ISSN 0974-3618 (Print)
0974-360X (Online)

www.rjptonline.org

RESEARCH ARTICLE

Development and Validation of Analytical Method for Simultaneous Estimation of Amlodipine Besylate and Celecoxib in Pure and Combined Dosage Form

Miss. Tejaswini Rajaram Mandale[1]*, Dr. Manish. S. Kondawar[2], Mr. Sandeep Dilip Kadam[3]
[1,3]Department of Quality Assurance, ABCP, Sangli - 416416, Maharashtra, India.
[2]Head of Department of Quality Assurance, ABCP, Sangli - 416416, Maharashtra, India.
*Corresponding Author E-mail: **tejaswinimandale12@gmail.com**

ABSTRACT:
A simple, rapid and reliable UV spectroscopic method was developed and validated for simultaneous estimation of Amlodipine besylate (Amlo) and Celecoxib (Celo) in pure and combined dosage form. Amlo and Celo shows absorption maximum in methanol at 237nm and 252nm respectively. According to ICH guidelines, this method was validated for linearity, precision, accuracy, limit of detection and Limit of quantitation. This method showed linearity and correlation coefficient for Amlo and Celo as 2 to 12µg/ml, 0.9981 and 0.9984 respectively. The recovery studies for Amlo and Celo was obtained to be 100.5 to 101.66% and 100.28% to 101% respectively. The precision of method were established by repeatability study. Limit of detection and Limit of quantitation are 0.21µg/ml and 0.64µg/ml for Amlo and 0.12µg/ml and 0.36µg/ml for Celo respectively. The statistical parameters and recovery data presented that the method may be engaged for efficient, rapid examination of both drugs the from tablet formulation. The percentage label claim present in formulation tablet was obtained to be 99% and 100.3% for Amlo and Celo respectively. These analysis is clearly specified that without there interference from excipients in the formulation.

KEYWORDS: Amlodipine besylate, Celecoxib, Methanol, Simultaneous equation method, UV Spectroscopy.

INTRODUCTION:
Amlodipine besylate (Amlo) chemically designed as 3-ethyl 5-methyl (4RS)-2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1, 4-dihydropyridine-3, 5-dicarboxylate benzene sulfonic acid (Fig.1a) is antihypertensive agent used for the treatment of hypertension. It comes under the class of calcium channel blocker[1,2].

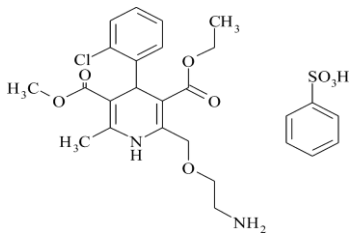

Fig.1a: Chemical Structure of Amlodipine besylate

Celecoxib (Celo) chemically known as 4-[5-(4- methyl phenyl) –3-(trifluoromethyl) pyrazol-1–yl] benzene sulphonamide (Fig.1b). Celo is a NSAID (Non-Steroidal Anti-inflammatory Disorder) that exhibits anti-inflammatory, analgesic and antipyretic activities. It comes under the class of selective cyclo-oxygenase-2 (COX-2) inhibitor. It is purpose for prevent the rheumatoid arthritis and osteoarthritis.

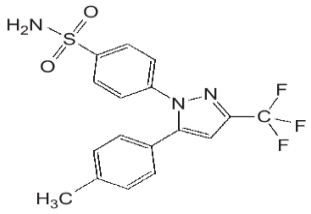

Fig.1b: Chemical Structure of Celecoxib

As per some cases, patient have both problems, hypertension and osteoarthritis. In that condition, they used to take both tablet and sometimes they may face the non- compliance for taking two tablet. Such

Received on 23.10.2019 Modified on 08.12.2019
Accepted on 31.01.2020
Research J. Pharm. and Tech 2020; 13(9):4280-4284.
DOI: 10.5958/0974-360X.2020.00756.8

conditions can be overcome in single tablet, i.e. combination, by administering both drug. Therefore, Amlo and Celo in a combination used for both condition. These combination were prepared to overcome the possibility of osteoarthritis in hypertension patients and vice versa.

