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Metoprolol succinate and Olmesartan medoxomil spiked in human plasma for simultaneous estimation of antihypertensive drugs using RP-HPLC

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ABSTRACT:

Introduction: *To create a quick, precise, and economical HPLC approach for the simultaneous quantification of metoprolol succinate and olmesartan medoxomil in a combination tablet formulation, a simultaneous equation technique has been devised.*

Materials and Methods: *The method uses acetonitrile as a solvent and is based on calculations that allow for simultaneous analysis of both medications. Olmesartan medoxomil and metoprolol succinate have absorption peaks in acetonitrile at 225 nm.*

Results: *Olmesartan medoxomil and metoprolol succinate showed linearity at concentrations of 5 and 25 µg/ml and 4 and 20 µg/ml, respectively. To find the medicine amounts, the simultaneous equations method was used. For metoprolol succinate, the average recovery time was 97.72 ± 2.009 hours, and for olmesartan medoxomil, it was 98.02 ± 1.28 hours..*

Conclusion*: This method makes it easy, accurate, and precise to find both and olmesartan medoxomil metoprolol succinate at the same time in pharmaceutical tablet format. Recovery studies and statistical evidence support the research's findings.*

KEYWORDS: *Acetonitrile, metoprolol succinate, HPLC, olmesartan medoxomil, recovery, validation, method development.*

INTRODUCTION:

Metoprolol succinate, a β-blocker as a cardio-selective, is utilized for the management of arrhythmia, heart failure, myocardial infarction, angina pectoris, and hypertension [1]. A lot of research has been done on different ways to measure metoprolol succinate in pharmaceutical formulations and biological matrices [2]. These include UV, HPLC and RP-HPLC, validated for metoprolol assessment in human plasma. Spectrophotometry and RP-HPLC techniques can also be used to measure metoprolol succinate along with other pharmaceuticals. Angiotensin II receptor antagonists, such as olemesartan medoxomil, are utilized for the management of hypertension. There is no approved pharmacoeia for olmesartan medoxomil [3]. There are many ways to find out how much olmesartan medoxomil is in pharmaceutical dosage forms and biological fluids. These include spectrophotometry, HPLC, and the RP-HPLC method for finding out how much is present while other drugs are being measured at the same time. For the treatment of hypertension, the market offers combination dose forms of metoprolol succinate and olmesartan medoxomil [4]. According to a literature study, simple spectroscopic techniques based on the simultaneous equation approach and methanol as a solvent can be used to quantify the concentrations of metoprolol succinate and olmesartan medoxomil in mixed dose forms [5, 6]. This study describes how to measure both olmesartan medoxomil and metoprolol succinate at the same time in tablet forms using acetonitrile as a solvent. It does this using a simple, accurate, quick, and cheap spectrophotometric method based on simultaneous equations [7-9].

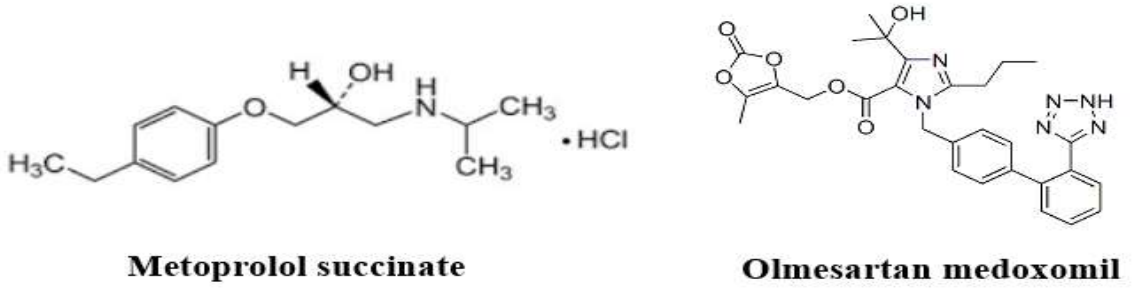

Figure 1: Structure of olmesartan medoxomil and metoprolol succinate

MATERIALS AND METHODS:

The current study has developed and validated a straightforward, quick, accurate, selective, and cost-effective bioanalytical approach for the simultaneous measurement of metoprolol succinate and olemesartan medoxomil in tablet formulation [10, 11].

Method Development:

Solubility: Solubility study of Metoprolol Succinate and Olmesartan medoxomil represented in table 1.

