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## **ANALYTICAL METHOD DEVELOPMENT FOR ESTIMATION OF S (-) AMLODIPINE BESYLATE & OLMESARTAN MEDOXOMIL BY UV SPECTROSCOPY**

**Nupur A Inamdar\*, Ambekar Abdul Wahid, Satyam Z Chemate, Kalyani A Jadhav,  
Mahesh S Digge, Gajanan V Pulgamwar**

Padmashri Dr. Vitthalrao Vikhe Patil Foundation's College of Pharmacy, Ahmednagar, Maharashtra, India 414111.

### **ABSTRACT**

For simultaneous estimation of S (-) amlodipine besylate & olmesartan medoxomil, the simple, accurate, precise, reproducible and economic UV spectroscopic methods have been developed. The method A-Area Under curve method, method B- Absorption ratio method and method C- Dual wavelength method were developed by using JASCO double beam UV Spectrophotometer (model-V630). Simultaneous estimation of S(-) amlodipine Besylate and olmesartan medoxomil was carried out at wavelength 238 nm and 257 nm respectively. Both the drugs were showing linearity at 238 nm and 257 nm and obey Beer's law in the concentration range of 5-20 µg/ml with good correlation coefficient. In Area under curve method, wavelength selected for S (-) amlodipine besylate was from 227 to 249 nm and for olmesartan medoxomil was from 244 to 269 nm. It gives peak area of 1.9585 for S (-) amlodipine besylate and 2.1252 for Olmesartan medoxomil. Absorption ratio method involves formation of isoabsorptive point at 237 nm.

**Key Words:** S(-) Amlodipine besylate, Olmesartan medoxomil, Methanol, Absorbance, Validation.

### **INTRODUCTION**

Hypertension is a progressive cardiovascular syndrome arising from complex and interrelated etiologies. Hypertension is the state in which systolic blood pressure (SBP) of 140 mm Hg or greater or a diastolic blood pressure (DBP) of 90 mm Hg or greater. Multiple mechanisms are involved in the pathogenesis of hypertension. Therefore it is multifactorial condition and combination therapy makes it more effective than monotherapy<sup>1</sup>. Combination of Calcium Channel Blockers and Angiotensin Receptor Blockers are emerging as convenient and rational options for antihypertensive treatment. S (-) amlodipine besylate, a calcium channel blocker (CCB) and olmesartan medoxomil, an Angiotensin

receptor Blocker (ARB) are the drugs used therein. S (-) amlodipine besylate selectively inhibits calcium influx across cell membranes in cardiac and vascular smooth muscle, with a greater effect on vascular smooth muscle. Olmesartan blocks the vasoconstrictor effects of Angiotensin II by selectively blocking the binding of Angiotensin II to the AT1 receptor in vascular smooth muscle.

S (-) amlodipine Besylate is 3-ethyl-5-methyl(±)-2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylate, mono benzene sulpho nate with empirical formula  $C_{20}H_{25}ClN_2O_5 \cdot C_6H_6O_3S$ . It is the dihydropyridine agent of antihypertensive class. Olmesartan medoxomil is a prodrug, hydrolysed to olmesartan during absorption from GIT. Chemically it is 2,3-dihydroxy-2-butenyl 4 (1-hydroxy-1 methylethyl) 2-propyl-1[p- (o-1H-tetrazol-5yl-phenyl) benzyl] imidazole

Corresponding Author

**Nupur A Inamdar**  
Email: [nupur.25692@gmail.com](mailto:nupur.25692@gmail.com)

5- carboxylate, cyclic 2,3 carbonate, with molecular formula  $C_{29}H_{30}N_6O_6$

## MATERIALS AND METHODS

JASCO V-630 double beam spectrophotometer with spectral bandwidth of 2 nm, wavelength accuracy of  $\pm 0.3$  nm and a pair of 10 nm matched quartz cell was used for the estimation. The API used i.e. S (-) amlodipine besylate and olmesartan medoxomil was obtained as a gift sample from Emcure Pharmaceuticals, Pune and methanol was obtained from the institute P.D.V.V.P.F's College of Pharmacy, Ahmednagar, Maharashtra -414003, India.