According to literature survey, no analytical method was developed and validated on Amlo and Celo in combination. Therefore, this particular combination is selected for development of suitable analytical method. These survey mentioned that the use of spectroscopic methods[4,5,6,7], high performance thin layer chromatography (HPTLC)[8,9,10,11] and high performance liquid chromatography (HPLC) methods[11,12,13] for separate and combination of other substance. Therefore, this was establish simple, rapid and accurate UV spectroscopic method procedure for simultaneous determination of Amlo and Celo.

It is the simple working analytical method used in the pharmaceutical laboratories and research. This analytical method applicable for qualitative and quantitative drugs analysis. These are mainly used for multi-component analysis thus overcomes the bulky task of separating interferons and agree to the estimation of an increasing number of analytes, therefore minimizing the time and cost of analysis.

The main objective is, to develop a specified, sensitive and accurate UV spectroscopic for simultaneous estimation of amlodipine besylate and celecoxib in combined dosage form by multicomponent analysis method.

MATERIAL AND METHOD:

Chemical and reagent:

Amlodipine besylate and Celecoxib was obtained as a gift sample from Smruthi Organics Limited, Solapur and Aarti Drugs Limited, Mumbai respectively. All reagents employed were of analytical grade ordered from Research Lab, (Mumbai, India). Stock solutions of Amlo and Celo (1 mg/ml) were prepared in Methanol.

Instrumentation:

Analytical balance was used. The analytical method was developed using UV Visible double beam spectrophotometer JASCO UV-V-730 with matched pair of quartz cuvette which consist of 1 cm path length and spectral bandwidth of 1nm.

Experimental work:

Preparation of Standard Stock Solution:

Standard stock solution having Amlo and Celo were prepared by dissolved 10mg of Amlo and 20mg of Celo in 10ml of methanol separately to acquire standard stock solution containing 1000µg/ml of Amlo and 2000µg/ml of Celo in different 10ml volumetric flasks.

Preparation of Working Standard Solution:

From standard stock solution further dilution was prepared by pipette out 1ml of Amlo and 0.5ml of Celo in 10ml of methanol separately to acquire working standard stock solution having 100µg/ml of Amlo and Celo in different 10ml volumetric flask.

Method-Simultaneous Equation Method:

Procedure for selection of analytical wavelength:-

Standard working solution of both standard drugs was suitably dilute with methanol, to acquire concentration of 10µg/ml of Amlo and 10µg/ml of Celo and they can scanned individually in a wavelength range of 200-400nm against blank as methanol, to maximum absorption of selected wavelength for drugs. From the overlain spectrum, 237nm and 252nm wavelength were selected for Amlo and Celo respectively.

Procedure for Plotting Calibration Curve:-

From working standard solutions, series of diluted standard solutions having Amlo and Celo are prepared. For this 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2ml of working standard solution were transferred to separately 10 ml of volumetric flask and addition of methanol up to the mark, to acquire concentration of 2, 4, 6, 8, 10 and 12µg/ml. The absorbance of beyond solutions was estimated at the certain wavelength and the calibration curves was produced by plotting absorbance vs concentration for both drugs. The calibration curve for Amlo was plotting by record the absorbance at 237nm and calibration curve for celecoxib was plotting by record the absorbance at 252nm. The absorptivity of both these drugs at both wavelengths was determined.

The absorbance and absorptivity values at the specific wavelength was calculated and substituted in the resulting equation, to achieve concentration.

