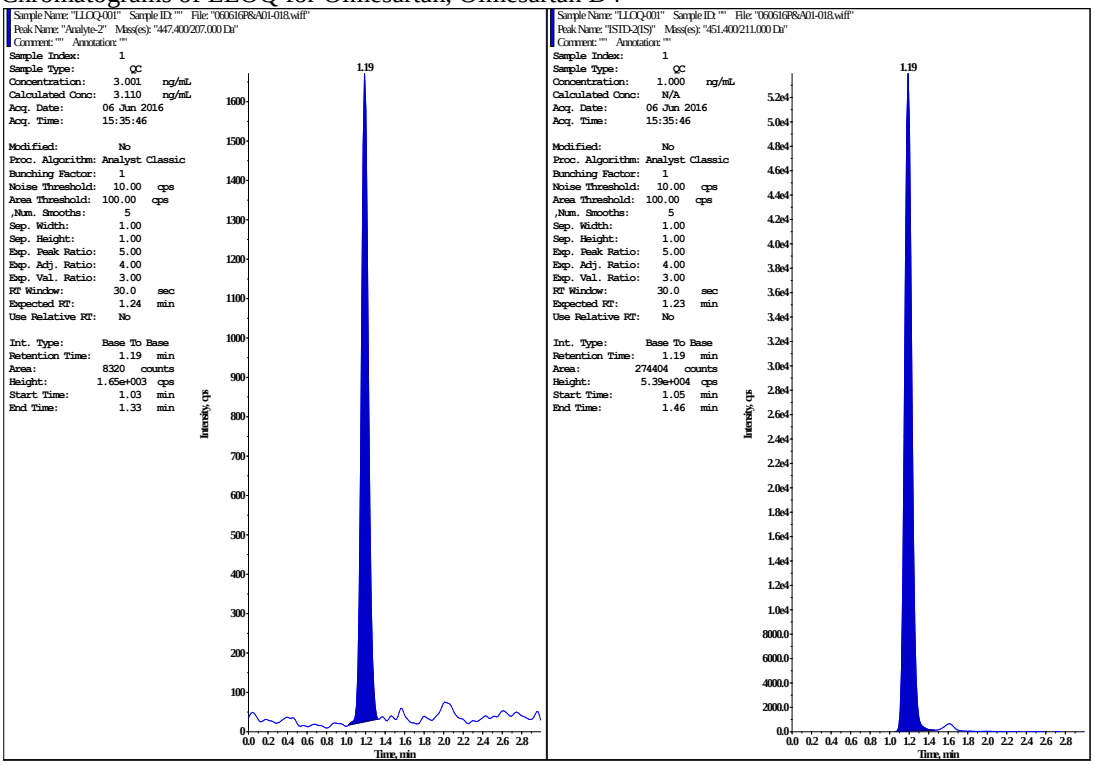
D. Chromatograms of STD Zero for Olmesartan, Olmesartan D4



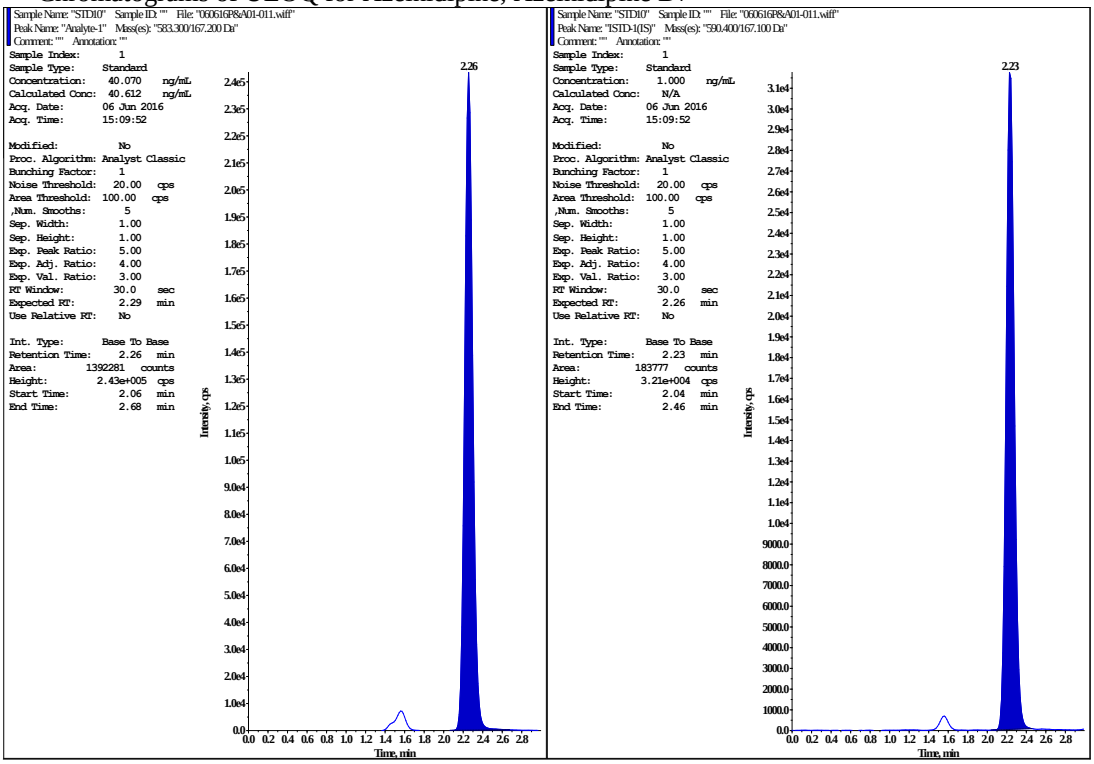
E. Chromatograms of LLOQ for Azelnidipine, Azelnidipine D7



