VOLUME 10 ISSUE 2 2024

ISSN 2454 – 3055

INTERNATIONAL JOURNAL OF ZOOLOGICAL INVESTIGATIONS

Forum for Biological and Environmental Sciences

Published by Saran Publications, India

International Journal of Zoological Investigations Vol. 10, No. 2, 652-663 (2024)

International Journal of Zoological Investigations
Contents available at Journals Home Page: www.ijzi.net
Editor-in-Chief: Prof. Ajai Kumar Srivastav
Published by: Saran Publications, Gorakhpur, India

ISSN: 2454-3055

Development and Validation of Novel Bioanalytical Method for Simultaneous Estimation of Simvastatin, Ramipril, Atenolol, Hydrochlorothiazide and Aspirin in Polycap™ Capsule using LC-MS/MS

Akula Sushma[1], Konda Shravan Kumar[1], Boggula Narender[2] and Duddagi Suchitra[1]*

[1]Vision College of Pharmaceutical Sciences and Research, RNS Colony, Boduppal, Hyderabad, Telangana, India
[2]Omega College of Pharmacy, Edulabad, Ghatkesar, Hyderabad, Telangana, India

**Corresponding Author*

Received: 15th November, 2023; **Accepted:** 16th August, 2024; **Published online:** 7th September, 2024

https://doi.org/10.33745/ijzi.2024.v10i02.061

Abstract: The present study describes a simple, specific, economical, novel liquid chromatographic-tandem mass spectrometric (LC-MS/MS) for simultaneous estimation of simvastatin, ramipril (RAM), atenolol, hydrochlorothiazide (HCTZ) and aspirin in polycap capsule used for cardiovascular therapy. This method made use of electrospray ionization in positive mode for RAM, simvastatin, atenolol using carbamazepine as internal standard with mobile phase composition of 100% acetonitrile and 5 mM ammonium formate in water and in negative mode for HCTZ, aspirin using triple quadrupole mass spectrometry where 7-hydroxy coumarin was used as an internal standard (IS) with mobile phase composition of 0.1% formic acid in acetonitrile and 0.1% formic acid in water. Separation of analytes was performed on Synergi Polar-RP C_{18} (75x2 mm, 4 µm) column using a gradient elution mode with a runtime of 3.5 min. The method was validated over a concentration range from 0.1-100 ng/ml, 0.1-100 ng/ml, 1-1000 ng/ml, 0.3-300 ng/ml and 2-2000 ng/ml for simvastatin, ramipril, atenolol, hydrochlorothiazide and aspirin, respectively. The method showed good linearity, precision (RSD values between 1.19% to 13.12%) and accuracy (+/-15%). Method validation was in accordance with USFDA bioanalytical method validation guidelines. The recoveries were within 73 to 100% range. The method showed good applicability for measuring these drugs in plasma samples.

Keywords: Bioanalytical method, Carbamazepine, Hydrochlorothiazide, LC-MS/MS, Ramipril, Simvastatin, 7-Hydroxy coumarin

Citation: Akula Sushma, Konda Shravan Kumar, Boggula Narender and Duddagi Suchitra: Development and validation of novel bioanalytical method for simultaneous estimation of simvastatin, ramipril, atenolol, hydrochlorothiazide and aspirin in Polycap™ capsule using LC-MS/MS. Intern. J. Zool. Invest. 10(2): 652-663, 2024.

https://doi.org/10.33745/ijzi.2024.v10i02.061

Introduction

Simvastatin (SMV) is (+)-{1S,3R,7S,8S,8aR}-1,2,3, 7, 8, 8a- hexahydro- 3, 7-dimethyl-8-[2-(2R,4R)-tetrahydro-4- hydroxy-6- oxo-2H-pyran-2-yl]-1-naphthyl-2,2-dimethyll;k butanoate (Fig. 1). It acts

by inhibiting the enzyme HMG Co-A reductase and is used for the treatment of hypercholesterolemia (Patil *et al*., 2007). Ramipril (RP), with the chemical name [(2S, 3aS, 6aS)-1-[(S)-2-[[(S)-1-(ethoxy carbonyl)-3-phenylpropyl] amino] propanoyl] octahydrocyclopenta [b] pyrrole-2-carboxylic acid (Fig. 1), is an angiotensin-converting enzyme inhibitor (ACEI)(Mitakos *et al*., 2002).

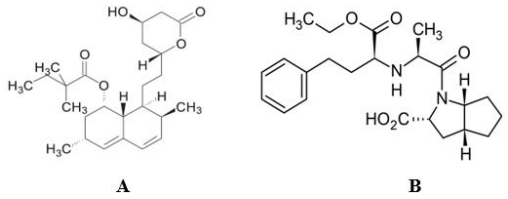

Fig. 1: Structure of A) simvastatin B) ramipril.

