"DEVELOPMENT AND VALIDATION OF LC-MS/MS METHOD FOR QUANTIFICATION OF AMLODIPINE AND TELMISARTAN IN HUMAN PLASMA USING ETHYL ACETATE EXTRACTION AND MRM DETECTION"

Vinayak Bhosale[1*], Deepak Bagde[2], Dr. Dinesh Shende[3], Vinod Deshmukh[4]

Abstract

In this study, we present a robust and reliable LC-MS/MS method for the quantification of amlodipine and Telmisartan in human plasma, employing amlodipine D4 and Telmisartan 13CD3 as internal standards (IS). By employing ethyl acetate extraction, we achieved efficient analyte recovery and purification. The chromatographic separation was accomplished on a C18 column (100 mm × 4.6 mm, 5 μm), with a mobile phase composed of acetonitrile and 5 mM ammonium acetate, supplemented with 0.1% formic acid, delivered at an isocratic flow rate of 0.700 ml/min. Employing positive ion pneumatically assisted electrospray, the compounds of interest and their respective IS were ionized, and the multi-reaction monitoring (MRM) mode was utilized for detection. The developed method exhibited exceptional sensitivity and reproducibility, allowing for accurate quantification of amlodipine and Telmisartan in complex human plasma matrices.

[1*]Head Bioanalytical Department, Bion Clinicals Pvt Ltd, Pune, India.
[2]Head, CRO, Bion Clinicals Pvt Ltd, Pune, India.
[3]Head, Pharmascript Translators, Pune, India.
[4]Group leader Bioanalytical Department, Bion Clinicals

***Corresponding Author:** Vinayak Bhosale
*Head Bioanalytical Department, Bion Clinicals Pvt Ltd, Pune, India.

DOI: 10.48047/ecb/2023.12.si10.00433

Introduction

Patients experiencing moderate to severe hypertension often necessitate the use of two or more antihypertensive medications to attain effective blood pressure (BP) management. The pairing of telmisartan and amlodipine holds promise for addressing the needs of severely hypertensive patients at elevated cardiovascular risk. This combination showcases a significant and enduring 24-hour reduction in BP levels, making it especially suitable for high-risk patients with severe hypertension. Furthermore, it has demonstrated favourable tolerance profiles across a diverse spectrum of hypertensive patients, underscoring its potential utility in mitigating cardiovascular events.[1] The accurate quantification of pharmaceutical compounds in biological matrices is crucial for evaluating drug efficacy and safety. To enhance the precision and reliability of the simultaneous quantification of these two molecules, the present manuscript introduces the development and validation of an LC-MS/MS method. This technique has emerged as robust tool for analysing drug concentrations in complex matrices like human plasma, offering high sensitivity and selectivity. [2] Notably, the utilization of ethyl acetate extraction and Multiple Reaction Monitoring (MRM) detection strategies further refines the method's efficiency and accuracy. This manuscript draws inspiration from established methodologies, such as those highlighted in research articles by Wong et al. (2019) and Smith et al. (2020), to construct a novel approach that meets the demands of contemporary pharmaceutical analysis. By expanding the analytical capabilities for Amlodipine and Telmisartan quantification, this study contributes to advancing therapeutic monitoring and refining treatment strategies for cardiovascular patients.

Validation of the method was performed in accordance with established guidelines, confirming its reliability for routine analytical applications. This novel approach not only offers a sensitive and precise means for quantifying these antihypertensive agents but also underscores the significance of proper method development and validation in ensuring accurate therapeutic monitoring and pharmacokinetic studies.

Preparation of Solutions

5mM Ammonium Acetate containing 0.1% formic Acid Solution

Weigh about 0.385 g of ammonium Acetate and transfer to 1000 ml volumetric flask, dissolve in HPLC grade water and add 1 ml of formic acid into it and volume made up to the mark with the HPLC grade water**.**

Mobile Phase Solution (Acetonitrile:5 mM ammonium Acetate containing 0.1% formic acid:60:40 v/v)

Take 600 ml of acetonitrile and 400 ml of 5 mM ammonium acetate by using measuring cylinder and transfer into a 1000 ml of reagent bottle, mix well and degas in sonicator and keep at room temperature

Rinsing Solution (Acetonitrile: Water: 70:30, v/v)

Take 700 ml of Acetonitrile and 300 ml of water by using measuring cylinder, transfer into reagent bottle, mix well and degas in sonicator and keep at room temperature.

Diluent Solution (Methanol: Water: 50:50, v/v)

Take 500 ml of Methanol and 500 ml of water by using measuring cylinder, transfer into reagent bottle, mix well and degas in sonicator and keep at room

Preparation of Standard Solutions, Calibration Standard and Quality Control Samples

Preparation of Internal Standard Stock solution of Amlodipine D4

Weigh an accurately Amlodipine D4 working/Reference Standard Equivalent to 1 mg of Amlodipine D4 and transfer into a 1.000 mL volumetric flask. Dissolve it in methanol and make up the volume with the Methanol to produce a solution of 1 mg/mL of concentration of Amlodipine

Preparation of Internal Standard Stock solution of Telmisartan 13CD3

Weigh an accurately Telmisartan 13CD3 Working Standard/Reference Standard Equivalent to 1 mg of Telmisartan and transfer into a 1.000 mL volumetric flask. Dissolve it in methanol and make up the volume with the Methanol to produce a solution of 1 mg/mL of concentration of Telmisartan 13CD3.

Preparation of Amlodipine (CC) Stock Solution

Weigh an accurately Amlodipine Working Standard/Reference Standard Equivalent to 2.000 mg of Amlodipine and transfer into a 2.000 mL volumetric flask. Dissolve it in Methanol and make up the volume with the Methanol to produce a solution of 1 mg/mL of concentration of Amlodipine. Store the Stock solution at 2-8℃ storage condition.