### Preparation of Stock Solution

S (-) amlodipine besylate 10mg was weighed by using electronic balance (Model-Shimadzu AUW-220D) and dissolved in the small amount of methanol. Volume was adjusted to 100 ml in volumetric flask to give the solution of 100 $\mu$ g/ml. Similarly Stock solution of olmesartan medoxomil was prepared by dissolved in the 10 mg drug in methanol in a volumetric flask and adjusting the volume to 100ml to get 100  $\mu$ g/ml solution.

### Preparation of Sample

Standard solutions were prepared from the stock solutions of both the drugs. For both the drugs standards were prepared of concentrations 5-30 $\mu$ g/ml. 0.5ml stock solution was adjusted to 10ml with methanol to get 5 $\mu$ g/ml solution. 1ml stock solution was adjusted to 10ml with methanol to get 10 $\mu$ g/ml solution. 1.5ml stock solution was adjusted to 10ml with methanol to get 15 $\mu$ g/ml solution. 2ml stock solution was adjusted to 10ml with methanol to get 20 $\mu$ g/ml solution. 2.5ml stock solution was adjusted to 10ml with methanol to get 25 $\mu$ g/ml solution. 3ml stock solution was adjusted to 10ml with methanol to get 30 $\mu$ g/ml solution. Similarly, standard solutions of olmesartan medoxomil were prepared

### Measurement of Readings

After the sample preparation readings were taken on JASCO make double beam spectrophotometer. Blank readings were taken for correction of baseline. For measurement of  $\lambda_{max}$ , 20 $\mu$ g/ml samples of S (-)amlodipine besylate and olmesartan medoxomil were taken.  $\lambda_{max}$  238 nm and 257 nm was obtained for S (-) amlodipine besylate and Olmesartan medoxomil respectively. Sample readings were taken by adjusting fixed wavelength at 238 nm and 257 nm.

Following 3 methods were used for the estimation of S (-)amlodipine besylate & olmesartan medoxomil.

### Method A: Area under curve Method

For the estimation of drugs by AUC method, suitable dilutions of standard stock solutions (100 $\mu$ g/ml) of S(-) amlodipine besylate and olmesartan medoxomil were

prepared separately in methanol. The solutions of drugs were scanned in the range of 200-400 nm. Wavelength ranges 227 to 249 and 244 to 269 were selected for the Amlodipine Besylate and Olmesartan medoxomil respectively. Area under curve was measured at the selected wavelength ranges.

Concentration of two drugs in mixed standard and the sample solution were calculated using equation (1) and equation (2).

$$C_x = A_2 ax_2 - A_1 ay_2 / ax_2 ay_1 - ax_1 ay_2 \dots \dots \dots \text{eq. (1)}$$

$$C_y = A_2 - ax_2 * C_x / ay_2 \dots \dots \dots \text{eq. (2)}$$

### Method B: Absorption Ratio Method

Method uses the absorbance at two selected wavelengths; of which one is isoabsorptive point and second is  $\lambda_{max}$  of any of the two drugs. Isoabsorptive point was obtained at 237 nm from the overlain spectra of both the drugs. The second wavelength is 257 nm which is the  $\lambda_{max}$  of olmesartan medoxomil.

The concentrations of two drugs were measured by using following equations:

$$C_x = \{(Q_m - Q_y) / (Q_x - Q_y)\} * A_1 / ax_1 \dots \dots \dots \text{eq. (3)}$$

$$C_y = \{(Q_m - Q_x) / (Q_y - Q_x)\} * A_1 / ay_1 \dots \dots \dots \text{eq. (4)}$$

Where A1 and A2 are absorbance's of mixture at 238 nm and 257 nm,  $ax_1$  and  $ay_1$  are absorptivities of S (-)amlodipine besylate and olmesartan medoxomil at 272 nm,  $ax_2$  and  $ay_2$  are absorptivities of S (-)amlodipine besylate and olmesartan medoxomil at 233nm,  $Q_m = A_2/A_1$ ,  $Q_x = ax_2/ax_1$ ,  $Q_y = ay_2/ay_1$ .

### Method C: Dual wavelength method

If sample contains two absorbing drugs of each of which absorbs at the  $\lambda_{max}$  of other, we can estimate both the drugs. Method is also known as simultaneous equation method. Estimation is done using following equation.

$$C_x = A_2 ay_1 - A_1 ay_2 / ax_2 ay_1 - ax_1 ay_2 \dots \dots \dots \text{eq. (5)}$$

$$C_y = A_1 ax_2 - A_2 ax_1 / ax_2 ay_1 - ax_1 ay_2 \dots \dots \dots \text{eq. (6)}$$

## RESULTS

### VALIDATION OF PROPOSED METHOD

All the methods were validated according to the International Conference of Harmonization (ICH) guidelines.