Atenolol is a beta (β_1)-selective (cardio selective) adrenoceptor blocking agent (Al-Ghannam *et al*., 2006). The chemical name of atenolol is 4-[2-hydroxy-3-(1-metyl ethyl amino) propyl] benzene acetamide (Fig. 2). Hydrochlorothiazide (HCT) is a thiazide derivative, IUPAC name is 6-chloro-1,1-dioxo 3, 4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide, is one of the oldest members of the thiazide class of diuretics (Fig. 2)(Haitao *et al*., 2003; Kurbanoglu *et al*., 2013). Aspirin (ASP) is chemically 2-(acetyloxy)-benzoic acid (Fig. 2). It is non-selective cyclooxygenase inhibitor used (Nanyan *et al*., 2004; Thaidala Sriveni *et al*., 2021).

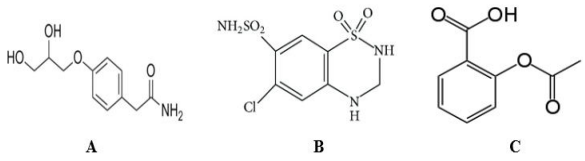

Fig. 2: Structure of (A) atenolol (B) hydrochlorothiazide and (C) aspirin.

Materials and Methods

Chemicals:

Pure sample of simvastatin is procured from Medreich Limited, Hyderabad, Telangana and API of ramipril, hydrochlorothiazide, dimethyl sulfoxide are obtained from Sigma-Aldrich, and all the chemicals used are AR grade.

Instruments:

Analytical HPLC (Shimadzu), Auto sampler (PAL HTS-xt), Mass Spectrometry (API 4000LC/MS/MS Systems, Applied Biosystems) were used.

Experimental work (*Sagar et al., 2008*; *Akiful et al., 2018*: Boggula *et al., 2019*):

Preparation of stock solutions: Primary stock solutions of simvastatin, ramipril, atenolol, hydrochlorothiazide and aspirin were prepared separately at 2 mg/ml by weighing 2mg of drug accurately and dissolved in 1ml of methanol: dimethyl sulfoxide (50:50) in 1.5 ml Eppendorf tubes, vortexed and sonicated to dissolve properly. This was split into 200 µl sub aliquots in 0.5 ml microfuge tubes and stored at 200°C.

Preparation of internal standard: Primary stock solution of 1 mg/ml was prepared for carbamazepine and 7-hydroxy coumarin. From this, working stock solution of 100 ng/ml was prepared by adding 5 µl of each concentrated stock solution to 50 ml of acetonitrile in 50ml Eppendorf tubes and stored at -80°C.

Preparation of mobile phase: 5 mM Ammonium formate, 0.1% formic acid in water, 0.1% formic acid in acetonitrile solutions were prepared.

Extraction solvent: Acetonitrile containing internal standards (carbamazepine at 100 ng/ml and 7-hydroxy coumarin at 100 ng/ml concentration) are used as extraction solvents.

Preparation of mass spectrometry optimization (infusion) solution: 100 ng/ml of tuning solutions for each analyte and internal standard were prepared separately by adding 0.2 µl of 2 mg/ml concentrated stock solution to 4 ml of methanol: water (50:50) in 5 ml centrifuge tubes and vortexed for few minutes.

Plasma preparation: The blood was treated with 2% w/v anti-coagulant (K_2EDTA)and centrifuged at 13000 rpm to separate the plasma. The supernatant plasma is separated into 15 ml centrifuge tubes and stored at -80°C. To this for each ml of plasma 10 µl of 50% w/v of sodium fluoride (esterase inhibitor) was added before

sample processing step.

Quantitative method for drug analysis in human plasma:

Preparation of calibration standards & quality control samples: The calibration standard range was chosen based on the literature reported for human plasma concentrations (C_{max}) of each drug. Initially calibration standards were prepared in acetonitrile: water (50:50). Later by using these working calibration standards, plasma (matrix) standards were prepared.

Preparation of neat solutions: 1.25 µl of Simvastatin, 1.25 µl ramipril, 12.5 µl atenolol, 25 µl aspirin and 3.75 µl hydrochlorothiazide were added to 956.25 µl of aqueous diluent for preparing neat solution ULOQ concentration level (SS-09). Later serial dilution was performed till LLOQ level (SS-01). Similarly, 1.05 µl simvastatin, 1.05 µl ramipril, 11 µl atenolol, 21 µl aspirin and 3.25 µl hydrochlorothiazide were added to 962.65 µl of aqueous diluent for preparing neat solution HQC concentration level. Later serial dilution was performed till LQC level.

Preparation of spiking solutions: These volumes were spiked separately from 2 mg/ml stock solutions of each drug into common main stock to get linearity ranges of 0.1-100 ng/ml, 0.1-100 ng/ml, 1-1000 ng/ml, 2-2000 ng/ml and 0.3-300 ng/ml simultaneously for simvastatin, ramipril, atenolol, aspirin and hydrochlorothiazide, respectively (gives cassette main stock).