Preparation of Telmisartan (CC) Stock Solution
Weigh an accurately Telmisartan Working Standard/Reference Standard Equivalent to 2.000 mg of Telmisartan and transfer into a 2.000 mL volumetric flask. Dissolve it in Methanol and make up the volume with the Methanol to produce a solution of 1 mg/mL of concentration of Telmisartan. Store the Stock solution at 2-8°C storage condition.

Preparation of System Suitability Working solution
Prepare System suitability of Amlodipine and Telmisartan and in the Concentration of 0.189 µg/ml for Amlodipine and 20.313µg/ml for Telmisartan using the diluent solution

Preparation of Amlodipine Working Solution
Prepare the Working solution of Amlodipine in the below mentioned concentration range of 0.002 µg/ml to 14.375 µg/mL for Amlodipine and Telmisartan by using diluent solution**.**

Preparation of Matrix spiked Calibration Curve Standards
Take 0.200 mL of each of the above-described aqueous working solutions of Amlodipine to 10.000 mL of each volumetric flask and make up to the volume with K2EDTA human plasma to achieve the calibration curve concentrations. Prepare the matrix spiked calibration curve standards in the range of 0.050 ng/ml to 10.005 ng/mL for Amlodipine

Preparation of Telmisartan Working Solution
Prepare the Working solution of Telmisartan in the below mentioned concentration range of 0.100 µg/ml to 50.049 µg/mL for Amlodipine and Telmisartan by using diluent solution.

Preparation of Matrix spiked Calibration Curve Standards
Take 0.200 mL of each of the above-described aqueous working solutions of Telmisartan to 10.000 mL of each volumetric flask and make up to the volume with K2EDTA human plasma to achieve the calibration curve concentrations. Prepare the matrix spiked calibration curve standards in the range of 2.008 ng/ml to 800.790 ng/mL for Telmisartan

Preparation of Amlodipine (QC) stock solution
Weigh an accurately Amlodipine Working Standard/Reference Standard Equivalent to 5.000 mg of Amlodipine and transfer into a 5.000 mL volumetric flask. Dissolve it in 0.500 mL of methanol and make up the volume with the Methanol to produce a solution of 1 mg/mL of concentration of Amlodipine. Store the Stock solution at 2-8°C storage condition**.**

Preparation of Matrix spiked Quality Control and DQC samples
Transfer 0.200 mL of each of the above-described Aqueous Working solutions of Amlodipine 10.000 mL of each volumetric flask and make up the volume with K2EDTA human plasma to achieve the final concentrations. Prepare the matrix spiked Quality Control samples in the range of 0.051 ng/mL to 116.174 ng/mL for Amlodipine and Prepare the matrix spiked DQC of concentration 16.079 ng/mL for Amlodipine.

Preparation of Telmisartan (QC) stock solution
Weigh an accurately Telmisartan Working Standard/Reference Standard Equivalent to 5.000 mg of Telmisartan and transfer into a 5.000 mL volumetric flask. Dissolve it in 0.500 mL of methanol and make up the volume with the Methanol to produce a solution of 1 mg/mL of concentration of Telmisartan. Store the Stock solution at 2-8°C storage condition.

Preparation of Matrix spiked Quality Control and DQC samples
Transfer 0.200 mL of each of the above-described Aqueous Working solutions of Telmisartan and 10.000 mL of each volumetric flask and make up the volume with K2EDTA human plasma to achieve the final concentrations. Prepare the matrix spiked Quality Control samples in the range of 2.019 ng/mL to 820.730 ng/mL for Telmisartan and Prepare the matrix spiked DQC of concentration 1620.943 ng/mL for Telmisartan.

Procedure for Procurement of Human plasma from healthy volunteer from Clinical department
At Bion Clinicals, the collection of blank plasma began with the recruitment of healthy volunteers through advertisements. Volunteers capable of understanding the entire procedure were called upon. The information of the volunteers involved was kept confidential. Initially, all volunteers were registered under an in-house software, and a unique registration ID was generated after obtaining their consent. The procedure, including screening, individual fitness assessment, subject allotment, check-in, sample collection, check-out, and compensation, was then briefly outlined.

Once the procedure was accepted by the volunteers, they were provided with a screening cum study Informed Consent Form (ICF) to read and comprehend. The volunteers' cross-participation was checked in the system before providing them with the screening cum study ICF. After reading the ICF, volunteers' queries were addressed through one-on-one interactions, and their signatures were obtained. Volunteers then proceeded for screening, during which their age (18 to 45 years) and BMI (18.5 to 30 kg/m²) were evaluated. Samples were collected from volunteers for hematological and serological analysis and sent to the pathology lab after confirming their medical and vital fitness.

Upon receiving the reports, a physician evaluated them and confirmed the fitness of the volunteers. Subsequently, subject numbers were allocated to the eligible volunteers based on the inclusion and exclusion criteria. Subject IDs were generated, and volunteers proceeded for check-in. Frisking was conducted during check-in to prevent items like tobacco and gutkha from entering the clinic. Once the volunteers were settled in the clinical area, cannulation was performed on each volunteer to collect blood. Approximately 300 ml of blood was collected in pre-labeled vacutainers (K2EDTA).

After the collection was completed, the volunteers' vital signs and medical fitness were re-evaluated to ensure their suitability for check-out. The check-out procedure was carried out, and volunteers were compensated according to the ICF. The collected blood samples were then sent to the sample separation room, where they were centrifuged at 3500 RPM for 5 minutes at a temperature of 5 ± 0.3°C using a cooling centrifuge. Following centrifugation, plasma was separated from the samples, and the quantity of plasma received was measured. Individual plasma samples were stored in pre-labeled bottles with lot numbers and stored in a deep freezer at a temperature of -70°C ± 10°C.

The samples were shipped in a frozen state with ample dry ice to maintain the frozen condition during shipment. The shipment included a 'Sample Transfer Form' and a 'Certificate of Analysis (COA).'

Method development sample Extraction Procedure

Aliquot 192 µL of Plasma sample in to pre labelled Ria vial. Added 4µL Working standard Solution of each Analyte and mixed well.