$C_{Amlo} = (A_1 ax_2 - A_2 ax_1)/ (ax_2 ay_1 - ax_1 ay_2)$

$C_{Celo} = (A_2 ay_1 - A_1 ay_2) / (ax_2 ay_1 - ax_1 ay_2)$

Where,

C_{Amlo} = Concentration of Amlodipine besylate

C_{Celo} = Concentration of celecoxib

A_1 = Absorbance of sample at 237nm

A_2 = Absorbance of sample at 252nm

ax_1 = Absorptivity of Amlodipine besylate at 237nm

ax_2 = Absorptivity of Celecoxib at 252 nm

ay_1 = Absorptivity of Amlodipine besylate at 252nm

ay_2 = Absorptivity of Celecoxib at 252 nm

Analysis of Sample Solution:-

A tablet formulation containing Amlo 10mg and Celo 200mg was used for sample preparation. Twenty tablet was weighed correctly, fine powdered and quantity equivalent to 0.5mg of Amlo and 10mg of Celo was weighed accurately and transferred in to previously

cleaned and dried 100ml of volumetric flask along with 9.5mg of Amlo. Added 30ml of methanol, flask were sonicated for 5 min, allowed to cool and volume was added upto mark by using methanol, to acquire stock solution containing 100µg/ml of Amlo and 100µg/ml of Celo. Flask contents were filtered using whatmman filter paper. After filtration the solution was properly diluted to obtain required concentration by using methanol. The absorbance of sample solutions were measured at 237nm and 252nm using solvent as a blank (Methanol). The results were calculated by the above equation.

Method validation:
The validation of developed method parameters like linearity, precision, accuracy, limit of detection and limit of quantitation was performed by ICH Q2 (R1) guidelines.

Linearity:
Linearity of the methods were checked by analysing the standard solutions separately, having Amlo and Celo (2, 4, 6, 8, 10 and 12µg/ml) in methanol and absorbance were noted at 237 and 252nm respectively. Calibration graphs were constructed using absorbance of standard solutions vs concentration. Regression analysis were performed by least squares method to estimate the values of intercept, slope and correlation coefficient.

Precision:
The precision of developed method was confirmed by repeatability studies. Repeatability of that methods were evaluated by analysing sample solutions (Amlo and Celo: 6µg/ml) for three times by assessing the absorbance of both these drug solution at 237 and 252 nm respectively and % RSD were calculated.

Accuracy:
In this method accuracy was tested by recovery study by standard addition method. In pre-analyzed sample solution of 6µg/ml a definite concentration of 4, 6 and 8µg/ml standard solution of Amlo and Celo were added and its recovery was studied. The absorbance of resultant solutions was estimated at their corresponding wavelengths. Recovery studies were determined by standard addition method at level of 80%, 100% and 120% and % recovery was determined.

Limit of detection:
It is the lowest quantity of analyte in a sample that may be detected but not essentially quantitated under the specified experimental conditions. It may be determined by using following equation,

$LOD = 3.3 \times \sigma / S$

Where,
σ = Responce of standard deviation
S = Calibration graph slope

Limit of quantitation:
It is the lowest quantity of analyte in a sample that can be quantitated with the acceptable precision and accuracy under specified experimental conditions. It is determined by using following equation,

$LOQ = 10 \times \sigma / S$

Where,
σ = Responce of standard deviation
S = Calibration curve slope

RESULT AND DISCUSSION:

Standard drug solutions in methanol containing 10 µg/ml of Amlo and Celo was scanned individually in a range of wavelength 200-400nm against blank as methanol and it showed the absorption maxima at 237 nm for Amlo and 252 nm for Celo and overlaid spectra was made.

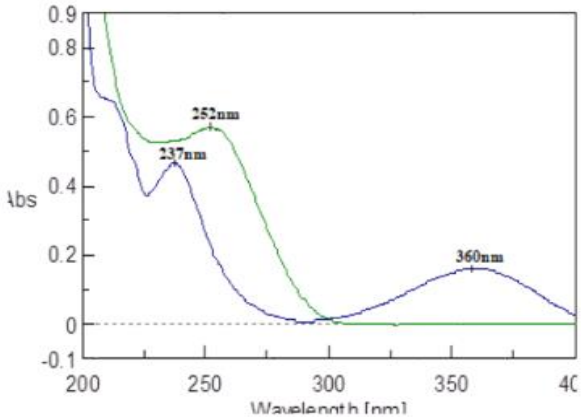

Figure No.2: Overlay spectra of Amlodipine besylate and Celecoxib

The series of dilutions of Amlo and Celo in the concentration ranges of 2-12µgl/ml was prepared and the absorbance of these solutions was measured at 237nm and 252nm. The calibration curves was produced by plotting the absorbance against the concentration for both the drugs.