F. Chromatograms of LLOQ for Olmesartan, Olmesartan D4



G. Chromatograms of ULOQ for Azelnidipine, Azelnidipine D7



H. Chromatograms of ULOQ for Olmesartan, Olmesartan D4

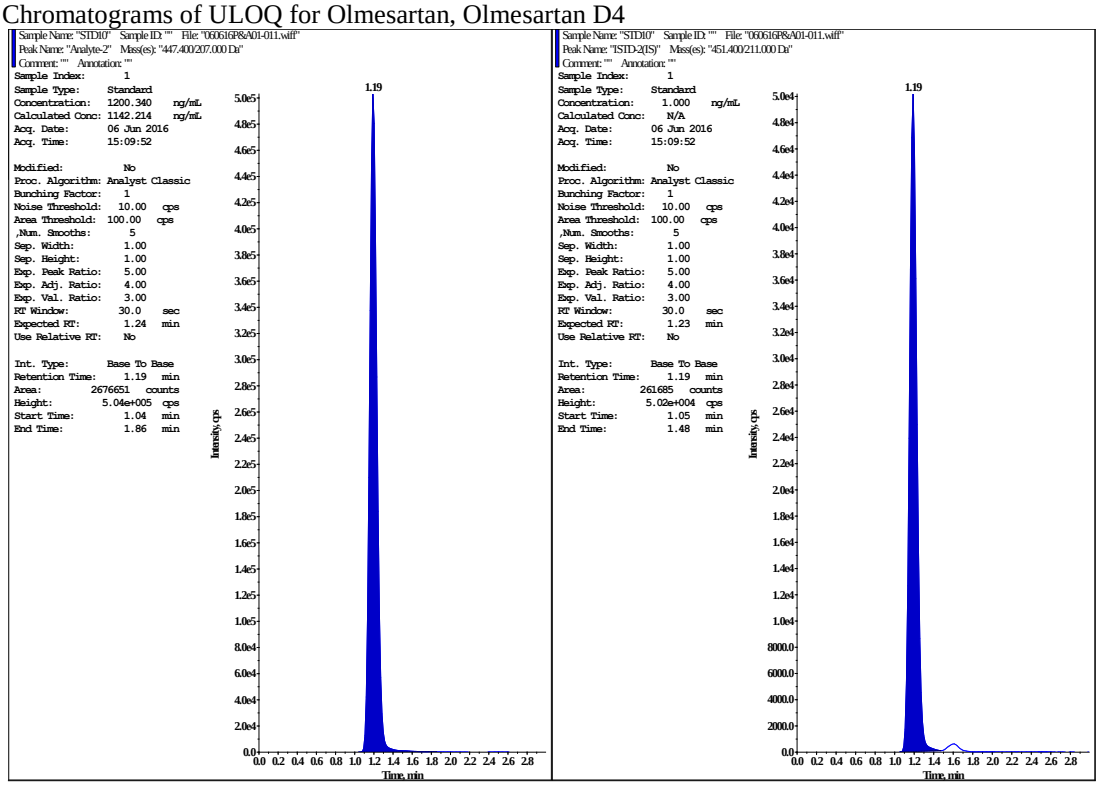


Table 1 A. ***Precision and accuracy of Azelnidipine***

Nominal concentration of Azelnidipine(ng/ml)	Intra-day (n=6)			Inter-day (n=6)		
	Mean	CV (%)	Accuracy (%)	Mean	CV (%)	Accuracy (%)
HQC(30.556)	29.0342	1.99	95.02	29.6442	3.05	97.02
MQC1(18.333)	17.4432	1.09	95.15	17.7778	2.59	96.97
MQC2(3.667)	3.6320	1.75	99.05	3.6618	2.46	99.86
MQC3(1.467)	1.4522	1.60	98.99	1.4708	2.21	100.26
LQC(0.300)	0.2817	1.24	93.90	0.2943	4.24	98.10
DQC(100.354)	98.6910	0.71	98.34	99.7339	1.46	99.38
LLOQ(0.100)	0.0852	4.15	85.20	0.0972	11.35	97.20