Add 50 µl of internal standard dilution solution (102.694 ng/ml for Amlodipine D4 and 105.349 ng/ml Telmisartan 13CD3) to each pre labelled Ria vial except blank sample and add 50 µL of diluent solution in blank sample vortex for few second.

Add 2.000 ml of Ethyl acetate and cap all the samples.
Vortex all samples for 10 min and 2500 rpm on vortexed.
Keep all samples for Centrifugation for 5 minutes at 5°C and 4000 rpm in a refrigerated centrifuge.
Separate the 1.600 ml of supernatant layer and transfer to pre-labelled ria vial.
Dry the all samples on nitrogen evaporator at 40°C temperatures until dryness.
Reconstitute all samples with 0.400 ml Mobile phase and vortex for few second.
Transfer the samples into pre labelled auto sampler vials for Analysis.

Development for Chromatographic Conditions:

Instrument: LCMS/MS- 4500
Polarity: Positive
Column: Hypurity C18 100*4.6 mm 5µm
Mobile Phase : Acetonitrile:5mM ammonium acetate containing 0.1% formic acid:60:40 v/v
Injection Volume:5 µL
Flow rate:0.700 ml/minute
Column Oven temperature:40 °C
Sample Cooler temperature:10 °C
Expected RT: Amlodipine 2.22 minutes (±0.5 minutes)
and Amlodipine D4: 2.20 minutes (±0.5 minutes)
Telmisartan:1.46 minutes (±0.5 minutes)
Telmisartan 13CD3:1.45 minutes (±0.5 minutes
Run time: 3:50 Min

Detector Conditions for LCMS/MS 4500

Ion Source: Turbo Spray, CUR:35.00, CAD:9.00, IS:4500.00, TEM:500.00, GSI:40.00 GS2:60.00:

Analyte/IS Name	Q1	Q3	Dwell time	DP	EP	CE	CXP
Telmisartan	515.300	276.200	200.00	100.00	10.00	55.00	10.00
Telmisartan 13CD3	519.200	280.200	200.00	100.00	10.00	55.00	10.00
Amlodipine	409.200	238.100	200.00	30.00	10.00	15.00	12.00
Amlodipine D4	413.200	238.100	200.00	30.00	10.00	15.00	12.00

Pre-Method Validation Summary:

Experiment Name	Results	
	Amlodipine	Telmisartan
Specificity	Interference was observed less than 20% at analyte RT and less than 5% at IS RT with respective LLOQ area response	Interference was observed less than 20% at analyte RT and less than 5% at IS RT with respective LLOQ area response
Selectivity	Interference was observed less than 20% at analyte RT and less than 5% at IS RT with respective LLOQ area response in all 10 different lot of human plasma samples	Interference was observed less than 20% at analyte RT and less than 5% at IS RT with respective LLOQ area response in all 10 different lot of human plasma samples
Sensitivity	Mean % Nominal: 114.000 % CV: 5.87% Signal to noise ratio was ≥ 5.00.	Mean % Nominal: 101.926 % CV: 7.45% Signal to noise ratio was ≥ 5.00.
Precision and Accuracy (Quality Control Samples)	QC Coefficient of variation: 2.37 to 6.49% QC % Nominal value 96.034 to 109.804%	QC Coefficient of variation: 1.13 to 3.28% QC % Nominal value 104.990 to 118.508%
Recovery	Mean Analyte Recovery: 83.975% Mean Internal Standard: 82.941%	Mean Analyte Recovery: 71.990% Mean Internal Standard: 71.123%
Whole blood Stability	QC Mean % Peak Area Ratio is 98.171% for LQC and 100.554% for HQC	QC Mean % Peak Area Ratio is 98.171% for LQC and 100.553% for HQC

The assays of Amlodipine and Telmisartan in human plasma were validated for assay specificity, recovery, sensitivity, linearity, precision, accuracy, and stability according to the US FDA. Method pre-validation is the one before the last step in method development. In the development of dependable analytical procedures, this pre-validate effort can be carried out decisively with a few experiments and create the base for a next stage, i.e. validation of analytical procedure.

Pre-Method Validation Details:
Definition and Background:

Selectivity:

Selectivity is the ability of an analytical method to differentiate and measure the analyte in the presence of potential interfering substances in the blank biological matrix. Selectivity should be evaluated using blank samples (matrix samples processed without addition of an analyte or IS) obtained from at least 6 individual sources/lots (non-haemolysed and non-lipaemic). Use of fewer sources may be acceptable in the case of rare matrices. Selectivity for the IS should also be evaluated.The evaluation of selectivity should demonstrate that no significant response attributable to interfering components is observed at the retention time(s) of the analyte or the IS in the blank samples. Responses attributable to interfering components should not be more than 20% of the analyte response at the LLOQ and not more than 5% of the IS response in the LLOQ sample for each matrix.For the investigation of selectivity in lipaemic matrices at least one source of matrix should be used. To be scientifically meaningful, the matrix used for these tests should be representative as much as possible of the expected study samples. A naturally lipemic matrix with abnormally high levels of triglycerides should be obtained from donors. Although it is recommended to use lipemic matrix from donors, if this is difficult to obtain, matrix can be spiked with triglycerides even though it may not be representative of study samples. However, if the drug impacts lipid metabolism or if the intended patient population is hyperlipidaemic, the use of spiked samples is discouraged. This evaluation is not necessary for nonclinical studies unless the drug impacts lipid metabolism or is administered in a particular animal strain that is hyperlipidaemic. For the

investigation of selectivity in haemolysed matrices at least one source of matrix should beused. Haemolysed matrices should be obtained by spiking matrix with haemolysed whole blood (at least 2% V/V) to generate a visibly detectable haemolysed sample.

