Nat. Volatiles & Essent. Oils, 2021; 8(4): 16787-16796

NVEO
Natural Volatiles &
Essential Oils

Geometrical Correction Method For Simultaneous Estimation Of Azilsartan Medoxomil And Amlodipine Besylate From Biological Sample

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Abstract

The geometrical correction method eliminates background irrelevant absorption that may be present in the sample from biological origin. Three-point geometrical correction method was used for quantification of Azilsartan medoxomil and Amlodipine besylate from biological sample. The method was developed at three wavelengths 240nm ,251nm ,262nm for Azilsartan Medoxomil and 226nm 239nm,245nm for Amlodipine besylate. The proposed method shows linear relationship at three wavelengths in the range of 4.4-44µg/ mL for Azilsartan Medoxomil and The concentration rang of 3.4-34 µg/mL for Amlodipine besylate with the regression coefficient of 0.9976 ,0.9961 ,0.9986 for Azilsartan Medoxomil at 251nm,262nm,240nm. and 0.9978 ,0.999, 0.9991 for Amlodipine besylate at 226nm 239nm,245nm. The accuracy at three wavelengths was found to be 98.53 ,98.01 ,99.02 for Azilsartan Medoxomil at 251nm,262nm,240nm. 97.33,98.51,97.5 for Amlodipine besylate at 226nm 239nm, 245nm.The % RSD at three wavelengths was found to be 0.23 ,0.6978 ,0.2374 for ruggedness and 0.5897 ,0.4503 ,0.7745 for robustness of Azilsartan Medoxomil at 251nm,262nm,240nm. and 0.5407 ,0.4923 ,0.2144 for ruggedness and 0.7530 ,0.2459,0.3482 for robustness of Amlodipine besylate at 226nm 239nm,245nm. The proposed method was found to be accurate, precise, rugged and robust. The proposed method was validated as per ICH guidelines. corrected absorbance D value was
calculated. D value showed linearity. Simultaneous estimation of Azilsartan Medoxomil and Amlodipine Besylate with egg albumin was 99.93% and 96%. also Absorbance ratio of Azilsartan Medoxomil and Amlodipine besylate by using egg albumin was found to be 99.93% and 96%

Keywords: Geometrical correction method, Azilsartan medoxomil, Amlodipine besylate, egg albumin, simultaneous and absorption ratio method.

1. INTRODUCTION

Azilsartan medoxomil (AZ) is a prodrug of Azilsartan marketed as "Edarbi" by Takeda. AZ is an angiotensin Ⅱ receptor antagonist used in the treatment of mild to moderate essential hypertension.

AZ has so far been shown to be superior to Olmesartan and valsartan in the lowering of blood pressure. AZ blocks the angiotensin Ⅱ type 1 receptor preventing angiotensin Ⅱ from binding and causing vasoconstriction.

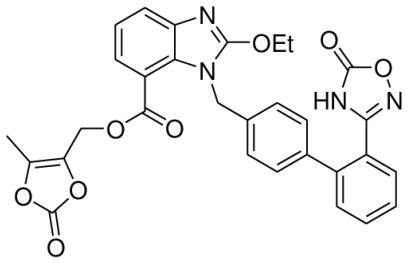

Figure 1: Structure of Azilsartan medoxomil

Amlodipine Besylate (AB) is commonly used in the treatment of high blood pressure and angina. It has antioxidant property and has an ability to enhance the production of nitric oxide (NO) which is an important vasodilator that is decreases blood pressure. It is initially approved by FDA in 1987, is an antihypertensive drug belonging to the group of drugs called as Dihydropyridine calcium channel blocker. Due their selectivity for the peripheral blood vessels, Dihydropyridine calcium channel blocker are associated with a lowering of myocardial depression and cardiac conduction abnormalities than other calcium channel blockers. AB is a peripheral arterial vasodilator that exerts its action directly on the vascular smooth muscles which leads to reduction in peripheral vascular resistance, causing a decrease in the blood pressure.