Table 1: Solubility study of Metoprolol Succinate and Olmesartanmedoxomil

Solvents	**Solubility**	
	Metoprolol Succinate	**Olmesartan Medoxomil**
Water	Freely soluble	Insoluble

0.1N NaOH	Freely soluble	Soluble
0.1N HCl	Freely soluble	Soluble
Acetonitrile (ACN)	Freely soluble	Freely soluble
Methanol (MeOH)	Freely soluble	Freely soluble

Selection of Solvent:

Acetonitrile (HPLC Grade) was selected as the solvent to determine the drug's spectral characteristics. After assessing the solubility of both drugs in different solvents, a decision was made [12].

Standard Solutions Preparation:

Stock solution (100 µg/ml) of Metoprolol Succinate Preparation:

To make a stock solution (1000 µg/ml), 10 mg of the drug was carefully measured, put into a 10 ml volumetric flask, and mixed with acetonitrile. In a 10-milliliter volumetric flask, 1 milliliter was taken out and diluted with acetonitrile to make the stock solution [13].

Preparation of stock solution of Olmesartan Medoxomil (100 µg/ml):

To make a stock solution with 1000 µg/ml, 10 milligrams of the API were weighed out correctly, moved to a 10 ml volumetric flask, and mixed with acetonitrile. In a 10-milliliter volumetric flask, 1 milliliter was taken out and diluted with acetonitrile to make the stock solution [14, 15].

Selection of Mobile Phase:

Various mobile phase compositions were evaluated to achieve a sharp peak and optimal resolution, considering drug solubility, stability, and compatibility. Initially, several mobile phases in different ratios were tested to quantify metoprolol succinate and olemesartan medoxomil, considering system appropriateness parameters such as retention time, HETP and tailing factor [16, 17].

Separation Variable:

In comparison to alternative mobile phases, the acetonitrile as to phosphate buffer ratio of 40:60% v/v, pH 2.4, modified with triethylamine, yields excellent peak resolution and satisfactory peak symmetry [18].

Calibration Curve:

Metoprolol succinate and Olmesartan medoxomil are solubilized in acetonitrile (ACN) to provide a stock solution with a concentration of 1 mg/ml for each compound. 100 mg of each medicine was dissolved in a sufficient volume of acetonitrile to prepare the solution, which was subsequently transferred to a 100 ml volumetric flask using the same solvent [19].

Multiple volumes were transferred to prepare the working standards solution. Introduce 0.5–2.5 ml of Olmesartan Medoxomil and 3.0–15.0 ml of stock Metoprolol succinate into a 10.0 ml volumetric flask. Acetonitrile is utilized to complete the volume [20].

150 µl of human plasma drug free was combined with 50 µl of working standard solution to attain drug concentration levels of 3.0 µg/ml to 15.0 µg/ml for metoprolol succinate and 0.5 µg/ml to 2.5 µg/ml for olemesartan medoxomil.

At three different concentration levels—low, medium, and high—three separate quality control

samples were made and mixed together. It was possible to make a calibration model [21].

Plasma sample preparation:

Blood is extracted from the rat's retroorbital plexus. K3 EDTA anticoagulant tubes are utilized for blood collection. Liquid-liquid extraction is employed to isolate the plasma from the blood sample. Acetonitrile was utilized as a solvent for extraction. The plasma sample was centrifuged at 5000 rpm for five minutes. Acetonitrile was introduced into a 10 ml polypropylene tube subsequent to the pipetting of an aliquot (0.5 ml) into the tube. Sample material was extracted for 15 minutes, after which the supernatant was collected in Eppendorf tubes. Following filtration through a syringe filter, plasma was promptly introduced into an HPLC system [22, 23].

Working Linearity:

The relationship between analyte concentration and instrument response is referred to as a calibration curve. A calibration curve should be established by incorporating known analyte concentrations into the biological matrix identical to that of the samples intended for the inquiry. We found linearity by showing the peak area ratio against the concentrations of metoprolol succinate and olemesartan medoxomil. The drugs were tested at five different concentrations, ranging from 3.0 µg/ml to 15.0 µg/ml for metoprolol succinate and 0.5 µg/ml to 2.5 µg/ml for olemesartan medoxomil [24].

LLOQ and ULOQ of Drugs: Both Drugs LLOQ and ULOQ value represented in table 2.