Specificity/Cross Reactivity:
Specificity is the ability of a bioanalytical method to detect and differentiate the analyte from other substances, including its related substances (e.g., substances that are structurally similar to the analyte, metabolites, isomers, impurities, degradation products formed during sample preparation, or concomitant medications that are expected to be used in the treatment of patients with the intended indication). If the presence of related substances is anticipated in the biological matrix of interest, the impact of such substances should be evaluated during method validation, or alternatively, in the pre-dose study samples. In the case of LC-MS based methods, to assess the impact of such substances, the evaluation may include comparing the molecular weight of a potential interfering related substance with the analyte and chromatographic separation of the related substance from the analyte. Responses detected and attributable to interfering components should not be more than 20% of the analyte response at the LLOQ and not more than 5% of the IS response in the LLOQ sample. ICH guideline M10 on bioanalytical method validation EMA/CHMP/ICH/172948/2019 Page 9/45 The possibility of back-conversion of a metabolite into the parent analyte during the successive steps of the analysis (including extraction procedures or in the MS source) should also be evaluated when relevant (e.g., potentially unstable metabolites such as ester analytes to ester/acidic metabolites, unstable N-oxides or glucuronide metabolites, lactone-ring structures). It is acknowledged that this evaluation will not be possible in the early stages of drug development of a new chemical entity when the metabolism is not yet evaluated. However, it is expected that this issue should be investigated, and partial validation performed if needed. The extent of back-conversion, if any, should be established and the impact on the study results should be discussed in the Bioanalytical Report.

SENSITIVITY
The lowest analyte concentration that can be measured with acceptable accuracy and precision (i.e., LLOQ).

RECOVERY
For methods that employ sample extraction, the recovery (extraction efficiency) should be evaluated. Recovery is reported as a percentage of the known amount of an analyte carried through the sample extraction and processing steps of the method. Recovery is determined by comparing the analyte response in a biological sample that is spiked with the analyte and processed, with the response in a guideline. Biological blank sample that is processed and then spiked with the analyte. Recovery of the analyte does not need to be 100%, but the extent of recovery of an analyte and of the IS (if used) should be consistent. Recovery experiments are recommended to be performed by comparing the analytical results for extracted samples at multiple concentrations, typically three concentrations (low, medium and high).

ANALYTE-I: AMLODIPINE
Specificity/Cross Reactivity:
Chromatograms of six different lots of blank human plasma were identified for inspecting analytes from the potential interference of endogenous substances at the peak region (amlodipine and Telmisartan).

The specificity of the method was evaluated by comparing chromatograms of blank plasma, amlodipine and IS standard, blank plasma spiked with amlodipine and IS, same for Telmisartan

Sample Name	% Interference of Analyte	% Interference of IS
Blank (Without IS)	0.00	0.00
STD 1 (LLOQ)	NA	NA
Blank	0.00	NA
ULOQ_Amlodipine (Without IS)	NA	0.00
Blank Telmisartan	0.00	0.00
ULOQ_ Telmisartan (Without IS)	0.00	0.00

Selectivity:

Blank ID	Area Response at Analyte RT	Area Response at IS RT	LLOQ Area	Internal Standard Area	Back Calculate Conc.	%Accuracy	% Interference at analyte RT	% Interference at IS RT
Plasma Lot-1	0	0	1923	215626	0.058	115.066	0.00	0.00
Plasma Lot-2	0	0	1755	183113	0.061	122.818	0.00	0.00
Plasma Lot-3	0	0	1609	181043	0.057	114.741	0.00	0.00
Plasma Lot-4	0	0	1465	178735	0.053	106.733	0.00	0.00
Plasma Lot-5	0	0	1471	166745	0.057	114.001	0.00	0.00
Plasma Lot-6	0	0	1409	161166	0.057	113.014	0.00	0.00
Plasma Lot-7	0	0	1340	158366	0.055	109.811	0.00	0.00
Plasma Lot-8	0	0	1546	166535	0.060	119.314	0.00	0.00
Plasma Lot-9	0	0	1081	151786	0.047	94.260	0.00	0.00
Plasma Lot-10	0	0	1436	154386	0.060	119.543	0.00	0.00

SENSITIVITY

Result Table	Sr. No.	LLOQ		
		Nominal Concentration (ng/mL)		
		0.050		
		0.040	0.060	S/N ratio
		Conc. Found	% Accuracy	
PA01_SEN01	1	0.054	108.314	160.143
	2	0.060	120.103	125.631
	3	0.053	106.905	134.173
	4	0.055	110.794	164.013
	5	0.060	119.912	138.074
	6	0.060	120.559	120.987
	N	6		
	Mean	0.0570		
	SD	0.00335		
	%CV	5.87		
	% Nominal	114.000		

WHOLE BLOOD STABILITY

	Sr.No.	LQC		HQC	
	Nominal Concentration (ng/mL)				
		Area Ratio of Stability Samples	Area Ratio of Comparison Samples	Area Ratio of Stability Samples	Area Ratio of Comparison Samples
	1	0.0293	0.0302	1.7284	1.6684
	2	0.0298	0.0307	1.6945	1.7062
	3	0.0321	0.0308	1.6595	1.6699
	4	0.0284	0.0310	1.6785	1.6790
	5	0.0310	0.0327	1.7101	1.6338
	6	0.0319	0.0305	1.6242	1.6823
N		6	6	6	6
Mean		0.03042	0.03098	1.68253	1.67327
SD		0.001488	0.000884	0.037300	0.023629
%CV		4.89	2.85	2.22	1.41
% PEAK AREA RATIO		98.171		100.554	