Preparation of matrix solution: 2 µl of the cassette mixture (from aqueous linearity) was spiked to 48 µl of plasma and vortexed. Samples were precipitated by adding 150 µl of acetonitrile containing internal standard. The MRM transitions of each analyte were shown to be selective and specific when used to analyze six individual lots of unspiked K_2EDTA human plasma.

Preparation of sample solution: 48 µl of clean reference plasma (blank plasma) was spiked into 1.5 ml centrifuge tubes for each standard (total of 9) and quality control (low, medium and high concentrations). 2 µl of each standard and quality control working stock solution was added separately to the plasma spiked to get 25 times dilution. The centrifuge tubes (Eppendorf) were capped and vortexed for a short time. 150 µl of Acetonitrile containing internal standard was added for each tube and vortexed at 1200 rpm for 5 min. using mix-mate. Then they were centrifuged at 13000 rpm for 10min at 4°C. After centrifugation 150 µl supernatant was spiked and transferred to a 96-well plate. To this 150 µl of milli-Q water was added to each sample well containing standards and quality controls and vortexed for 5 min. The samples are ready for injection.

Development/optimization of MS/MS method: Analytes and internal standards were manually infused separately at 10 µl/min using the Harvard syringe apparatus. Detection was performed using an API 4000 triple quadrupole mass spectrometer (Applied Biosystems/MDS Sciex). Optimum compound dependent parameters such as declustering potential, entrance potential, collision energy, collision cell exit potential were tuned for mass spectrum followed by the identification of precursor mass and corresponding fragment ions. Source/gas parameters were optimized through FIA mode. This process was repeated for each of the individual drugs tested and also for internal standards.

Optimized chromatographic conditions for simvastatin, ramipril, carbamazepine (ISZ), atenolol, hydrochlorothiazide, aspirin and 7-hydroxy coumarin (ISZ):

Column used was Synergi 4µ Polar-RP 80A (C_{18}), 30 x 2 mm with the mobile phase composition of 5 mM ammonium formate and 100% Acetonitrile with a flow rate of 1 ml/min and the Injection volume of about 20 µl. Good chromatogram was observed for all analytes after changing the flow rate. Temperature was maintained at 600°C and results are depicted in Figures 3 and 4.

Validation:

Linearity:

The calibration curve for analytes was generated

using quadratic regression and performed by the Analyst 1.5 software. Standards with a back-calculated accuracy outside the range of 85-115% of the nominal concentration were excluded from the regression statistics. The correlation coefficients for all calibration curves were more than 0.99.

Accuracy and precision:

The results show acceptable accuracies, especially when taking into account the multistep sample preparation procedure, with most between 90-110% of the nominal concentration. The coefficients of variation for each analyte, both intra- and inter-day, are also well below the nominal criteria of less than 15%.

Recovery:

The recovery for analytes from K_2EDTA human plasma from the LQC, MQC and HQC level was determined by comparing with their respective aqueous quality control samples.

Stability studies:

Freeze-thaw stability:

Freeze-thaw stability of low, mid and high-level quality control samples was validated for three cycles of freezing and thawing. The calculated values for the freeze-thaw stability samples were within the precision and accuracy range determined by the inter-day QC samples, leading to the conclusion that the analytes showed no instability due to freezing and thawing over the three cycles.

Auto-sampler stability:

Auto-sampler stability of K_2EDTA human plasma quality control samples was validated by keeping the samples for 24 h duration in auto-sampler (temperature condition at 4^{o}C) and then compared with fresh samples. The calculated values for auto-sampler stability (n=6 at low, mid and high QC levels) were within the precision and accuracy range determined by the inter-day QC samples, leading to the conclusion that the analytes showed no instability due to auto-sampler storage of the samples throughout the run time.

Bench-top stability:

Bench-top stability of K_2EDTA human plasma quality control samples was validated by keeping the samples for 4 h at room temperature where the sample processing is carried out and then they were compared with fresh samples. The calculated values for bench-top stability (n=6 at low, mid and high QC levels) were within the precision and accuracy range determined by the inter-day QC samples, leading to the conclusion that the analytes showed no instability due to auto-sampler storage of the samples throughout the run time.

Results and Discussion

The LC-MS procedure was optimised with a view to develop an accurate assay method for simultaneous determination. The present study describes a novel liquid chromatographic-tandem mass spectrometric (LC-MS/MS) method for the simultaneous estimation of simvastatin, aspirin, ramipril, atenolol, and hydrochlorothiazide in plasma. A simple, specific, sensitive, validated method was developed using liquid chromate-graphy with tandem mass spectrometry with electrospray ionization of human plasma for the simultaneous estimation of drugs (simvastatin, ramipril, atenolol, hydrochlorothiazide, and aspirin) of Polycap™ capsule used in cardio-vascular therapy.

