Stability Indicating HPLC Method for Development and Validation of Simultaneous Estimation of Amlodipine and Celecoxib from Bulk and Marked Formulation

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A B S T R A C T

The current work manages the stability indicating RP-HPLC techniques for concurrent assurance of amlodipine and celecoxib in pharmaceutical formulation Attempts were made to develop RP-HPLC method for simultaneous estimation of amlodipine and celecoxib from tablet. For the RP HPLC method, Agilent (S.K) Gradient System UV Detector, chemstation software and C18 column with 250mm x4.6 mm i.d and 5µm particle size, C-18 (coxmosil) stationary phase.For amlodipine and celecoxib using mobile phase of MEOH + Water (0.1% OPA) & (70:30 v/v) 235 nm, 1ml, 3+60 mcg presented acceptable RT 3.953 minute & 6.587 min. with good peak shape And validation parameter like, Accuracy, Liniarity, LOD, LOQ, Precision, Robustness within limit according to ICH guideline.Marketed formulation % Lable Claim was found to be within 98-100% and %RSD less than 2% and Study is to determine and generate inheriting stability data for drug through stress degradation studies under ICH recommended stress conditions and develop stability indicating assay.

Keywords: HPLC, Method development, Validation, Stress testing, label claim.

1. Introduction

Amlodipine Besilate is a bleached transparent triturate without the nuclear heap at 678.2. The aforementioned exists to some degree dissolvable within H2O as well as parsimoniously dissolvable in methanol. AMLO medicines remain figured by way of silver pills indistinguishable from 3.0, 10 & 20 miligram of AMLO aimed at uttered group. Notwithstanding the dynamic fixing, amlodipine besylate, each tablet contains the going with lethargic fixings: smaller crystal-like fibre, di basic CaSO4, Amlodipn is utilized in the administration of high blood pressure as well as artery course ailment in individuals through whichever constant chest pain (wherever heart torment happens for the most part afterward somatic or enthusiastic pressure) or vaso spastic chest pain (wherever it happens with sequences) then deprived of cardiovascular breakdown. It very well may remain utilized as whichever mono rehabilitation otherwise mix treatment to administration of high blood pressure conduit infection. AMLO canister stay managed near grown-ups and youngsters. It is utilized to organization of high blood pressure in addition to heart hall syndrome in individuals through whichever unchanging chest pain Celecoxib is the NASID quieting medication utilized for the cure of osteo inflammation, and old joint inflammation, intense torment, excruciating monthly cycle in addition to catamenial indications, plus remains additionally utilized for lessen quantities to large intestine as well as and anus with affected persones. The situation remains promoted through Cadila pharma underneath trademark tag as celebrex. Celecb stays open thru arrangement with case structure.

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Celecoxib is utilized for osteoarthritis, rheumatoid joint inflammation, intense agony, musculoskeletal torment, agonizing period, ankylosing spondylitis, and to decrease the quantity of large intestine as well as rectal pulp inside individuals through ancestral colonal polypsis. This might likewise be utilized in youngsters with adolescent rheumatoid joint inflammation who are more seasoned than two years old.
Side Effects- stomach torment, clogging, looseness of the bowels, gas, acid reflux, sickness, regurgitating, discombobulation, migraine, respiratory tract disease

2. Methodology

i. **Material-** API- Amlodipine (Amlo) & Celecoxib (Cel)

ii. **Reagent and chemicals**- Orthophopsphoric acid(OPA), Acetonitrile, Water were used of HPLC grade

iii. **Chromatographic condition**- The analysis of the drug was carried out on Agilent (1100) Gradient System UV Detector. Equipped with Reverse Phase (Cosmosil) C18 column (4.6mm x 250mm; 5µm), a SP930D pump, a 20µl injection loop and UV730D Absorbance detector and running Chemstation software.

A .Preparation of standard stock solution:- (stock-I)

An appropriately weighed quantity, 10 mg of Amlodipine (AMLO) changed into thawed with MEOH within a 100 ml volumetric flask as well as quantity ended as much as 10.0 ml to produce a solution of 100 ug/ml

B. Preparation of standard stock solution:- (stock-II)

An appropriately weighed quantity, 200 mg of Celecoxib (CLB) turned into liquefied in Methanol in 100mililiter volumetric flask & volume make up with 10 ml to produce a solution of 2000 ug/ mililiter.