WITHIN AND BETWEEN BATCH PRECISION AND ACCURACY

Sr. No.	LLOQQC		LQC		M1QC		MQC		HQC	
	0.051		0.145		0.501		4.005		8.010	
	0.041	0.061	0.123	0.167	0.123	0.167	3.404	4.606	6.809	9.212
	Conc. Found	% Accuracy	Conc. Found	% Accuracy	Conc. Found	% Accuracy	Conc. Found	% Accuracy	Conc. Found	% Accuracy
11	**0.058**	**113.791**	**0.134**	**92.720**	**0.474**	**94.587**	**3.764**	**93.986**	**7.810**	**97.501**
2	**0.055**	**106.903**	**0.154**	**106.466**	**0.478**	**95.348**	**3.887**	**97.045**	**8.049**	**100.482**
3	**0.052**	**101.344**	**0.142**	**98.081**	**0.497**	**99.240**	**3.866**	**96.528**	**7.734**	**96.557**
4	**0.061**	**119.997**	**0.145**	**99.916**	**0.475**	**94.869**	**3.998**	**99.825**	**7.974**	**99.549**
5	**0.058**	**114.496**	**0.154**	**105.903**	**0.501**	**99.979**	**3.805**	**94.997**	**8.273**	**103.287**
6	**0.052**	**102.677**	**0.144**	**99.422**	**0.468**	**93.371**	**3.757**	**93.803**	**8.180**	**102.120**
N	**6**		**6**		**6**		**6**		**6**	
Mean	0.0560		0.1455		0.4822		3.8462		8.0033	
SD	0.00363		0.00764		0.01350		0.09112		0.20823	
%CV	6.49		5.25		2.80		2.37		2.60	
% Nominal	109.804		100.345		96.241		96.034		99.917	

RECOVERY OF ANALYTE

Sr. No.	LQC		MQC		HQC	
	Nominal Concentration (ng/mL)					
	Area of extracted samples	Area of Post extracted samples	Area of extracted samples	Area of Post extracted samples	Area of extracted samples	Area of Post extracted samples
1	5743	6170	147003	184063	312592	386464
2	5558	6480	147780	186446	292763	391533
3	5928	5519	149999	184507	304038	403628
N	3	3	3	3	3	3
Mean	5743.0	6056.3	148260.7	185005.3	303131.0	393875.0
SD	185.00	490.48	1554.76	1267.25	9945.57	8818.42
%CV	3.22	8.10	1.05	0.68	3.28	2.24
% Recovery	94.826		80.139		76.961	
Average	83.9753					
S.D.	9.53035					
% C.V.	11.35					

RECOVERY OF INTERNAL STANDARD

Sr. No.	LQC		MQC		HQC	
	Nominal Concentration (ng/mL)					
	Area of extracted samples	Area of Post extracted samples	Area of extracted samples	Area of Post extracted samples	Area of extracted samples	Area of Post extracted samples
1	197723	234221	202355	240720	209771	256313
2	195019	235200	204163	248644	197359	257451
3	200297	218701	205819	242729	206372	262810
N	3	3	3	3	3	3
Mean	197679.7	229374.0	204112.3	244031.0	204500.7	258858.0
SD	2639.27	9256.04	1732.56	4119.33	6414.11	3469.51
%CV	1.34	4.04	0.85	1.69	3.14	1.34
% Recovery	86.182		83.642		79.001	
Average	82.9417					
S.D.	3.64137					
% C.V.	4.39					

ANALYTE-II: TELMISARTAN:

Specificity/Cross Reactivity:

Chromatograms of six different lots of blank human plasma were identified for inspecting analytes from the potential interference of endogenous substances at the peak region (amlodipine and Telmisartan).

The specificity of the method was evaluated by comparing chromatograms of blank plasma, amlodipine and IS standard, blank plasma spiked with amlodipine and IS, same for Telmisartan

Sample Name	Analyte Area	IS Area	% Interference of Analyte	% Interference of IS
Blank (Without IS)	0	0	0.00	0.00
STD 1 (LLOQ)	5818	38338	NA	NA
Blank+IS	314	38689	5.40	NA
ULOQ_Telmisartan (Without IS)	2425049	0	0.00	0.00
Blank+IS Amlodipine	168	0	2.89	0.00
ULOQ (Without IS)	0	0	NA	0.00

Selectivity

Blank ID	Area Response at Analyte RT	Area Response at IS RT	LLOQ Area	Internal Standard Area	Back Calculate Conc.	%Accuracy	% Interference at analyte RT	% Interference at IS RT
Plasma Lot-1	0	0	7974	228101	2.174	108.270	0.00	0.00
Plasma Lot-2	0	0	6402	177110	2.263	112.718	0.00	0.00
Plasma Lot-3	0	0	6141	172596	2.221	110.592	0.00	0.00
Plasma Lot-4	0	0	5673	176779	1.959	97.579	0.00	0.00
Plasma Lot-5	0	0	4917	149234	2.023	100.770	0.00	0.00
Plasma Lot-6	0	0	5057	144601	2.175	108.333	0.00	0.00
Plasma Lot-7	0	0	4773	136642	2.172	108.165	0.00	0.00
Plasma Lot-8	0	0	5589	154322	2.268	112.964	0.00	0.00
Plasma Lot-9	0	0	5008	140915	2.218	110.444	0.00	0.00
Plasma Lot-10	0	0	4167	132516	1.911	95.163	0.00	0.00

SENSITIVITY			
Sr. No.	**LLOQ**		
	Nominal Concentration (ng/mL)		
	2.008		
	1.606	2.410	**S/N**
	Conc. Found	**% Accuracy**	**ratio**
1	1.964	97.824	395.578
2	2.002	99.700	291.464
3	1.833	91.261	415.035
4	2.040	101.596	467.899
5	2.246	111.829	585.609
6	2.195	109.314	521.824
N	6		
Mean	2.0467		
SD	0.15249		
%CV	7.45		
% Nominal	101.926		

WHOLE BLOOD STABILITY

	Sr.No.	LQC		HQC	
		Nominal Concentration (ng/mL)			
		Area Ratio of Stability Samples	Area Ratio of Comparison Samples	Area Ratio of Stability Samples	Area Ratio of Comparison Samples
	1	0.3603	0.3588	50.8600	51.3263
	2	0.3604	0.3476	50.7028	50.7916
	3	0.3597	0.3556	50.5618	51.0996
	4	0.3631	0.3624	51.4205	49.9774
	5	0.3677	0.3655	51.4816	51.0480
	6	0.3656	0.3480	51.7613	50.2817
N		6	6	6	6
Mean		0.03042	0.36280	0.35632	51.13133
SD		0.001488	0.003273	0.007391	0.486784
%CV		4.89	2.85	2.22	1.41
% PEAK AREA RATIO		98.1711		100.5538	