This validated bioanalytical method has the potential for future fixed dose combination based preclinical and clinical studies that can save analysis time. The proposed method was validated as per ICH guidelines and it can also be used for routine quality control analysis of these drugs in biological samples either alone or in combined pharmaceutical dosage forms. The simple procedure involved in sample preparation and the short runtime (3.5 min) added the important property of high throughput to the method.

Positive ion mode (for simvastatin, ramipril and atenolol) and negative ion mode (for

Table 1: Positive ion mode (for simvastatin, ramipril and atenolol) and negative ion mode (for hydrochlorothiazide and aspirin)

Parameter	**Value (simvastatin, ramipril and atenolol)**	**Value (hydrochlorothiazide and aspirin)**
Ionization type and polarity	ESI, positive ion mode	ESI, negative ion mode
Ion source	Turbo spray	Turbo spray
Scan type	MRM	MRM
Ion spray voltage	5500V	4500V
Q1 Resolution	Unit (0.7)	Low (1.1)
Q3 Resolution	Unit (0.7)	Low (1.1)
Temperature	500^{o}C	550^{0}C
Gas1	50	55
Gas2	50	50
CUR gas	20	10
CAD gas	4	4
Ihe	ON	ON

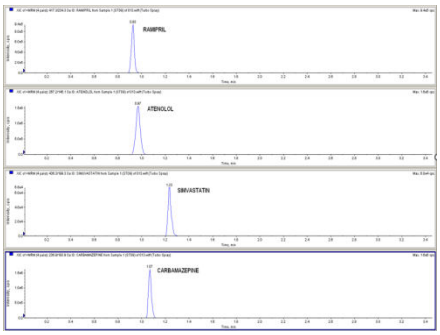

Fig. 3: Optimized curves of simvastatin, ramipril, carbamazepine and atenolol.

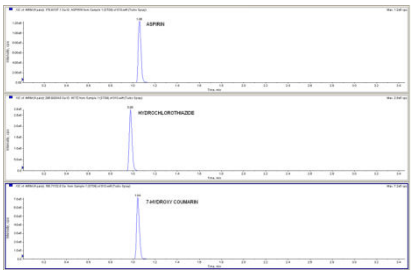

Fig. 4: Optimized curves of hydrochlorothiazide, aspirin and 7-hydroxy coumarin (ISZ).

Table 2: Calibration curve data for simvastatin in human plasma

Concentration (ng/ml)							
Std. conc.	**Batch-1**	**Batch-2**	**Batch-3**	**Mean**	**SD**	**% CV**	**% Accuracy**
0.1	0.09	0.11	0.10	0.10	0.01	8.46	101.00
0.2	0.23	0.16	0.20	0.20	0.03	16.14	98.50
1	1.04	0.88	0.97	0.96	0.08	8.24	96.40
5	4.93	4.99	5.02	4.98	0.04	0.85	99.57
20	19.31	20.95	20.73	20.33	0.89	4.37	101.64
50.01	47.36	53.76	49.00	50.04	3.32	6.64	100.07
80.01	84.03	75.74	79.25	79.67	4.16	5.22	99.58
90	86.31	86.61	88.03	86.98	0.92	1.06	96.64
100	100.40	105.78	103.03	103.07	2.69	2.61	103.07

Table 3: Calibration curve data for ramipril in human plasma

Concentration (ng/ml)							
Std. conc.	**Batch-1**	**Batch-2**	**Batch-3**	**Mean**	**SD**	**% CV**	**% Accuracy**
0.1	0.10	0.11	0.10	0.10	0.00	2.26	102.33
0.2	0.14	0.18	0.10	0.14	0.04	28.58	69.33
1	0.91	1.00	0.87	0.93	0.06	6.91	92.73
5	4.85	4.89	5.29	5.01	0.24	4.83	100.16
20	20.35	20.00	21.33	20.56	0.69	3.35	102.80
50.01	49.84	51.52	47.18	49.52	2.19	4.42	99.01
80.01	85.37	85.46	79.59	83.47	3.36	4.03	104.33
90	84.13	86.99	88.71	86.61	2.31	2.67	96.23
100	99.40	100.74	106.83	102.32	3.96	3.87	102.32

Table 4: Calibration curve data for atenolol in human plasma

Concentration (ng/ml)							
Std. conc.	**Batch-1**	**Batch-2**	**Batch-3**	**Mean**	**SD**	**% CV**	**% Accuracy**
1	1.00	0.99	1.01	1.00	0.01	0.90	100.20
2	1.76	2.03	1.94	1.91	0.14	7.25	95.38
10	9.99	9.93	10.33	10.08	0.22	2.16	100.85
50.01	52.20	51.63	52.99	52.27	0.69	1.31	104.52
200.03	214.16	208.38	189.49	204.01	12.90	6.32	101.99
500.06	496.38	500.85	458.10	485.11	23.50	4.84	97.01
800.1	826.37	792.35	784.10	800.94	22.41	2.80	100.11
900	796.10	888.39	907.78	864.09	59.68	6.91	96.01
1000	1038.89	947.23	1112.03	1032.72	82.57	8.00	103.27