Table 2: Both Drugs LLOQ and ULOQ value

Drug/ API Name	**LLOQ**	**ULOQ**
Metoprolol Succinate	3.020 µg/ml	15.004 µg/ml
Olmesartan Medoxomil	0.4969 µg/ml	2.493 µg/ml

Linearity: Linearity of Metoprolol Succinate represented in table 3 and table 4.

Table 3: Linearity of Metoprolol Succinate

Metoprolol Succinate						
Conc. µg/ml	**Area Under Curve (AUC)**					**Mean Area**
	R1	**R2**	**R3**	**R4**	**R5**	
3.0	24751998	2478115	2474450	2472709	2478120	24751998
6.0	4947125	4949210	4948135	4947130	4947198	4947125
9.0	7323921	7324205	7321195	7319998	7323216	7323921
12.0	9650493	9652116	9649115	9651116	9650199	9650493
15.0	12372427	12392517	12390150	12381511	1231016	12372427

Table 4: Linearity of Olmesartan Medoxomil

Olmesartan Medoxomil						
Conc. µg/ml	Area Under Curve					MeanArea
	R1	R2	R3	R4	R5	
0.5	11599720	11611015	11579875	11601598	11600015	11599720
1.0	23221379	23231128	23201998	23229898	23219895	23221379
1.5	34877439	34891125	34879970	34881127	34869991	34877439
2.0	46503841	46511516	46509810	46499196	46521001	46503841
2.5	57772351	57791123	57761315	57779815	57779811	57772351

System Suitability Parameters:

The goal of this test is to see if the resolution and repeatability of the chromatographic machine are good enough for analysis. Five identical doses of a common drug solution were used to collect data for the tests. It was left for the mobile phase and fixed phase to balance out until a stable baseline was reached. Six injections of 20 µl each from a normal stock solution that mixed both drugs (table 5 & table 6) [25, 26].

Table 5: System Suitability Test of Metoprolol Succinate

Replicate Conc.6.0µg/ml	R.T.	AUC	Tailing Factor	HETP	Resolution
R1	3.86	49661474	1.22	3587	00
R2	3.85	49672115	1.20	3585	00
R3	3.87	49670198	1.23	3589	00
R4	3.96	49662615	1.21	3588	00
R5	3.84	49651991	1.19	3583	00
Mean	3.856	49663678	1.21	3586.4	00
SD	0.0114	8004.17	0.015811	2.4	00
% RSD	0.2956	0.020	1.3066	0.0948	00

Table 6: System Suitability Test of Olmesartan Medoxomil

Replicate Conc.2.0µg/ml	R.T.	AUC	Tailing Factor	HETP	Resolution
R1	12.91	46759001	0.97	9463	22.94
R2	12.90	46769861	0.96	9462	22.91
R3	12.93	46749598	0.98	9465	22.96
R4	12.91	46780016	0.99	9468	22.99
R5	12.91	46751216	0.94	9460	22.89
Mean	12.91	46761938	0.968	9463	22.93
SD	0.01095	12898.17	0.01923	3.049	0.03962
% RSD	0.0848	0.01931	1.986	0.0322	0.172

Selectivity:

Selectivity is the ability of an analysis method to find and measure the analyte while leaving other parts of the sample alone. The blanks from six samples were analyzed in the relevant biological matrix. At the LLOQ, selectivity must be guaranteed, and interference should be checked for in every blank sample. To make sure the measurements are correct, more than one analyte must be checked to make sure there is no confusion. To test how selective the method is, six different blank plasma samples from rats were used. Using this method for analysis, each blank sample was checked for interference and compared to samples that had been spiked with analyte amounts at the LLOQ level [27].

Accuracy:

You can figure out how much analyte was recovered in an experiment by comparing the detector reaction to the concentration of the analyte in the solvent to the concentration of the analyte that was added to and taken out of the biological matrix. To find out how much of the analyte and internal standard was recovered, you should compare the results of the tests on samples that were extracted at three different amounts (low, medium, and high) with the results of the test on the standard that wasn't extracted, which means that 100% recovery happened. The recovery of all the analytes does not have to be 100% [28].

Precision:

To find out how accurate the biological matrix volume was, five different sample preparations of the same batch of bulk medicine and formulation were made. It is suggested to use at least three amounts that are within the allowed range of the study sample concentration. The accuracy is based on each quantity. The level shouldn't be more than 15% of the CV, except for the LLOQ, which shouldn't make up more than 20% of the CV [29].