WITHIN AND BETWEEN BATCH PRECISION AND ACCURACY										
Sr. No.	LLOQQC		LQC		M1QC		MQC		HQC	
	2.019		5.848		102.591		410.365		820.730	
	1.615	2.423	4.971	6.725	87.202	117.980	348.810	471.920	697.621	943.840
	Conc. Found	% Accuracy	Conc. Found	% Accuracy	Conc. Found	% Accuracy	Conc. Found	% Accuracy	Conc. Found	% Accuracy
1	2.326	115.222	6.792	116.140	114.739	111.841	443.200	108.002	870.220	106.030
2	2.391	118.448	6.317	108.022	111.123	108.317	0.227*	0.055	860.252	104.815
3	2.407	119.232	6.351	108.593	111.978	109.150	447.971	109.164	847.884	103.309
4	2.369	117.314	6.552	112.038	112.110	109.278	459.050	111.864	871.914	106.236
5	2.492	123.408	6.398	109.403	113.466	110.600	445.384	108.534	866.966	105.634
6	2.371	117.420	6.785	116.017	114.423	111.533	442.512	107.834	852.891	103.919
N	6		6		6		5		6	
Mean	2.3927		6.5325		112.9732		447.6234		861.6878	
SD	0.05577		0.21399		1.45750		6.73416		9.74908	
%CV	2.33		3.28		1.29		1.50		1.13	
% Nominal	118.508		111.705		110.120		109.079		104.990	

RECOVERY OF ANALYTE

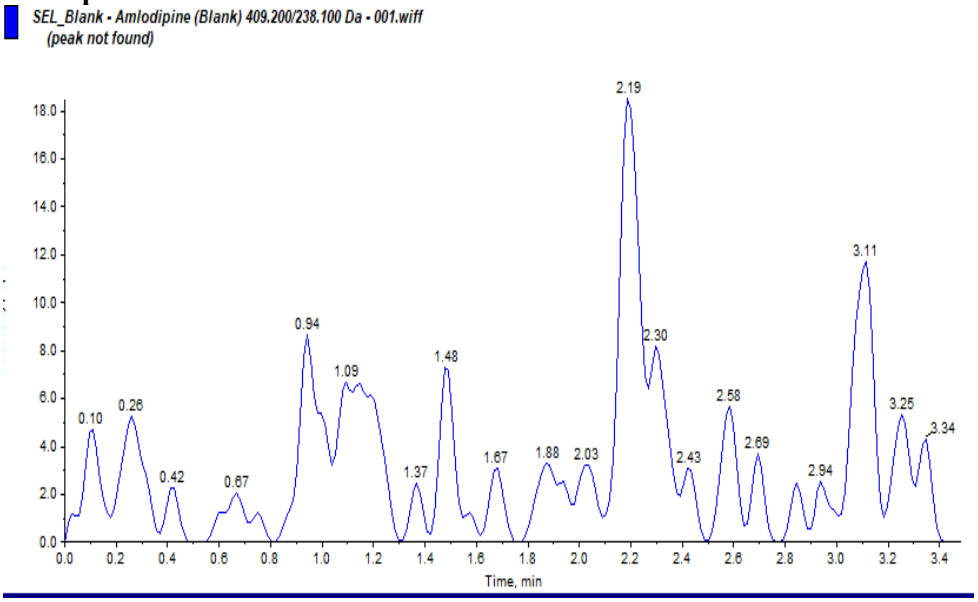
	LQC		MQC		HQC	
	Area of extracted samples	Area of Post extracted samples	Area of extracted samples	Area of Post extracted samples	Area of extracted samples	Area of Post extracted samples
1	20949	26636	1177793	1667535	2198552	3112516
2	20525	25980	1168746	1654438	2099916	3202792
3	19884	25937	1161676	1680472	2184739	3256723
N	3	3	3	3	3	3
Mean	20452.7	26184.3	1169405.0	1667481.7	2161069.0	3190677.0
SD	536.17	391.75	8078.68	13017.08	53408.49	72862.85
%CV	2.62	1.50	0.69	0.78	2.47	2.28
% Recovery	78.110		70.130		67.731	
Average	71.9903					
S.D.	5.43383					
% C.V.	7.55					

RECOVERY OF INTERNAL STANDARD

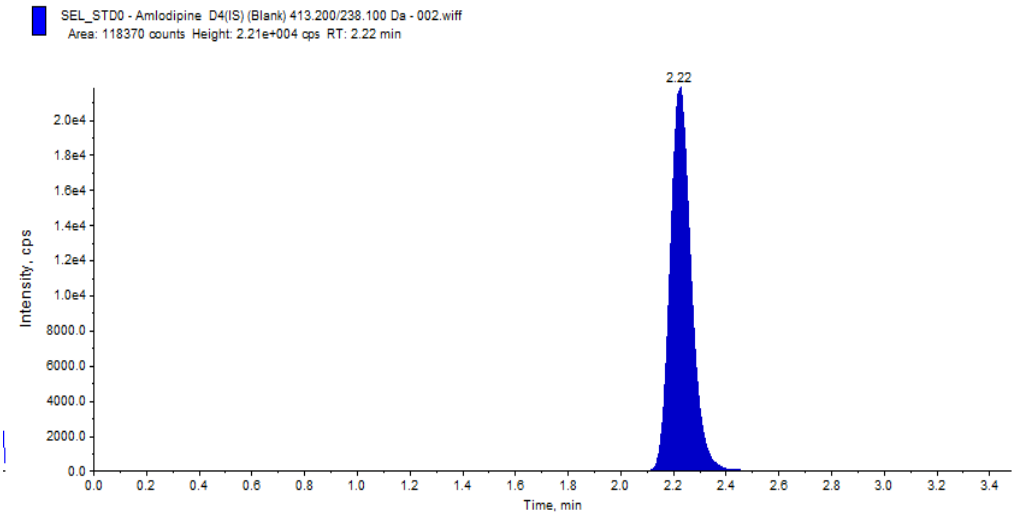
	LQC		MQC		HQC	
	Area of extracted samples	Area of Post extracted samples	Area of extracted samples	Area of Post extracted samples	Area of extracted samples	Area of Post extracted samples
1	205839	278013	202200	278662	194277	279131
2	202021	284773	202322	285107	185106	283308
3	206380	265810	206396	287418	195834	290140
N	3	3	3	3	3	3
Mean	204746.7	276198.7	203639.3	283729.0	191739.0	284193.0
SD	2375.94	9610.81	2388.12	4537.74	5796.86	5557.60
%CV	1.16	3.48	1.17	1.60	3.02	1.96
% Recovery	74.130		71.772		67.468	
Average	71.1233					
S.D.	3.37804					
% C.V.	4.75					