Table 5: Calibration curve data for hydrochlorothiazide in human plasma

Concentration (ng/ml)							
Std. conc.	**Batch-1**	**Batch-2**	**Batch-3**	**Mean**	**SD**	**% CV**	**% Accuracy**
0.30	0.30	0.30	0.30	0.30	0.00	0.38	100.89
0.60	0.26	0.87	0.84	0.66	0.35	52.61	109.44
3.00	2.75	2.67	2.75	2.72	0.05	1.75	90.80
15.00	16.80	13.61	15.35	15.25	1.60	10.47	101.68
60.01	65.40	60.24	62.97	62.87	2.58	4.11	104.76
150.02	152.79	148.35	151.52	150.89	2.28	1.51	100.58
240.03	206.50	255.68	248.54	236.90	26.57	11.22	98.70
270.00	269.12	279.23	263.28	270.54	8.07	2.98	100.20
300.00	297.77	313.11	294.31	301.73	10.01	3.32	100.58

Table 6: Calibration curve data for aspirin in human plasma

Concentration (ng/ml)							
Std. conc.		Batch-1	Batch-2	Batch-3	Mean	SD	% CV
2.00		1.89	2.02	1.90	1.94	0.07	3.70
4.00		4.05	3.94	4.40	4.13	0.24	5.81
20.00	19.37	19.25	19.74	19.46	0.25	1.30	97.28
100.01	83.86	99.04	96.66	93.19	8.17	8.76	93.18
400.05	428.12	405.54	404.46	412.71	13.36	3.24	103.16
1000.13	1038.12	989.67	955.99	994.59	41.28	4.15	99.45
1600.20	2066.70	1651.65	1657.79	1792.05	237.87	13.27	111.99
1800.00	1728.20	1855.26	1727.17	1770.21	73.65	4.16	98.34
2000.00	1906.19	1968.70	2070.52	1981.81	82.94	4.19	99.09

Table 7: Precision and accuracy for simvastatin in human plasma

		Concentration (ng/ml)		
		LQC (0.3)	MQC (50.4)	HQC (84.0)
Batch-1 (n=6)	Intra-run mean	0.32	47.31	84.56
	Intra-run SD	0.03	3.19	8.80
	Intra-run % CV	8.93	6.73	10.41
	Intra-run % accuracy	108	93.87	100.66
Batch-2 (n=6)	Intra-run mean	0.29	47.96	78.14
	Intra-run SD	0.02	3.13	4.43
	Intra-run % CV	7.45	6.53	5.67
	Intra-run % accuracy	97.00	95.17	93.02
Batch-3 (n=6)	Intra-run mean	0.30	46.09	73.71
	Intra-run SD	0.03	2.46	5.39
	Intra-run % CV	9.62	5.33	7.31
	Intra-run % accuracy	98.33	91.44	87.75
Inter-batch (n=18)	Inter-run mean	0.30	47.12	78.80
	Inter-run SD	0.02	0.95	5.45
	Inter-run % CV	5.94	2.02	6.92
	Inter-run % accuracy	101.11	93.49	93.81

Table 8: Precision and accuracy for ramipril in human plasma

		Concentration (ng/ml)		
		LQC (0.3)	MQC (50.4)	HQC (84.0)
Batch-1 (n=6)	Intra-run mean	0.33	52.54	85.44
	Intra-run SD	0.03	2.41	3.62
	Intra-run % CV	10.46	4.59	4.24
	Intra-run % accuracy	108.39	104.25	101.71
Batch-2 (n=6)	Intra-run mean	0.28	49.50	89.27
	Intra-run SD	0.01	5.23	2.11
	Intra-run % CV	4.76	10.57	2.37
	Intra-run % accuracy	94.28	98.21	106.27
Batch-3 (n=6)	Intra-run mean	0.27	47.80	83.10
	Intra-run SD	0.02	0.97	7.54
	Intra-run % CV	6.62	2.02	9.07
	Intra-run % accuracy	90.28	94.83	98.93
Inter-batch (n=18)	Inter-run mean	0.29	49.94	85.94
	Inter-run SD	0.03	2.41	3.11
	Inter-run % CV	9.74	4.82	3.62
	Inter-run % accuracy	97.65	99.10	102.30