#### Linearity

To check the degree of repeatability of these methods, suitable statistical evaluation was carried out. Calibration curve for S(-) amlodipine besylate & olmesartan medoxomil were taken at 238 nm and 257 nm. Both the drugs show linearity and obey Beer's law in the concentration range 5-20  $\mu$ g/ml. The correlation coefficient of calibration curves were observed 0.9941 and 0.9831.

#### Precision

Relative standard deviations (% RSD) for intraday and interday were calculated as a part of precision study.

The Interday and intraday precision was determined by assay of the sample.

#### Limit of Detection

The limit of detection was determined using formula:

$$\text{LOD} = 3.3\sigma/S$$

Where,

LOD -Limit of Detection,

$\sigma$  - Standard Deviation and

S- Slope of the calibration curve.

#### Limit of Quantitation

The limit of Quantitation was calculated using formula:

$$\text{LOQ} = 10\sigma/S$$

Where,

LOQ -Limit of Quantitation,

$\sigma$  - Standard Deviation and

S- Slope of the calibration curve.

LOQ was found

**Table 1. Peak Areas of S (-) amlodipine besylate and olmesartan medoxomil by AUC**

Sr. no	Area under curve for S (-)amlodipine besylate			
	Peak	Wavelength range	Area	Area ratio
1	Peak 1	227-249 nm	1.95851	1
2	Peak 2	227-249 nm	1.95851	1
Sr. No	Area under curve for Olmesartan medoxomil			
	Peak 1	244-269 nm	2.12524	1
4	Peak 2	244-269 nm	2.12524	1

**Table 2. Optimized Method Parameters for dual wavelength Spectroscopy**

Sr. No	Method Parameters	Optimized Parameters
1	Solvent	methanol
2	Scanning Range	200 nm-400 nm
3	Scan Speed	Medium
4	Analytical Wavelength for determination of S (-) amlodipine besylate	238 nm-257 nm
5	Analytical Wavelength for determination of Olmesartan medoxomil	238 nm-257 nm

**Table 3. Linearity**

Sr. No	S (-) amlodipine besylate	Absorbance		Olmesartan medoxomil	Absorbance	
	(Conc. $\mu\text{g/ml}$ )	238 nm	257 nm	(Conc. $\mu\text{g/ml}$ )	238 nm	257 nm
1	5	0.1431	0.0500	5	0.2447	0.2663
2	10	0.2551	0.0981	10	0.4003	0.4547
3	15	0.4087	0.1548	15	0.5164	0.5921
4	20	0.5827	0.2115	20	0.6860	0.8080

**Table 4. Intraday Precision Studies**

Sr. No	Conc. Of drug	Absorbance		SD		%RSD	
	$\mu\text{g/ml}$	238 nm	257 nm	S (-) amlodipine besylate	Olmesartan medoxomil	S (-) amlodipine besylate	olmesartan medoxomil
1	10	0.2451	0.4546	0.00582	0.0001	2.30	0.02
		0.2551	0.4547				
		0.2552	0.4545				
2	15	0.4086	0.5921	0.0001	0.0057	0.02	0.98
		0.4087	0.5821				
		0.4085	0.5920				
3	20	0.5826	0.8079	0.0001	0.0058	0.02	0.72
		0.5825	0.7978				
		0.5827	0.8078				

**Table 5. Interday precision studies**

Sr. No	Conc. Of drug	Absorbance		SD		%RSD	
	µg/ml	238 nm	257 nm	S (-) amlodipine besylate	Olmesartan medoxomil	S (-) amlodipine besylate	olmesartan medoxomil
1	10	0.2355	0.4546	0.0055	0.01	2.30	2.20
		0.2451	0.4645				
		0.2452	0.4445				
2	15	0.4085	0.5722	0.0057	0.01	1.42	1.72
		0.3985	0.5821				
		0.4084	0.5922				
3	20	0.5725	0.8076	0.0058	0.0056	1.01	0.70
		0.5826	0.7979				
		0.5725	0.8077				