C.Preparation of Stock Standard Combination Solution :(Stock III) [AML+CLB]

Accurately weight and transfer 10 miligram AMLO and 200 miligram CELCB , working standard hooked on 10 & 100 mililiter volumetric flask by way of around diluent Methanol absolutely & mark capacity match with speed with the equal solvent to get 100 +2000 µg/ml respectively standard & 15minute sonicate to liquefy it & cast off the unsolicited air, similarly an cohert slice of each API stock solution in ratio of 1:20 and have been blended in a volumetric flask in 10miliL & bulk has been poised as abundant as an imprint with a mobile phase since the subsequent arrangement 0.1 miliL changed into moved to 10miliL volumetric flask and the size has been ended in control with Methanol:H2O arranged in 7.0 ml methanol : 3.0 ml Water (0.1% OPA) for amlodipine : celecoxib

D.HPLC used for chromatographic condition apply on the Preparation of standard solution:-

a.Preparation of std. Amlodipine solution: (Stock I)

From the freshly prepared standard stock solution 100 ug/ml, 0.1ml stock solution was suck out in 10ml volumetric flask & degree got finished up to 10 ml thru the mobile phase to contract the last fixation of 1µg/ml

b. Preparation of std. Celecoxib solution: (Stock II)

From the freshly prepared standard stock solution 200 ug/ml and 0.1 ml stock solution changed hooked on sucked out in 10ml volumetric flask & capacity got complete up to 10 ml through a mobile phase to get the final conc. 20 ug/ml.

iv. Selection of mobile phase: Each mobile phase was vacuum degassed and filtered through 0.45µ membrane filter. The mobile phase was allowed to equilibrate until OPA by baseline was obtained. The standard solution containing mixture of API was run with different individual solvents as well as combinations of solvents were tried to get a good separation and stable peak. From the various mobile phases tried, mobile phase containing MeOH and Water (0.1% OPA) was selected since it gave sharp, well resolved peaks with symmetry within the limits and significant reproducible retention time for API.

v. Selection of detection Wavelength: Usual arrangements have been examined inside the scope of 200-400 nanometer, towards 10 miliL Methanol & volume made by H2O dissolvable framework as orientation, Amlodipine and Celecoxib had been confirmed range of maxima at 237 nanometer & 222 nanometer correspondingly If Dual Amlodipine and Celecoxib, sample Interrelate by this fact is called Isobestic area Isobestic area in 235nm, had been selection wavelength is HPLC Method may be used.

vi. Validation parameter

A .Linearity:

a. Preparation of standard stock solution for linearity:

Average weight of tablet sample equivalent to 10 mg of Amlodipine and 200 mg of Celecoxib , were weighed and transferred to 10 mL volumetric flask & diluent was added to make up the volume. Sonicated for 10 min with occasional swirling. 0.1 ml of this solution diluted up to 10 ml volumetric flask with diluents was added to make up the volume.

b. Preparation of linearity solution:

A series of standard preparations of working standard of were prepared.

Table of linearity

AMLO	CELO
1	20
2	40
3	60
4	80
5	100

B. Accuracy

The accuracy was determined by 10 mg of Amlodipine and 200 mg of Celecoxib , (80 %, 100 % and 120 % of the label claimed, respectively) to quantity equivalent to average weight of marketed tablets. This powder mixture containing API in mg were triturated and then subjected to chromatographic analysis using the described method. The resulting mixtures were analyzed in triplicates over three days. The % recovery of added drug was taken as a measure of accuracy.

sample	Amount added in mg	
	AMLO	CELE
Accuracy 80 %	0.8	16
Accuracy 100%	1.0	20
Accuracy 120 %	1.2	24

C. Precision-

a. Repeatability: Precision of the system was determined with the sample of RP-HPLC& UV Method for. Six replicates of sample solution containing 10 mg of Amlodipine and 200 mg of Celecoxib , were injected and peak areas were measured and %RSD was calculated

b. Inter-day and Intra-day precision: Sample solutions containing 10 mg of Amlodipine and 200 mg of Celecoxib three different concentration (2µg/ml, 3µg/ml, 4µg/ml) Amlodipine and (40µg/ml, 60µg/ml, 80µg/ml) Celecoxib. Amlodipine and Celecoxib were analyzed three times on the same day and %R.S.D was calculated.

D. Robustness:

The mobile phase composition was changed in (±1 ml/ min^{-1}**)** proportion of Methanol: Water (0.1 % OPA) in the mobile phase composition and the flow rate was (±1 ml/ min^{-1}**)** and the change in detection wavelength (±1 ml/ min^{-1}**)** and the effect of the results were examined it was performed using using 4 µg/ml and 80 µg/ml solution of Amlodipine and Celecoxib,

E. Detection Limit

Based on the S.D. of the response and the slope of calibration curve, the detection limit was calculated as,

$DL = \frac{3.3\sigma}{S}$

F. Quantitation Limit

Based on the S.D. of the response and the slope of calibration curve, the quantitation limit was calculated as,

$QL = \frac{10\sigma}{S}$

vii. Forced degradation studies

Std .Amlodipine 10 mg and Celecoxib 200 mg in 100 ml MeOH = 100 µg/ml Amlodipine & 2000 µg/ml Celecxib ---------- stock-1