Table 9: Precision and accuracy for atenolol in human plasma

		Concentration (ng/ml)		
		LQC (3.17)	**MQC (528)**	**HQC (880)**
Batch-1 (n=6)	Intra-run mean	3.43	541.23	835.72
	Intra-run SD	0.33	21.46	26.38
	Intra-run % CV	9.60	3.96	3.16
	Intra-run % accuracy	108.17	102.51	94.97
Batch-2 (n=6)	Intra-run mean	3.15	494.87	809.82
	Intra-run SD	0.30	17.12	9.65
	Intra-run % CV	9.57	3.46	1.19
	Intra-run % accuracy	99.37	93.73	92.03
Batch-3 (n=6)	Intra-run mean	3.36	491.72	868.90
	Intra-run SD	0.24	23.31	54.79
	Intra-run % CV	7.06	4.74	6.31
	Intra-run % accuracy	106.07	93.13	98.74
Inter-batch (n=18)	Inter-run mean	3.31	509.28	838.15
	Inter-run SD	0.15	27.72	29.62
	Inter-run % CV	4.39	5.44	3.53
	Inter-run % accuracy	104.54	96.45	95.24

Table 10: Precision and accuracy for hydrochlorothiazide in human plasma

		Concentration (ng/ml)		
		LQC (0.94)	**MQC (156)**	**HQC (260)**
Batch-1 (n=6)	Intra-run mean	0.96	165.00	274.47
	Intra-run SD	0.12	5.23	20.44
	Intra-run % CV	12.40	3.17	7.45
	Intra-run % Accuracy	101.76	105.77	105.57
Batch-2 (n=6)	Intra-run mean	0.97	158.64	265.91
	Intra-run SD	0.06	3.92	5.72
	Intra-run % CV	6.60	2.47	2.15
	Intra-run % Accuracy	102.68	101.69	102.27
Batch-3 (n=6)	Intra-run mean	0.98	165.35	290.63
	Intra-run SD	0.04	3.56	3.69
	Intra-run % CV	3.88	2.15	1.27
	Intra-run % Accuracy	104.27	106.00	111.78
Inter-batch (n=18)	Inter-run mean	0.97	163.00	277.00
	Inter-run SD	0.01	3.78	12.55
	Inter-run % CV	1.24	2.32	4.53
	Inter-run % Accuracy	102.90	104.49	106.54

Table 11: Precision and accuracy for aspirin in human plasma

		Concentration (ng/ml)		
		LQC (6.05)	**MQC (1008)**	**HQC (1680)**
Batch-1 (n=6)	Intra-run mean	6.15	1112.26	1786.96
	Intra-run SD	0.44	28.99	57.73
	Intra-run % CV	7.13	2.61	3.23
	Intra-run % accuracy	101.70	110.34	106.37
Batch- (n=6)	Intra-run mean	5.82	1119.54	1832.43
	Intra-run SD	0.40	37.76	25.67
	Intra-run % CV	6.93	3.37	1.40
	Intra-run % accuracy	96.25	111.07	109.07
Batch-3 (n=6)	Intra-run mean	5.41	880.27	1628.46
	Intra-run SD	0.21	18.77	45.81
	Intra-run % CV	3.84	2.13	2.81
	Intra-run % accuracy	89.49	87.33	96.93
Inter-batch (n=18)	Inter-run mean	5.80	1037.35	1749.29
	Inter-run SD	0.37	136.09	107.08
	Inter-run % CV	6.39	13.12	6.12
	Inter-run % accuracy	95.81	102.91	104.12

Table 12: Recovery data of simvastatin, ramipril, atenolol, hydrochlorothiazide and aspirin

Analyte	**Concentration**			**Avg. recovery**	**SD**	**% CV**
	LQC	**MQC**	**HQC**			
Simvastatin	73.36	74.74	72.32	73.47	1.22	1.66
Ramipril	98.81	100.70	100.04	99.85	0.96	0.96
Atenolol	80.84	84.08	86.07	83.66	2.64	3.16
HCTZ	99.31	93.98	97.71	97.00	2.73	2.82
Aspirin	82.26	81.51	81.50	81.76	0.43	0.53

Table 13: Stability data of analytes

Analyte name	**Fresh samples**	**Freeze-thaw stability samples**	**Auto-sampler stability samples**	**Bench-top stability samples**
Simvastatin				
LQC	8.77	13.28	12.26	5.39
MQC	4.87	11.81	4.36	8.97
HQC	4.76	5.99	2.96	11.21
Ramipril				
LQC	5.75	12.26	11.98	6.93
MQC	6.05	5.21	5.48	7.52
HQC	4.48	2.57	3.08	3.02
Atenolol				
LQC	4.56	9.35	8.45	13.97
MQC	3.99	3.65	5.33	5.57
HQC	2.76	2.92	7.57	5.86
Hydrochlorothiazide				
LQC	12.53	14.73	7.76	4.22
MQC	2.75	2.44	1.67	9.03
HQC	1.54	2.64	1.86	6.42
Aspirin				
LQC	6.09	10.88	6.47	14.52
MQC	3.70	3.26	1.67	12.35
HQC	1.00	4.05	1.41	7.74

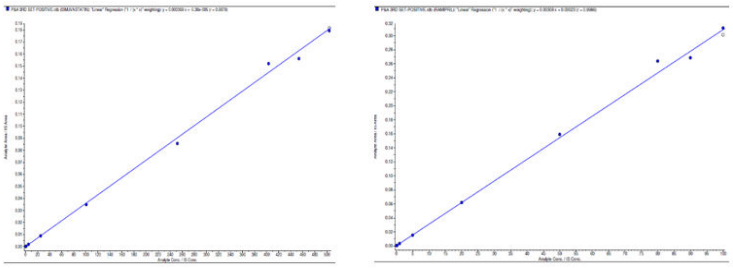

Fig. 5: Calibration curve for (A) simvastatin and (B) ramipril.