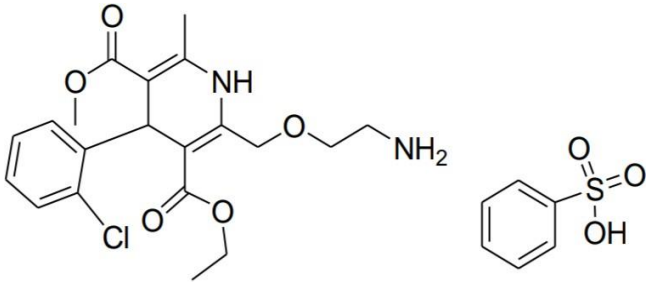

Figure 2: Structure of Amlodipine Besylate

The literature survey revealed many methods for AZ individual and with combination of other drugs including LC-MS voltammetry, spectrophotometry, capillary electrophoresis and HPLC. AB was determined by spectrophotometric, fluorimetry, colorimetry, HPLC, LC-MS, combined gas chromatography/ electron capture negative ion chemical ionization mass spectrometry. A three-point geometrical correction method was developed for estimation of AZ and AB in egg albumin as biological sample. Purpose of geometrical correction method was to analyze drugs in biological sample with corrected background absorbance. In the present study three-point geometrical correction UV Spectroscopic method was developed and validated as per ICH guidelines, for estimation AZ and AB. Geometric correction method A number of mathematical correction procedures have been developed for elimination of background irrelevant absorption in biological samples. The three-point geometrical procedure is one of the method in which the absorbance was measured at three different wavelengths. The corrected absorbance (D) of the drug was calculated at three absorbance A1, A2 and A3 of the sample solution at 31, 32 and 33 with background absorbance B1, B2 and B3. The v and w are the absorbance ratios vD/D and wD/D, y and z are the wavelength intervals.

2. MATERIAL AND METHODS

2.1 Materials:

All the chemicals and solvents used were of analytical grade and Azilsartan medoxomil and Amlodipine Besylate was obtained gift sample from Pharmaceutical Industry. The Tablets were purchased from local market of Kopargaon.

2.2 INSTRUMENTATION

A Shimadzu model 1800 PC double beam UV/visible spectrophotometer with spectral width of 2nm, wavelength accuracy of 0.5 nm and a pair of 10mm matched quartz cell were used to measure absorbance of all the solutions. Spectra were obtained by UV- Probe system software. An analytical balance, an ultrasonic bath was used in the study.

2.3 Selection of Common Solvent

After solubility analysis, Methanol AR grade and distilled water in the proportion of (10:90) was used as a solvent.

2.4 Selection of Biological Media

Egg albumin was used as the biological medium. The egg albumin consists of 40 different types of proteins.

2.5 Preparation of stock solution:

10mg of Azilsartan medoxomil and Amlodipine Besylate were accurately weighted and transferred in 100 ml volumetric flask. The drug was dissolved in 10ml of Methanol and was sonicated and then volume was adjusted up to mark with water to obtained final concentration 100ml.

2.6 Selection of analytical wavelength:

From stock solution appropriate dilution were prepared and scanned in wavelength range of 200-800nm. The 10ppm concentration of Azilsartan Medoxomil and Amlodipine besylate were scanned and showed absorbance maxima at 251nm for Azilsartan Medoxomil and239nm for Amlodipine besylate. Hence 251nm and 239nm was selected as max. The wavelengths 240nm ,251nm ,262nm for Azilsartan Medoxomil and 226nm 239nm,245nm for Amlodipine besylate were selected for geometrical correction method.

2.7 Selection of analytical concentration range and preparation of calibration curve for Azilsartan Medoxomil and Amlodipine besylate:

Appropriate concentration was pipette out from standard stock solution into 10ml volumetric flask. The volume was made up with Methanol: Water (10:90) to get a set of solution having the concentration range of 4.8-48µg/ mL for Azilsartan Medoxomil and Absorbance ratio were measured at240nm ,251nm ,262nm. The concentration rang of 3.7-37 µg/mL for Amlodipine besylate and Absorbance ratio were measured at 226nm 239nm, 245nm.The calibration curve of absorbance against concentration was plotted. The regression equation and coefficient was determined at the wavelengths 240nm ,251nm ,262nm for Azilsartan Medoxomil and 226nm 239nm,245nm for Amlodipine besylate.