1) **1 N HCL:-**take 0.4ml from std stock solution add 5 ml 0.1N HCL & makeup volume 10miliL of mobile phase, inject 20 µl in system after1 hr and 1 hr without heat

2) **0.1 N NAOH:-** take 0.4 ml from std stock solution add 5 ml 0.1N NAOH & makeup volume of 10 miliL of mobile phase inject 20 µl in

system after 1 hr and 1hr without heat

3) 3 % H_2O_2take 0.4 ml from std stock solution add 5 ml 3 % H2O2 and makeup volume 10 ml with mobile phase inject 20 µl in system after 1 hr and 1 hr without heat

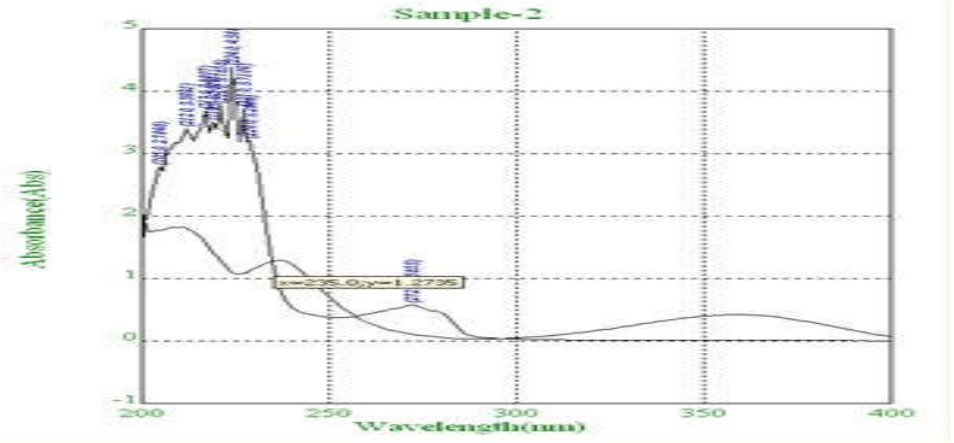
4) THERMAL :- take 0.4 ml from std stock solution and makeup volume 10 ml with mobile phase and stored in oven at 80˚C for 6 hr, then volume adjusted with diluent and inject 20 µl in system.

3. Result And Discussion

A. Melting point - Amlodipine 199-201^{0}C, celecoxib 157-159 ^{0}C,

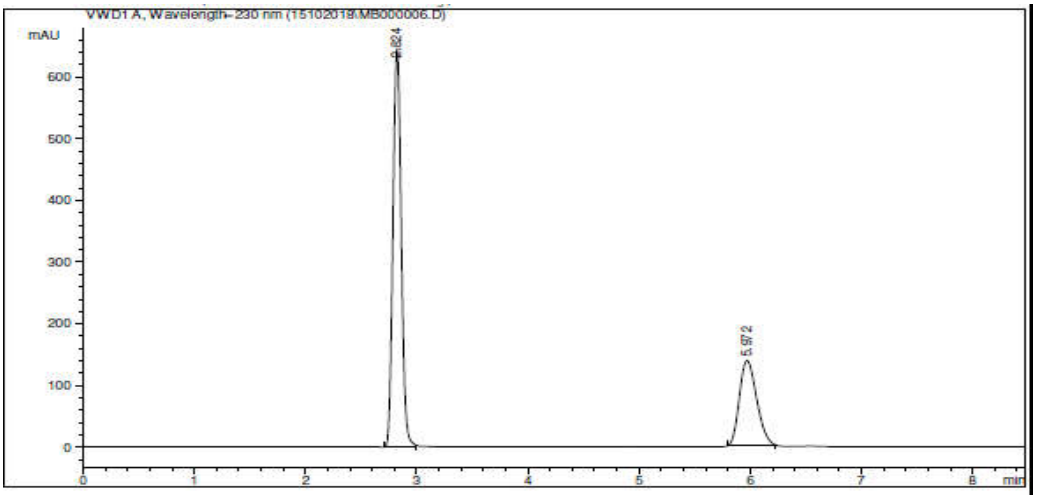
B. Solubility -Amlodipine-It is slightly soluble in water and sparingly soluble in ethanol, Celecoxib is soluble in organic solvents such as ethanol, DMSO, and dimethyl formamideCelecoxib is sparingly soluble in aqueous buffers,

C. UV Spectroscopy- Usual arrangements have been examined inside the scope of 200-400 nanometer, towards 10 miliL Methanol & volume made by H2O dissolvable framework as orientation, Amlodipine and Celecoxib had been confirmed range of maxima at 237 nanometer & 222 nanometer correspondingly If Dual Amlodipine and Celecoxib, sample Interrelate by this fact is called Isobestic area Isobestic area in 235nm, had been selection wavelength is HPLC Method may be used.