Representative Chromatogram

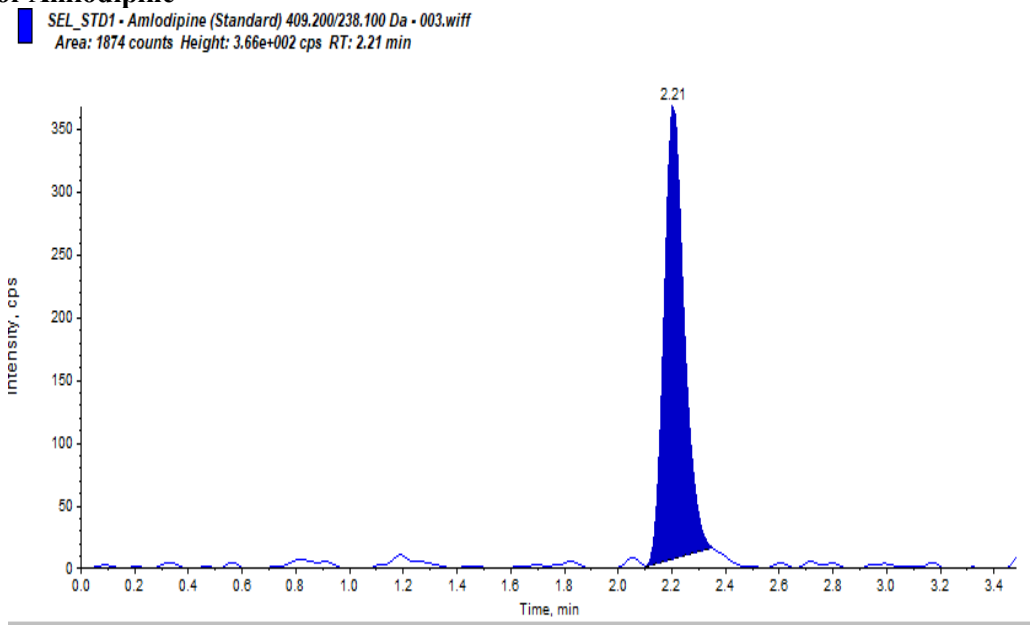
Blank For Amlodipine



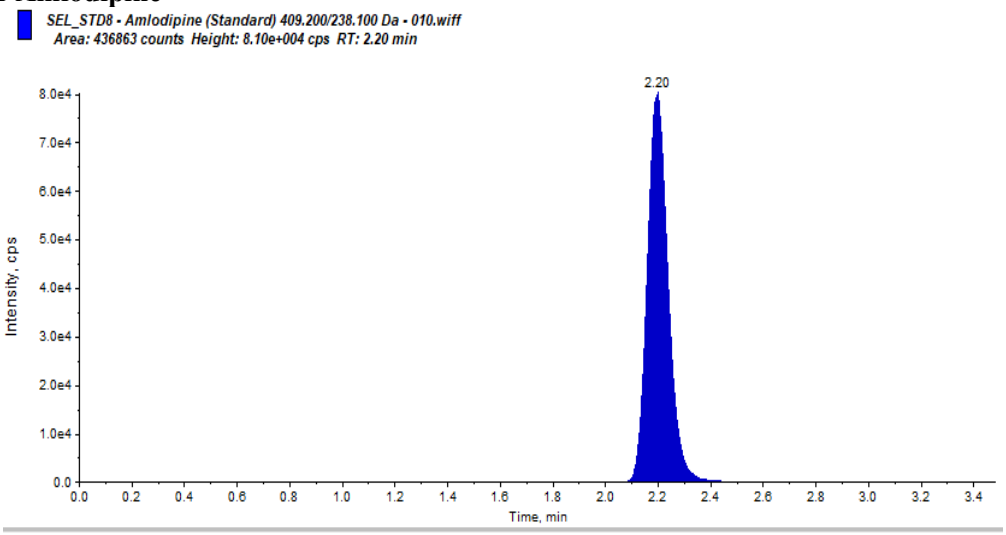
STD0 For Amlodipine



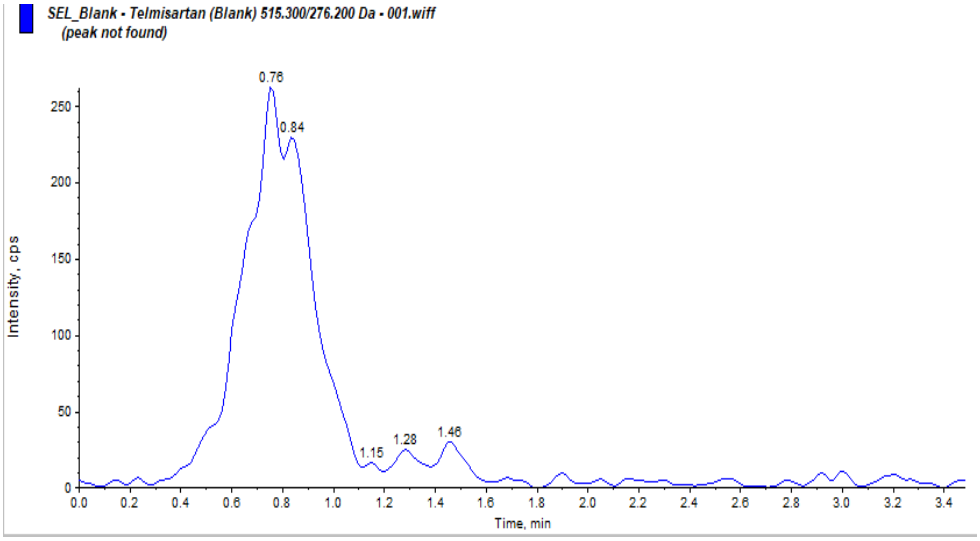
LLOQ For Amlodipine



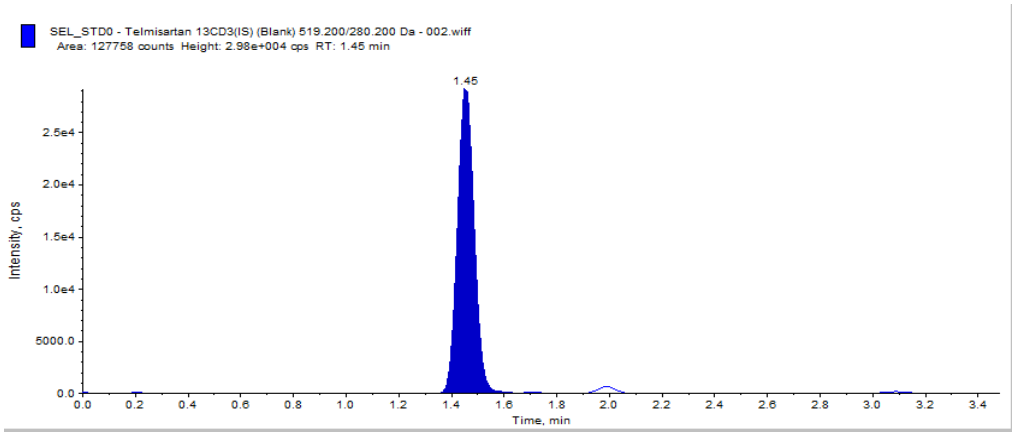
ULOQ For Amlodipine



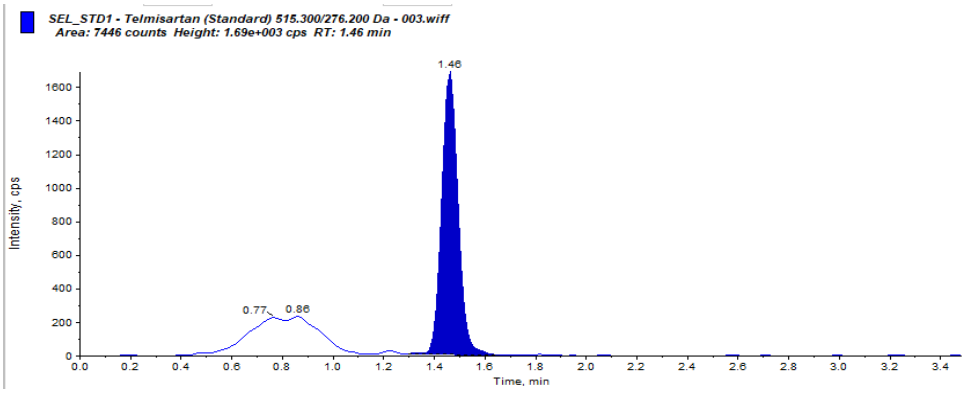
Blank For Telmisartan



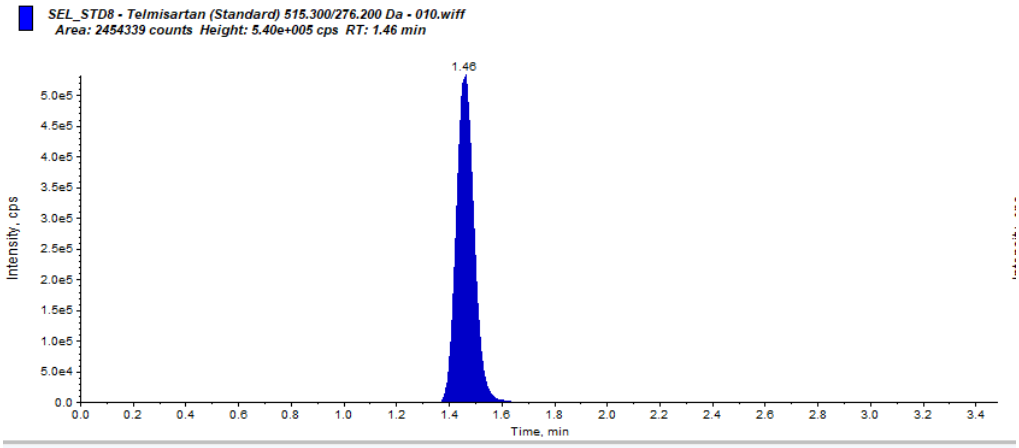
STD0 For Telmisartan



LLOQ For Telmisartan



ULOQ For Telmisartan



Conclusion:

Based on the study's findings for the selected combination, it can be concluded that the sample recoveries across all formulations align with the labeled claims for each drug. The proposed method demonstrates accuracy, sensitivity, speed, and cost-effectiveness in detecting Amlodipine and Telmisartan in combined pharmaceutical formulations. Through rigorous validation in accordance with ICH guidelines and comparison with standard values, the results have consistently met expectations. Consequently, this technique stands as a reliable approach for quantifying the combined dosage form of Amlodipine and Telmisartan.

BIBLIOGRAPHY

1. Ahrens K, Bramlage P. Importance of a fixed combination of telmisartan and amlodipine for the treatment of hypertension. Drugs Today (Barc). 2010 May;46(5):339-50.
2. Pitt JJ. Principles and applications of liquid chromatography-mass spectrometry in clinical biochemistry. Clin Biochem Rev. 2009 Feb;30(1):19-34