Table No.1: Calibration curve of Amlodipine besylate at 237nm

Sr. No	Conc. (µg/ml)	*Mean Abs.	Absorp.	± SD	% RSD
1.	2	0.0838	419	0.001308	1.56
2.	4	0.1559	389.91	0.00281	1.80
3.	6	0.2352	392.11	0.004583	1.94
4.	8	0.2962	370.29	0.002859	0.96
5.	10	0.3849	384.96	0.001484	0.38
6.	12	0.4638	386.55	0.001747	0.37

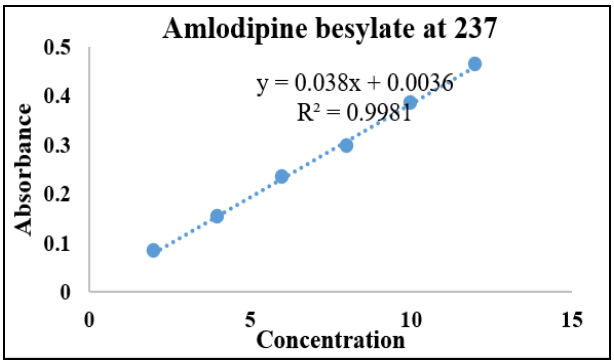

Figure No.3: Calibration curve of Amlodipine besylate at 237nm

Table No. 2: Calibration curve of Celecoxib at 252nm

Sr. No	Conc. (µg/ml)	*Mean Abs.	Absorp.	± SD	% RSD
1.	2	0.1467	733.5	0.00072111	0.49
2.	4	0.2730	682.58	0.00156950	0.57
3.	6	0.3665	610.88	0.00260832	0.71
4.	8	0.4855	606.87	0.00415812	0.85
5.	10	0.5971	597.13	0.00225018	0.37
6.	12	0.7278	606.52	0.00120968	0.16

*(n=3- three times determination)

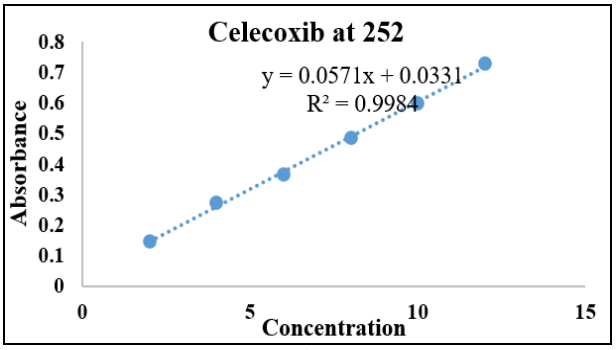

Figure No.4: Calibration curve of Celecoxib at 252nm

Method Validation:

Table No. 3: Validation Parameters

Parameters	Amlodipine Besylate	Celecoxib
λ_{max} (nm)	237nm	252nm
Beer's law limit (µg/ mL)	2-12µg/ml	2-12µg/ml
Molar absorptivity (L mol^{-1} cm^{-1})	22141.60	24391.85
Correlation coefficient (r)	0.9981	0.9984
Regression equation (y = mx + c)	Y= 0.038x+0.0036	Y= 0.057x + 0.0331
Slope(s)	0.038	0.057
Intercept(c)	0.0036	0.0331
Mean Standard deviation of response (σ)	0.002465	0.002086
LOD (µg /mL)	0.21	0.12
LOQ (µg/ mL)	0.64	0.36