**Table 6. Limit of Detection and Limit of Quantification**

Sr. No	Parameters	S (-)amlodipine besylate		Olmesartan medoxomil	
		238 nm	257 nm	238 nm	257 nm
1	LOD(µg/ml)	0.7361	1.5122	0.0089	0.010
2	LOQ(µg/ml)	0.6346	0.4582	0.0271	0.0321

**Table 7. Regression Characteristics**

Sr.No	Parameters	At 238 nm	At 257 nm
1	Beer's law range	5-20 µg/ml	5-20 µg/ml
2	Regression Equation	$y=0.026x+0.0082$	$y=0.0369x+0.0539$
3	Slope (m)	0.026	0.0369
4	Intercept (c)	0.0082	0.0539
5	Correlation Coefficient	0.9941	0.9831

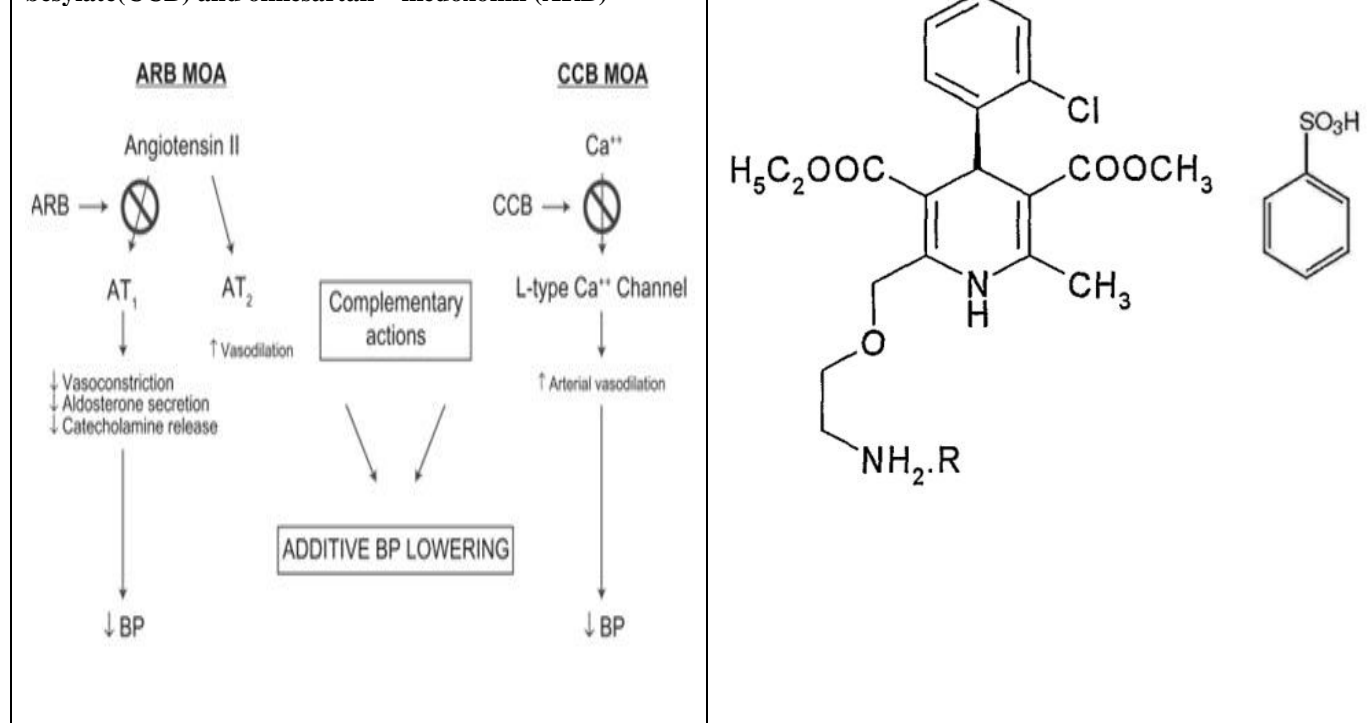
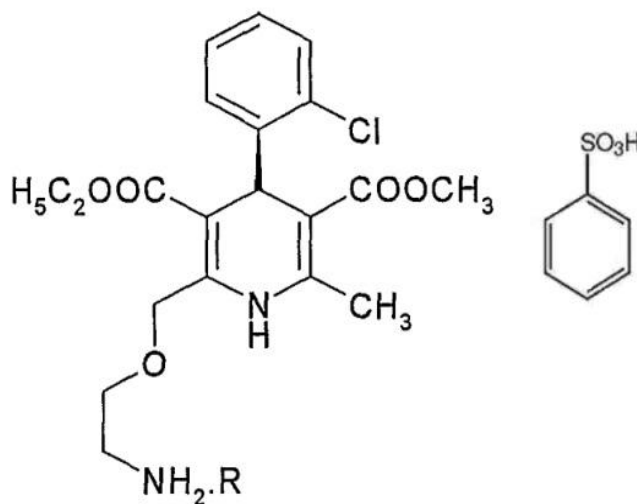
**Fig 1. Mechanism of action (MOA) of S (-)amlodipine besylate(CCB) and olmesartan medoxomil (ARB)****Fig 2. S (-) amlodipine besylate**