Limit of Detection and Limit of Quantification:

LOD is the smallest amount of an analyte that can be found in a sample without being able to give an accurate number. LOQ, on the other hand, is the smallest amount of an analyte that can be measured with the right level of accuracy and precision [30].

Robustness

Capacity to endure deliberate slight alterations in method parameters and demonstrates its reliability under standard operating settings. Measurements must be meticulously maintained when influenced by variations in analytical conditions [31].

Analysis of Tablet Formulation:

The twenty tablets were weighed out correctly and then ground up into a fine powder. The mix was put into a 100 ml volumetric flask after 25 mg of MPS and 20 mg of OLM tablet powder were carefully measured out. Ultrasound waves were used on the material for 15 minutes. After that, the amount was diluted enough and mixed very carefully. A small amount was taken out and put through a 0.25µm filter to make sure there was no particle waste. After that, more dilution was looked at [32-36].

RESULT:

The point of this study was to test and build a bioanalytical method for Olmesartan Medoxomil and Metoprolol Succinate in the form of medicinal tablets using RP-HPLC in human plasma.

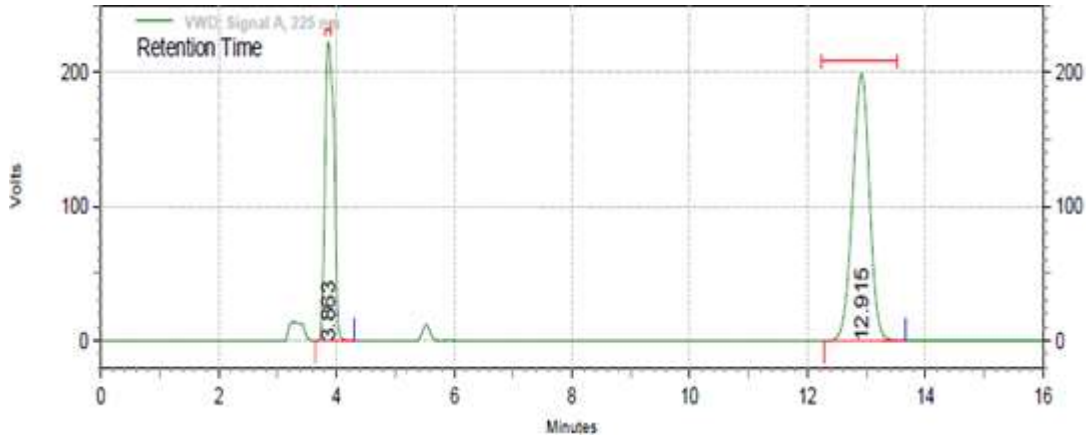

Figure 2: Standard Olmesartan Medoxomil with Metoprolol Succinate Chromatogram

This method is commonly employed for the quantitative analysis of medicines in body fluids derived from their formulations. This approach offers the capability to quantify nanoquantities and to assess the components of a multicomponent system without prior separation.

After considering the solubility, Stability and spectral feature of the drug 100% Acetonitrile selected as diluent and after a number of trialsPhosphate buffer: Acetonitrile (P^H-2.4) in isocratic flow was selected asa mobile phase (table 7).

Table 7: **Selection of Mobile Phase**

Mobile Phase	**Solvent Ratio**	**Flow rate**	**Observation**	**Remark**
Phosphate buffer: Acetonitrile (P^{H}-2.4)	60:40	0.8ml/min	Good resolution	Method Accepted

225 nm seems to be the optimal detection wavelength considering the drug's overlay spectra. The results clearly demonstrate that the RP-HPLC technology can effectively quantify the concentrations of Olmesartan Medoxomil and Metoprolol Succinate in human plasma spiked with respective dosage formulation.

Validation:

Linearity:

The results of the linearity analysis indicate that the drug's components exhibit linearity concerning the specified concentration range in the table 8, figure 3 and 4.