B. Precision and accuracy of Olmesartan

Nominal concentration of Olmesartan (ng/ml)	Intra-day (n=6)			Inter-day (n=6)		
	Mean	CV (%)	Accuracy (%)	Mean	CV (%)	Accuracy (%)
HQC(915.952)	873.7495	1.31	95.39	886.7056	1.41	96.81
MQC1(549.571)	534.6093	0.80	97.28	538.8327	1.48	1.48
MQC2(109.914)	109.3162	1.11	99.46	111.4439	1.93	1.93
MQC3(43.966)	44.6988	1.32	101.67	45.1284	2.42	2.42
LQC(8.987)	8.9817	0.60	99.94	9.0567	1.64	1.64
DQC(3008.275)	2993.5073	1.01	99.51	3046.8478	1.55	1.55
LLOQ(3.001)	3.0648	4.45	102.13	3.0834	4.63	4.63

Table 2 ***Recovery of analyte and ISTD***

Analyte	HQC	MQC1	LQC	Over all mean accuracy	CV (%)
Azelnidipine	69.08	62.41	61.95	64.48	6.19
Azelnidipine-D7	70.32	68.35	67.33	68.67	2.21
Olmesartan	61.89	57.41	49.12	56.14	11.54
Olmesartan D4	64.67	63.56	55.41	61.21	8.26

Table 3 ***Matrix effect of Azelnidipine***

	HQC	LQC
Overall mean (%)	30.556	0.300
CV (%)	0.46	1.29

Matrix effect of Olmesartan

	HQC	LQC
Overall mean (%)	915.952	8.987
CV (%)	0.74	1.00

Table 4 Stability data of Azelnidipine and Olmesartan

Name of the Experiment	Condition	Stability Period
Freeze Thaw Stability	-28 ± 5 °C	6 Cycles
	-70 ± 10 °C	6 Cycles
Bench Top Stability	Room Temperature	08 hours 37 minutes
Auto sampler Stability	5±3°C	43 hours 41 minutes
Wet Extract Stability	Room Temperature	03 hours 37 minute
	2-8 °C	43 hours 47 minutes
Dry Extract Stability	Room Temperature	03 hours 46 minutes
Blood Stability (Analyte)	Room Temperature	04 hours 14 minutes
	2-8 °C	04 hours 06 minutes
Short Term Stock Solution Stability for Analyte's & ISTD's	Room Temperature	06 hours 52 minutes
Short Term Spiking/Working Solution Stability for Analyte's & ISTD's	Room Temperature	06 hours 50 minutes
Long Term Stock Solution Stability for Analyte & ISTD	2-8 °C	09 days 15 hours
Long Term Spiking/Working Solution for Analyte & ISTD	2-8 °C	11 days 21 hours
Long Term stability in Matrix	-28 ± 5 °C	36 days
	-70 ± 10 °C	36 Days

Table 5 Pharmacokinetic data of Azelnidipine and Olmesartan

Parameters (Units)	Olmesartan		Azelnidipine	
	Test Product (T) n=12	Reference Product (R) n=12	Test Product (T) n=12	Reference Product (R) n=12
*T_{max} (hr)	4.75	4.75	1.67	2.00
C_{max} (ng/mL)	1080.476±204.3363	1260.982±242.8924	21.822±6.6323	9.408±3.5513
AUC_{0-t} (ng.hr/mL)	14833.357±3587.9033	15897.338±2100.2249	238.453±57.7397	178.675±56.7144
AUC_{0-inf} (ng.hr/mL)	15134.662±3806.6162	17757.718±6739.5410	247.213±59.2476	182.324±59.1756
K_{el} (1/hr)	0.166±0.0573	0.145±0.058	0.112±0.0025	0.166±0.0573
$t_{½}$ (hr)	4.738±1.8500	8.709±13.2366	1.626±1.2500	2.01±1.1523
AUC Ratio (%)	97.16±5.008	93.59±16.485	90.16±3.008	93.55±4.128

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