Fig 3. Olmesartan medoxomil

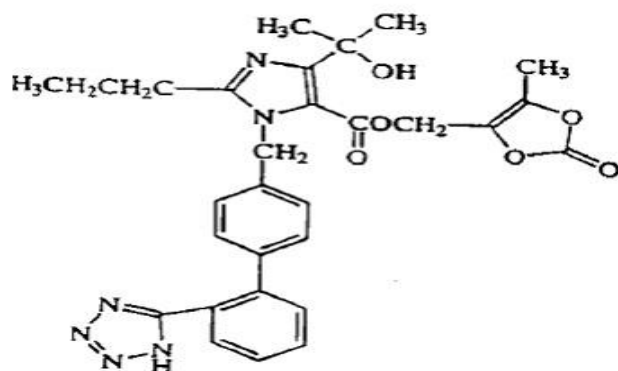


Fig 4. Spectra of S (-) amlodipine Besylate in methanol

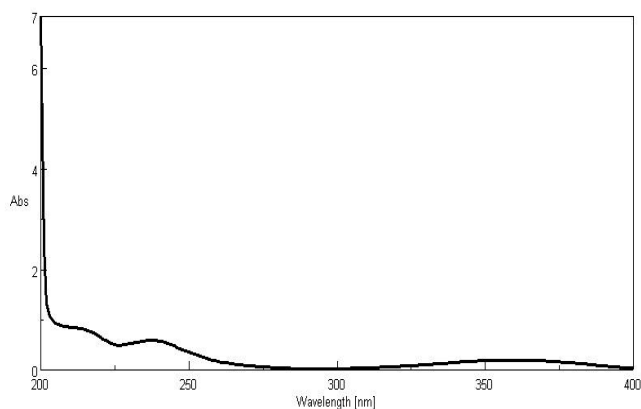


Fig 5. Spectra of Olmesartan medoxomil in methanol

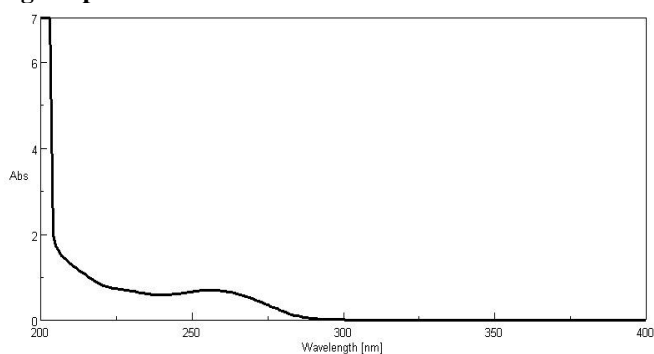


Fig 6. Overlain absorption spectra of S (-)amlodipine besylate and Olmesartan medoxomil showing isoabsorptive point (237 nm) in Methanol