Table 8: Linearity Statistical Value

Statistical Data for Linearity	**MetoprololSuccinate**	**OlmesartanMedoxomil**
Correlation Coefficient(r2)value	1.0	1.0
Slope (m)	816641.73	23125544
Y-intercept	3914.60	106628.80
Linearity Range	3.0 µg/ml to 15.0µg/ml	0.5 µg/ml to 2.5 µg/ml

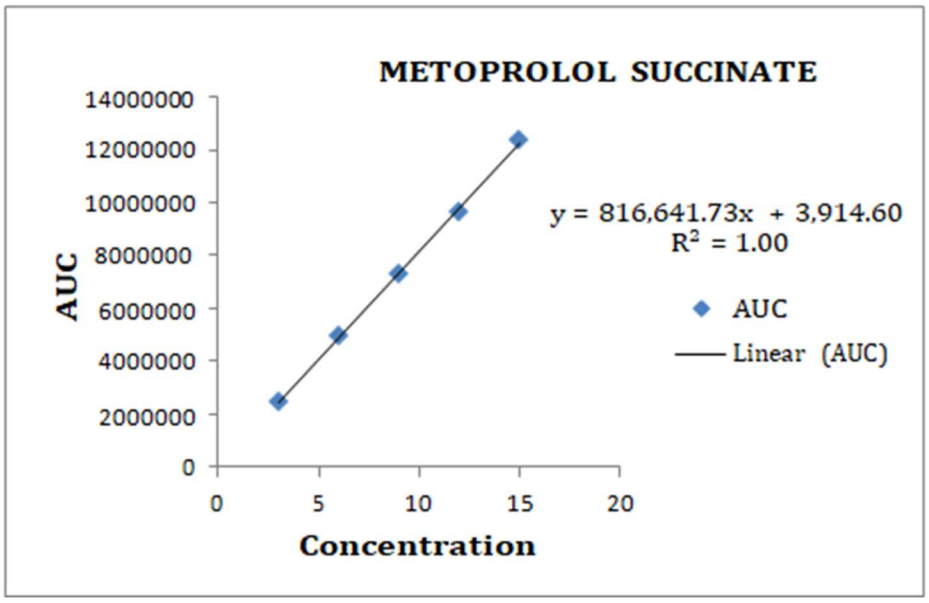

Figure 3: Linearity graph of Metoprolol Succinate drug

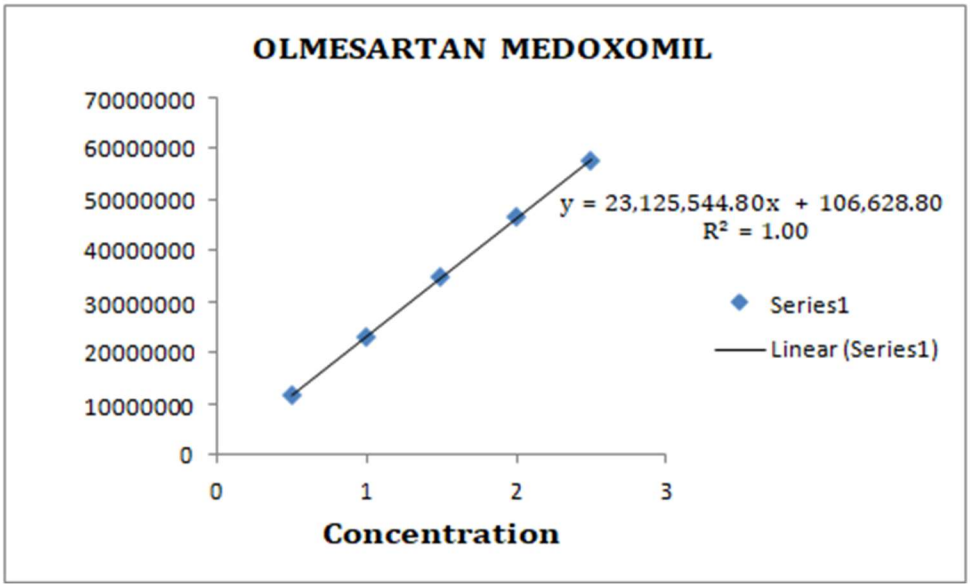

Figure 4: Linearity graph of Olmesartan Medoxomil drug

Accuracy:

Three accuracy levels—80%, 100%, and 120%—were analyzed utilizing the standard addition method, and the findings demonstrated that the recovery percentage fell within an acceptable range. The data is presented in the table 9.

Table 9: **Accuracy Statistical values**

Statistical Data	**Metoprolol Succinate**	**OlmesartanMedoxomil**
Mean	97.80	97.99

SD	2.011	1.31
%RSD	2.07	1.29

Precision:

Precision was also set up to make sure that the method could be used again and again. Less than 2.0% R.S.D. was found; the results are shown in the table 10.