Linearity:

A calibration curve was plotted between concentration and absorbance. Amlo and Celo was detected linear with the concentration range of 2-12µg/ml at wavelength of 237nm and 252nm. The correlation coefficient of the linearity was observed to be 0.99 at each wavelength for both the drugs. [Table 1-3 and Fig. 2-3]

Precision:

Precision study of Amlo and Celo illustrated that the developed method is precise. The result showed that the %RSD value was less than 2.0%. There was indicates without interference from the present of excipients in tablet. [Table 4]

Table No. 4: Precision studies

Drug	Conc.(µg/ml)	*Mean Abs.	±SD	%RSD
Amlo	**6**	0.2549	0.000902	0.35
Celo	**6**	0.3665	0.00063639	0.17

*(n= 3- three times determination)

Accuracy:

Accuracy was estimated by performing recovery studies by standard addition method. The recovery study of Amlo and Celo was described in terms of ±SD and %RSD. Percentage recovery values were observed in the range of 100.5% to 101.66% and 100.28% to 101 % for Amlo and Celo respectively. The results of recovery study are presented in Table 5.

Table No.5: Recovery study

Drug	Conc. (%)	Amt. present (µg/ml)	Amt. added (µg/ml)	*Amt. recovered (µg/ml)	% RSD	%Drug Recovery
	80	6	4	10.05	0.034	100.5
Amol	100	6	6	12.32	0.044	101.66
	120	6	8	14.07	0.056	100.5
	80	6	4	10.1	0.039	101
Celo	100	6	6	12.09	0.033	100.75
	120	6	8	14.04	0.044	100.28

Limit of detection and limit of quantitation:

It is determined by standard deviation of response and slope of calibration curve. These was observed to be 0.21µg/ml and 0.64µg/ml for Amlo and 0.12µg/ml and 0.36µg/ml for Celo respectively.

Analysis of sample solution:

A sample solution containing Amlo 10mg and Celo 200mg was required for analysis. Sample solution were analyzed three times and concentration of drug determined by UV Spectroscopy for simultaneous equation method was in good with the label claim. The results for the sample solution were establish to be 99% and 100.3% for Amlo and Celo respectively. The result for analysis of formulation showed % RSD values is < 2%, which indicates within limit. The results obtained from analysis of dosage forms are presented in Table 6.

Table No.6: Analysis of sample solution

Drug	Label claim (mg/tab)	Mean* Abs.	%Drug content	±SD	% RSD
Amlo	**10**	0.5445	99	0.0004	0.073
Celo	**200**	0.7813	100.3	0.00055	0.071

*(n= 3)

CONCLUSION:

It is stated that this developed method is simple, new, safe, accurate and precise. This method is successfully used for simultaneous estimation of Amlo and Celo. This method is successfully validated by using ICH Q2 (R1) guidelines for analytical method validation. It is suitable for application in pharmaceutical drugs analysis of the pure and combined dosage form with on interference of excipient and with good sensitivity.

AKNOWLEDGEMENT:

We grateful to the principle of the Appasaheb Birnale college of Pharmacy, Sangli. Institute for providing excellent laboratory facilities. We are grateful to Smruthi Organics Limited, Solapur and Aarti Drugs Limited, Mumbai for a gift sample of Amlo and Celo respectively. We are thankful to Dr. Manish S. Kondawar, HOD of Department Quality Assurance, Assistance professor for guidance and also thanks to laboratory technician and laboratory assistance for helping.