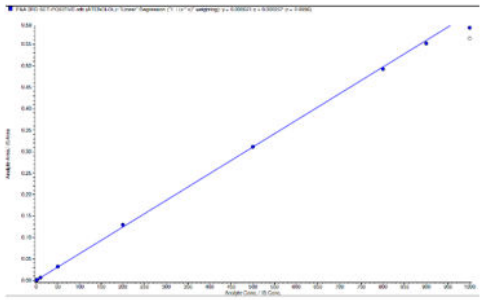

Fig. 6: Calibration curve for atenolol.

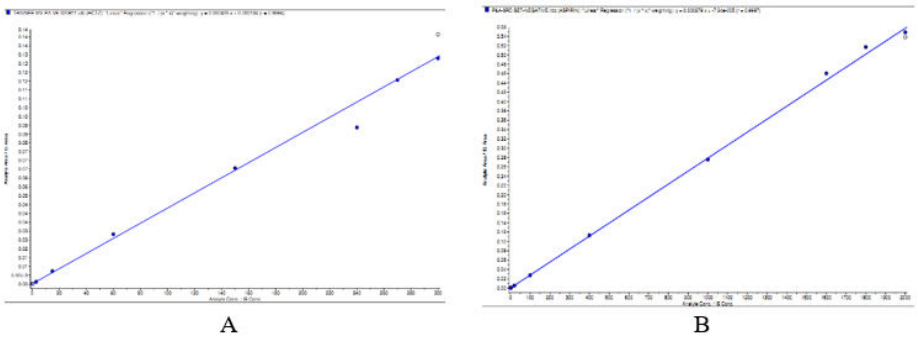

Fig. 7: Calibration curve for A) hydrochlorothiazide B) aspirin.

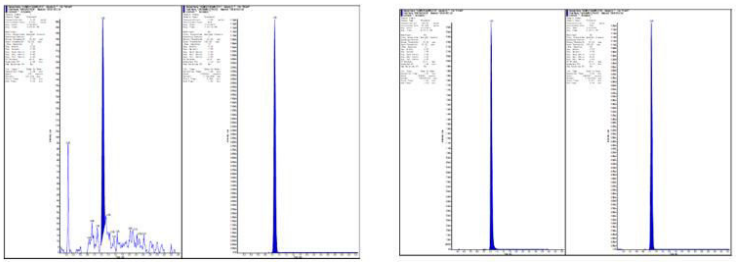

Fig. 8: LLOQ and ULOQ chromatograms for simvastatin.

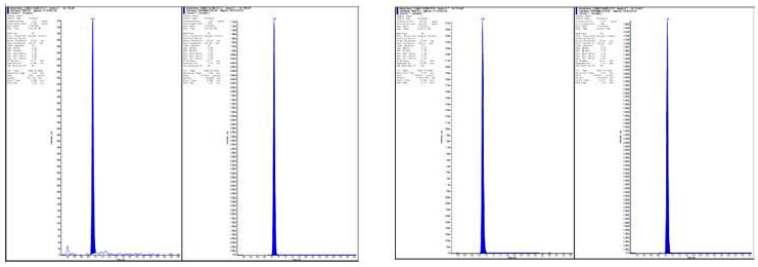

Fig. 9: LLOQ and ULOQ chromatograms for ramipril.

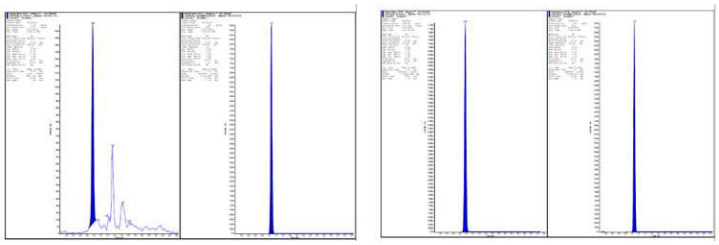

Fig. 10: LLOQ and ULOQ chromatograms for atenolol.