Repeatability:

Table 10: Precision Statistical value

StatisticalParameter	Metoprolol Succinate	OlmesartanMedoxomil
Mean	100.204	99.66
SD	0.9493	0.4988
%RSD	0.9473	0.5005

Intermediate precision:

Table 11: **Day to day precision**

StatisticalParameter	Metoprolol Succinate	OlmesartanMedoxomil
Mean	100.44	99.45
SD	0.7593	0.9826
%RSD	0.75597	0.9890

Analyst to Analyst:

Table 12: **Day to day precision**

StatisticalParameter	Metoprolol Succinate	OlmesartanMedoxomil
Mean	100.44	99.89
SD	0.7565	0.5100
%RSD	0.7531	0.51056

Limit of Detection and Limit of Quantitation:

Olmesartan medoxomil and Metoprolol succinate had respective LODs of 0.002414 µg/ml and 0.002162 µg/ml and LOQs of 0.007318 µg/ml and 0.006563 µg/ml.

Robustness:

The deliberate changes in pH of Mobile phase were carried out (table 13 &14).

Table 13: **Robustness Statistical value**

Statistical Parameters	MetoprololSuccinate	OlmesartanMedoxomil
Mean	100.44	99.89
SD	0.7565	0.5100
%RSD	0.7531	0.5160

Table 14: Analysis of Tablet formulation

Statistical Parameters	Metoprolol Succinate	OlmesartanMedoxomil
MEAN	20.55	15.97
SD	0.1021	0.04041
%RSD	0.4968	0.2530

DISCUSSION:

The proposed methods for quantifying olemesartan medoxomil and metoprolol succinate in tablets were found to be accurate, user-friendly, and efficient. The established approach is suitable for standard drug analysis in tablet form. The recovery study findings of the tablet ranged from 94.0 to 102.0 percent. The excipients in the formulation do not cause any interference. Implementing it for regular quality control analysis is straightforward and efficient. This method complies with USFDA standards, being accurate, straightforward, rapid, exact, reliable, sensitive, reproducible, and verified.

225 nm seems to be the optimal detection wavelength considering the drug's overlay spectra. The peak absorbance of Olmesartan Medoxomil and Metoprolol Succinate occurs at 225 nm.

In contrast, plasma, metoprolol succinate, and olemesartan medoxomil exhibited satisfactory resolution at 2.7, 3.86, and 12.96, respectively.

The best mobile phase was found by looking at a number of system suitability parameters, such as retention time, tailing, number of theoretical plates, and HETP. It was a 60:40 mixture of phosphate buffer and acetonitrile (pH 2.4), which allowed metoprolol succinate and olemesartan medoxomil to be found at 20.0 minutes with good resolution.

Metoprolol succinate and Olmesartan medoxomil exhibit a linearity correlation coefficient of 1.000 within the operational range, signifying exceptional linearity. The maximum relative standard deviation identified in terms of precision, accuracy, and robustness was below 2. Consequently, all validation parameters fall within the acceptable range. Consequently, Olmesartan Medoxomil and Metoprolol Succinate can be systematically evaluated utilizing this methodology.

CONCLUSION:

This study successfully found metoprolol succinate and olemesartan medoxomil in a pharmaceutical tablet preparation by using RP-HPLC (reversed phase high-performance liquid chromatography) on human plasma. The method was created by testing it, using information from a book review as a guide, and following the statistical principle of sampling. The study's goal was fully met because the method was easy to use, worked well, and could be repeated. This study

employed HPLC Agilent Technologies (1260 INFINIT) with a UV-Vis detector to effectively apply RP-HPLC for the examination of a selected medicine formulation. The suggested RP-HPLC approach was employed to quantify metoprolol succinate and olemesartan medoxomil, with validation based on linearity, accuracy, precision, robustness, and specificity. For each parameter, the %RSD was below two. The validation data for the proposed method indicates that Olmesartan Medoxomil and Metoprolol Succinate demonstrate satisfactory system adaptability.

DECLARATIONS:

Ethics approval and consent to participate:

Not applicable.

Consent for publication:

All the authors approved the manuscript for publication.

Availability of data and material:

All required data is available.

Competing interests:

All authors declare no competing interests.

Funding:

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