2.8 Analysis of Tablet formulation:

Twenty tablets of Azilsartan medoxomil and Amlodipine besylate were weighed. The tablets were crush to get fine powder. tablet powder equivalent to 10mg were weighed and transferred to 100ml volumetric flask and dissolved in 10ml methanol and the content was sonicated for 30 min. finally volume was made up to mark with water to obtained final concentration of 100µg/mL. The solution was filtered through Whatmann filter paper.

2.9 Preparation of biological sample:

10 mg of egg albumin powder was weighed accurately and transferred to 100ml volumetric flask. The egg albumin was dissolved in distilled water by sonication and then volume was made up to mark with water to obtained a final concentration of 100 µg/ml. Calibration sample were preparing by spiking 1ml of egg albumin with appropriate standard solution of each analyte. Calibration curve of standard solutions consist a set of concentration ranging from 4.4-44µg/ mL for Azilsartan Medoxomil and The concentration rang of 3.4-34 µg/mL for Amlodipine besylate.

2.10 Sample processing:

1ml of egg albumin solution was added to 5-40 ppm Azilsartan medoxomil and 4-36 ppm Amlodipine besylate. The absorbance was taken at three wavelengths.

3. METHOD VALIDATION

The method was validated as per ICH guidelines for linearity, precision, ruggedness, robustness, accuracy, limit of detection, and limit of quantitation.

3.1 Linearity

The linearity of AZ and AB with egg albumin was obtained in the concentration range of 4.4-44 µg/ml and 3.4-34 µg/ml respectively. The calibration curves of AZ and AB were obtained at three wavelengths 240nm ,251nm ,262nm and 226nm 239nm,245nm. The regression coefficients at three wavelengths were 0.993, 0.999 and 0.995 for AZ and for AB was found to be 0.992, 0.993, and 0.985.

3.2 Limit of detection (LOD)

It is the lowest concentration of analyte in the sample that can be detected, but not necessarily be quantified under the stated experimental condition. Different concentrations of standard drug solution were used and minimum detectable limit was found. It was within the limit.

3.3 Limit of quantitation (LOQ)

It is the lowest concentration of analyte in the sample that can be determined with the acceptable precision and accuracy under stated experimental condition. The LOQ was calculated at three wavelengths of both the drugs and was within the range.

Table 1: Statistical data of AZ with egg albumin at three different wavelengths

Sr. No.	Parameters	Observation		
		240nm	**251nm**	**262nm**
	Linearity range	4.8-48	4.8-48	4.8-48
	Intercept	0.999	0.9996	0.995
	Slope	0.0196	0.0172	0.0193

	Correlation coefficient	0.0155	0.0208	0.0037
	LOD	0.0909%	0.314%	0.581%
	LOQ	0.3139%	0.95%	1.761%

Table no. 2: Statistical data of AB with egg albumin at three different wavelengths

Sr. No.	Parameters	Observation		
		226nm	**239nm**	**245nm**
	Linearity range	3.7-37	3.7-37	3.7-37
	Intercept	0.9992	0.9994	0.9994
	Slope	0.0169	0.0217	0.019
	Correlation coefficient	0.0027	0.0063	0.0013
	LOD	0.3202%	0.1271%	0.0950%
	LOQ	0.9704%	0.3852%	0.2878%

3.4 Precision

The precision was performed by using the same concentration six times of concentration 10ppm for AZ and 6ppm for AB from egg albumin. The result was found to be within range of SD and %RSD. The method was found to be precise at three wavelengths of both the drugs. **3.5 Ruggedness**

The ruggedness is defined as the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of normal test conditions such as different reagents, labs and analysis. Ruggedness is a measure of reproducibility of the test results under normal expected operational conditions from laboratory to laboratory and from analyst to analyst. In the present study, determination of AZ and AB by using six series of dilutions of 10ppm and 6ppm was carried out by using different analysts. The % RSD was calculated.

3.6 Robustness

Robustness was studied by changing parameters like change in system. The robustness was performed by changing the ratio of methanol and water. The ratio selected was methanol: water (15: 85). The % RSD was calculated.