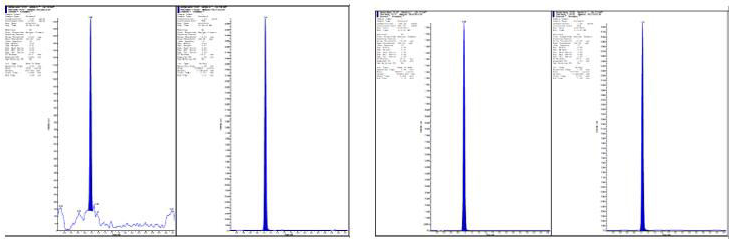

Fig. 11: LLOQ and ULOQ chromatograms for hydrochlorothiazide.

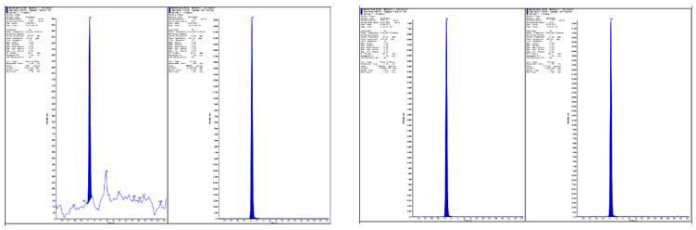

Fig. 12: LLOQ and ULOQ chromatograms for aspirin.

hydrochlorothiazide and aspirin) are reported in above Table 1.

Linearity:

The back calculated intra-batch data for standard curves is presented in Tables 2-6 and Figures 3-4. The correlation coefficients for all calibration curves were more than 0.99.

Accuracy and precision:

Intra-batch accuracy and precision for each analyte are shown in Tables 7-11. The results show acceptable accuracies between 90-110%. The coefficients of variation for each analyte are less than 15%. The average recovery for simvastatin, ramipril, atenolol, hydrochloro-thiazide and aspirin were found to be 73.47%, 99.85%, 83.66%, 97% and 81.76% (n=6), respectively. Recovery data are represented in Table 12. Stability data is shown in the Table 13.

Conclusion

A simple, rapid, sensitive and selective LC-MS/MS

method was developed and validated for the simultaneous estimation. The present study represents the report that, deals with the employment of Synergi Polar-RP C_{18} (75x2 mm, 4 µm) column for simultaneous estimation of simvastatin, ramipril, atenolol, hydrochloro-thiazide and aspirin in polycap capsule. The proposed method showed acceptable accuracy, precision, selectivity and linear concentration ranges. Statistical analysis of the results proved that the method is suitable without any interference from the excipients.

References

Akiful Haque M, Shanthi Priya DK, Dibyalochan M, Vasudha B and Boggula N. (2018) Analytical method development and validation of amlodipine in human plasma by using liquid chromatography–mass spectrometry/mass spectrometry. Asian J Pharm Clin Res. 11(7): 393-397.

Al-Ghannam SM. (2006) A simple spectrophotometric method for the determination of beta-blockers in dosage forms. J Pharm Biomed Anal. 40: 151–156.

Boggula N, Shanmuga Pandiyan P. (2019) Development and validation of opicapone in human plasma using tolcapone as internal standard by liquid chromatography-tandem mass spectrometry. J Emerging Technol Innov Res. 6(1): 1075-1089.

Haitao Y, Yan F and Yiwen L. (2003) Determination of simvastatin in human plasma by liquid chromatography–mass spectrometry. J Chromatogr B 785: 369-375.

Kurbanoglu S, Gumuştaş M and Ozkan SA. (2013) Simultaneous estimation and validation of some binary mixtures of antihypertensive drugs by RP-LC methods using two new generation silica columns. J Pharm Biomed Anal. 72: 198-201.

Mitakos A and Panderi I. (2002) A validated LC method for the determination of ramipril in pharmaceutical preparations. J Pharm Biomed Anal. 28(3-4): 431-438.

Nanyan Z, Amy Y, John Douglas R and Jamie JZ. (2004) Quantitative analysis of simvastatin and its beta-hydroxy acid in human plasma using automated liquid–liquid extraction based on 96-well plate format and liquid chromatography-tandem mass spectrometry. J Pharm Biomed Anal. 34: 175-187.

Patil P, Patil V and Paradkar A. (2007) Formulation of a self-emulsifying system for oral delivery of simvastatin: *In vitro* and *in vivo* evaluation. Acta Pharm. 57: 111-122.

Sagar AP, Ashutosh P, Santosh SJ, Vikas VV, Noel AG. Sudhir SK. (2008) Simultaneous determination of hydrochlorothiazide, quinapril and quinaprilat in human plasma by liquid chromatography-tandem mass spectrometry. J Chromatogr B 873: 59–69.

Thaidala S, Vanamala N, Vemula SR, Aeruva R, Sunil P, Akiful Haque M, Vasudha B and Boggula N. (2021) Development and validation of dolutegravir in bulk and formulation: An anti-retroviral drug using uv-spectroscopy. Int J Pharmaceut Quality Assurance 12(1): 57-60.