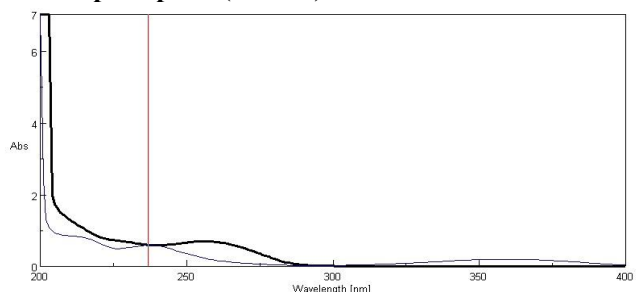


Fig 7. Area under curve of S (-)amlodipine besylate

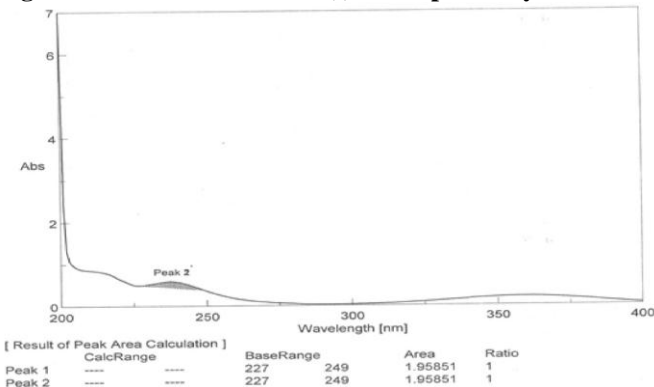


Fig 8. Area under curve of Olmesartan medoxomil

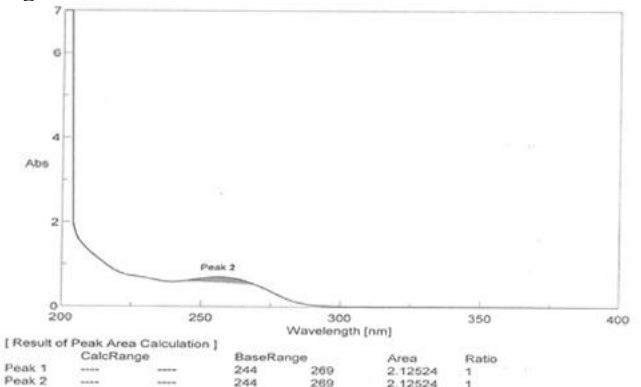


Fig 9. Standard calibration plot of (-) amlodipine besylate in methanol

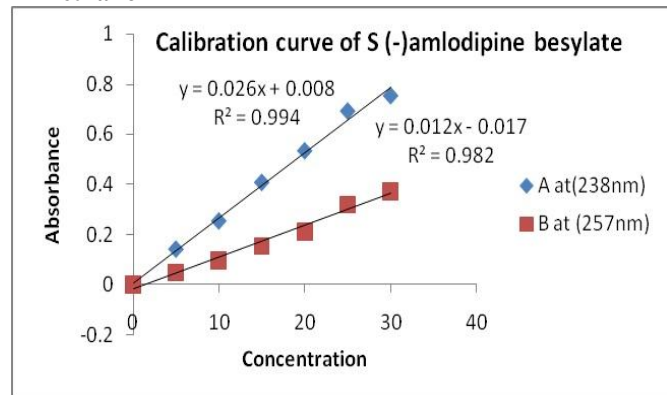
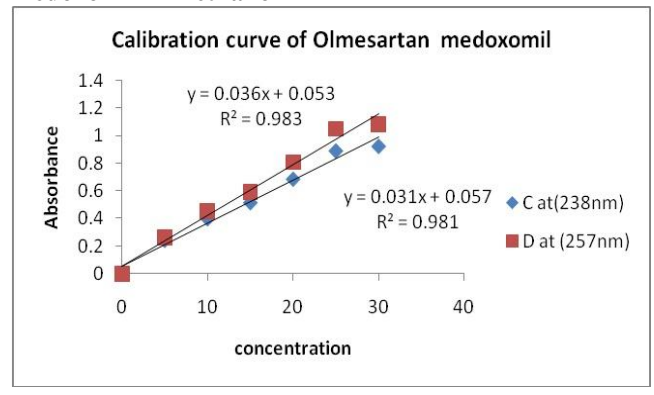


Fig 10. Standard calibration plot of Olmesartan medoxomil in methanol



## DISCUSSION

The use of S (-) amlodipine besylate and olmesartan medoxomil are showing better effectiveness in the treatment of hypertension. The present work allows simultaneous estimation of both the drugs. Methods are simple, accurate and economic. Both the drugs shows linearity at the selected wavelength i.e. 238 nm and 257 nm for S (-) amlodipine besylate and olmesartan medoxomil

respectively. The proposed method is validated according to the ICH guidelines.

## CONCLUSION

The new, sensitive, simple and economic methods were developed for the simultaneous analysis of S (-) amlodipine besylate and olmesartan medoxomil.

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