Table no. 3: Results of precision, ruggedness and robustness of AZ from egg albumin

Parameters		Observation		
		240nm	**251nm**	**262nm**
Precision Inter-day(n=6) Intraday(n=6)	 %RSD %RSD	 0.36% 0.42%	 0.73% 0.85%	 0.55% 0.72%
Ruggedness Analyst-I Analyst-II (n=6)	 % RSD % RSD	 1.6% 1.45%	 0.47% 0.35%	 0.172% 0.150%
Robustness (Solvent change) (n=6)	% RSD	0.84%	0.41%	0.094%

Table 4: Precision, Ruggedness and Robustness data of AB from egg albumin.

Parameters		Observation		
		226nm	**239nm**	**245nm**
Precision Interday(n=6) Intraday(n=6)	 %RSD %RSD	 0.48% 0.62%	 0.45% 0.75%	 0.80% 0.92%
Ruggedness Analyst-I Analyst-II (n=6)	 % RSD % RSD	 1.36% 1.75%	 0.64% 0.98%	 0.75% 0.168%
Robustness (Solvent change) (n=6)	% RSD	1.28%	0.72%	0.75%

3.7 Accuracy

The accuracy of the analytical method is closeness of the result obtained by that method to the true value. Accuracy may often be expressed as percent recovery by the assay of known, added amount of analyte. Accuracy is measure of the exactness of the analytical method. Standard solutions of AZ and AB were added to the unknown formulation of AZ and AB. The percent recovery was determined at three different levels (50%, 100% and 150%). The method was accurate at three wavelengths for AZ and AB at 240nm ,251nm ,262nm and 226nm 239nm,245nm respectively.

Table 5: Results of accuracy for AZ from egg albumin.

Sr.No	Levels	Std	Test	Total	%Purity	SD	RSD	%RSD
1	50%	5	10	15	99.31%	0.4878	0.0049	0.4926
2	100%	10	10	20	98.46%			
3	150%	15	10	25	99.3%			

Table 6: Results of accuracy for AB from egg albumin.

Sr.No	Levels	Std	Test	Total	%Purity	SD	RSD	%RSD
1	50%	4	8	12	97.13%	0.2050	0.0021	0.2106
2	100%	8	8	16	97.33%			
3	150%	12	8	20	97.54%			

3.8 Corrected Absorbance - (D) value:

For geometrical correction method the absorbances of both the drugs at all three wavelengths were measured and the D value was calculated using following formulas. [12]

B1 = A1 – vD

B2 = A2 – D

B3 = A3 – wD

Table 7: D value Amlodipine besylate from biological sample.

Sr.No	Conc	D value
1	4	0.11397
2	8	0.2149
3	12	0.3289
4	16	0.4589
5	20	0.6027
6	24	0.7218
7	28	0.8328
8	32	0.9897
9	36	1.098

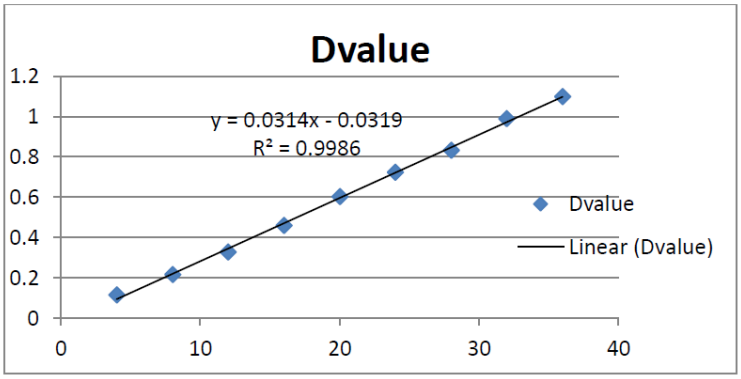

Figure 3: Calibration curve for D Value of AB

Table No 8: D value of Azilsartan medoxomil from biological sample.