REFERENCES:

1. Logoyda Liliya, Korobko Dmutro and Saprun Stanishav, "Development of methods for identification of calcium channel blockers in medicine", International Journal of research Ayurveda pharm, 2016; 7(2): 88-91.
2. Indian Pharmacopoeia 2007, Vol I and II, 143 and 96-97
3. Primo Fabián Teixeira and Pedro E. Froehlich, "Celecoxib Identification Methods", Acta Farm. Bonaerense, 2005; 24 (3): 421-425.
4. Naveed Safila., Qamar Hina, Jawaid Wardha, and Bokhariet Urooj, "Simple UV spectrophotometric assay of amlodipine", American Journal of Chemistry and application, 2014; 1(4): 66-69.
5. Saha R. N. Sajeev C., Jadhav P.R., Patil S.P., and Srinivasan N., "Determination of celecoxib in Pharmaceutical formulations using UV spectrophotometry and liquid chromatography", Journal of pharmaceutical and Biomedical analysis, 2002; 28: 741-751.
6. Vichare Vijaya., Vrushali Tambe, Vrushali Kashikar And Dhole S.N, "Spectrophotometric simultaneous determination of amlodipine besylate and hydrochlorothiazide in combined tablet dosage form by simultaneous equation, absorption ratio and first order derivative spectroscopy methods", International Journal of chemistry Research,2011; 2(1): 7-10.
7. Ingale. Pramod. L., Patil Laxmileena D., Gudi Sachin V., Jadav Deepali D., Kadam Yogita A. and Sampada D. Dalvi and "Development and validation of UV-spectrophotometric methods for simultaneous estimation of amlodipine besylate and clopidogrel bisulfate in bulk and tablet dosage form", Der Pharma Chemica, 2013, 5 (4):282-287.
8. Jain P. S., Patel M. K., Bari S. B. and Surana S. J., "Development and Validation of HPTLC Method for Simultaneous Determination of Amlodipine Besylate and Metoprolol Succinate in Bulk and Tablets", Indian Journal of Pharmaceutical Sciences, 2012; 74 (2): 152-156.
9. Patel V. B., Sahu Rajnikanta, and Patel Bhumika M, "Simultaneous determination of Amlodipine besylate and Atorvastatin calcium in Pharmaceutical tablet formulation by High Performance Thin Layer Chromatographic method", International Journal of ChemTech Research, 2011; 3(2): 695-698.
10. Shaalan R. A., Belal Tarek S., El Yazbi Fawzy A. and Elonsy Sohila M., "Validated HPTLC methods for determination of some selected antihypertensive mixtures in their combined dosage forms", Bulletin of Faculty of Pharmacy, Cairo University, 2014; 52: 225–237.
11. Kamble Asmita Y., Mahadik Mahadeo V., Khatal Laxman D. and Dhaneshwar Sunil R., "Validated HPLC and HPTLC Method for Simultaneous Quantitation of Amlodipine Besylate and Olmesartan Medoxomil in bulk drug and formulation", Analytical Letters, 2010; 43(2): 251-258.
12. Chandana OSS and Ravichandrababu R., "Stability indicating HPLC method for celecoxib related substances in solid dosage forms", International Journal of Research in Pharmacy and Science , 2017; 7(1): 10-18.
13. Jadhav Priyanka S., Jamakar Priti M. and Avachat Amelia M., "Stability indicating method development and validation for simultaneous estimation of atorvastatin calcium and celecoxib in bulk and niosomal formulation by RP-HPLC", Brazilian Journal of Pharmaceutical sciences, 2015; 51 (3).
14. Chanudhary Jasmine, Jain Akash and Vipin Saini, "Simultaneous estimation of multicomponent formulations by UV-Visible Spectroscopy: on Overview", International research Journal of Pharmacy, 2011; 2(12): 81-83.
15. Kamal Amir, El-Malla Samah F. and Hammad Sherin F., "A Review on UV Spectrophotometric Methods for Simultaneous Multicomponent Analysis", European Journal of Pharmaceutical and Medical Research, 2016; 3(2): 348-360.
16. Redasani K. V. Patel P. R., Marathe D. Y., Chudhari S. R., Shrikhedkar A. A. and Surna S.J., " A review Derivative of UV spectrophotometry analysis of drugs in pharmaceutical formulation and biological samples review", Journal of the Chilean Chemical society, 2018; 63 (3).