Sr.No	Conc	D value
1	5	0.103969
2	10	0.2249
3	15	0.31282
4	20	0.4369
5	25	0.5375
6	30	0.6312
7	35	0.8071
8	40	0.9204

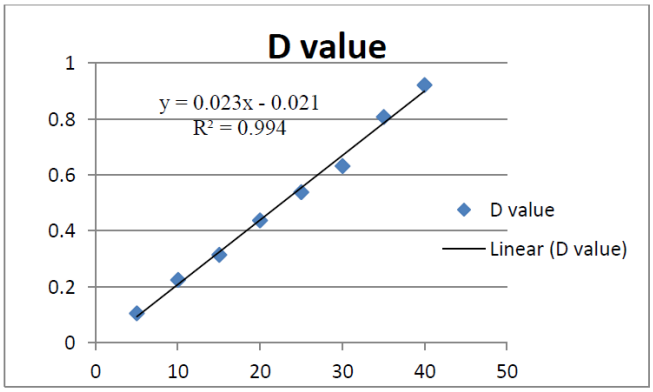

Figure 4: Calibration curve for D Value of AZ

Table No 9: Simultaneous estimation of Azilsartan medoxomil and Amlodipine besylate

Sr.No	Drug	Label claim (ppm)	Amount found (ppm)	Label claim (%)
1	Azilsartan medoxomil	30 ppm	30.08	100.2%
2	Amlodipine besylate	3.75 ppm	3.74	99.73%

4. RESULTS AND DISCUSSION

A validated three-point geometrical correction UV Spectrophotometric method was developed for determination AZ and AB from biological matrix using egg albumin. The solvent used for analysis was mixture of methanol: water (10: 90).

The Geometric correction method was found to be simple method to eliminate background irrelevant absorption in case of samples of biological origin. In this method the absorption spectra of Amlodipine besylate and that of background absorption were taken at the three wavelength λ_1, λ_2, and λ_3 i.e. 226nm ,239nm, 245nm.The corrected absorbance of drug were calculated from sample solution at 3 wavelengths using general equation of D value. The ratio of D value remains constant. The method was found to be rapid and simple for determination of Amlodipine besylate from biological sample.

Geometrical correction method is the simplest method to eliminate background irrelevant absorption in case of biological sample. UV – visible spectrometry method for quantification of Azilsartan medoxomil and Amlodipine besylate by using egg albumin as biological sample. The UV-visible method was performed for Azilsartan medoxomil and Amlodipine besylate by using Methanol: Water (10:90) as solvent. In this method three wavelengths were selected. The wavelengths 240nm ,251nm ,262nm for Azilsartan Medoxomil and 226nm 239nm,245nm for Amlodipine besylate were selected. In the geometrical correction method validation was performed by using these three wavelengths. The geometrical correction method was developed with sample egg albumin The method was found to be simple, accurate, precise, rugged and robust. The proposed method can be used for bioavailability and bioequivalence studies.

The simultaneous estimation method of Azilsartan medoxomil and Amlodipine besylate was developed by using egg albumin. The proposed method for simultaneous estimation of Azilsartan medoxomil and Amlodipine besylate was found to be simple, accurate and precise. The percentage label claim was found to be 100.2% and 99.73%. These methods gave good results and it can be used

for routine analysis of two drug Azilsartan medoxomil and Amlodipine besylate in mixture dosage form.

The absorbance ratio method the entire spectra follows the beer's law at all the wavelengths. The two wavelengths were used for analysis of the drug were 251 nm (Azilsartan medoxomil) and 242 nm (isoabsorptive point). The overlain UV absorption was found to be simple, accurate, sensitive. Hence this method can be employed for routine analysis of these two drug in combined dosage form.

5. CONCLUSION

The geometrical correction method was found to be simple ,rapid method for the determination of Azilsartan medoxomil and Amlodipine besylate in biological sample such as egg albumin .The three point geometrical correction was simplest method to eliminate background irrelevant absorption .Three point geometrical correction UV spectrophotometric method was developed and validated as per ICH guidelines with repeatability ,specificity ,accuracy ,Precision ,LOD ,LOQ, ruggedness and robustness for estimation of Azilsartan medoxomil and Amlodipine besylate. Simultaneous estimation and absorbance ratio method of Azilsartan medoxomil and Amlodipine besylate was developed by using egg albumin. These methods gave good results and it can be used for routine analysis of two drug Azilsartan medoxomil and Amlodipine besylate in combined dosage form.

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