Studies on the chromatographic behaviour of Amlodipine and Celecoxib

MEOH+ Water OPA 0.1% (70:30 % v/v) , 1 ml, 235 nm, 3+60 mcg gives Resolve peak and sharp at RT 3.953 and 6.587



Std drug chromatogram-MEOH+ Water OPA 0.1% (70:30 % v/v) , 1 ml, 235 nm, 3+60 mcg

D. System suitability parameters

Parameter	Amlo.	Cele.
No. of theoretical plates	9186	10969
Retention time (min).	3.953	6.587
Tailing factor/symmetry	0.76	0.72
Resolution		12.63

E. Validation results

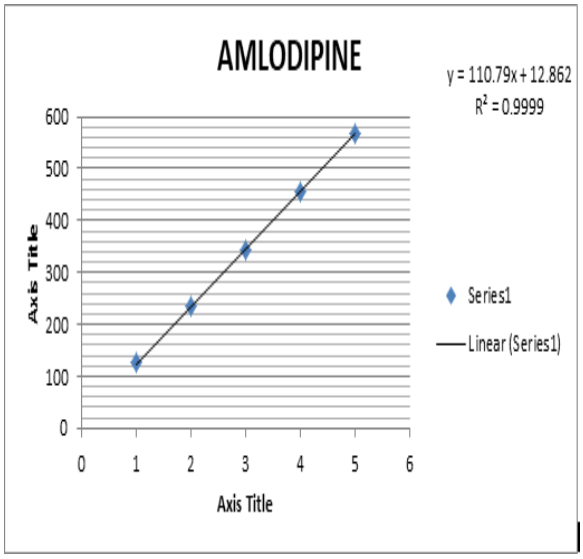
i. Linearity- Linearity of of amlodipine & celecoxib was observed in range 1-5 µg/ml & 20-100 µg/ml, Detection wavelength used was 235 nm

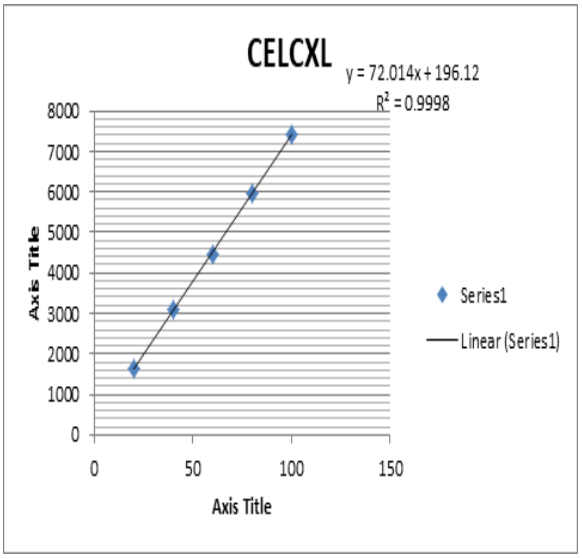
The regression equation was found to be y =110.7 x + 12.86, y = 72.01x + 196.1, amlodipine & celecoxib respectively with correlation coefficient (R^2) 0.999 to all above concentration of drug.

The plot should be linear passing through the origin, Correlation Coefficient should not be less than 0.999.that concluded

ii. LOD value were found to be 0.0152 µg/ml, 0.6276 µg/ml, for amlodipine & celecoxib so analytical method that concluded.

iii. LOQ value were found to be 0.046 µg/ml, 0.6276 µg/ml, for amlodipine & celecoxib respectively so analytical method that concluded





iv. Accuracy Recovery studies were performed to validate the accuracy of developed method. To pre analyzed tablet solution, a definite concentration of standard drug (80%,100%, and 120%) was added and then its recovery was analysed. The % recovery was found to be within 98-102%

Amlo.	80%	1	0.8	1.79± 0.01	0.79± 0.01	98.36 ±1.22	1.24
	100%	1	1.0	1.98± 0.001	0.98±0.001	98.62± 0.23	0.23
	120%	1	1.2	2.19 ±0.01	1.19± 0.01	101.58 ±0.38	0.38
Cel.	80%	20	16	39.07± 0.01	16.07± 0.01	100.45 ±0.06	0.06
	100%	20	20	40.17± 0.21	20.58 ±0.21	100.87± 1.05	1.04
	120%	20	24	43.92± 0.03	23.92 ± 0.03	101.58± 0.13	0.13

v. Precision

a. Repeatability- Reapetability studies on RP-HPLC method for amlodipine & celecoxib were analysed and The %RSD was less than 2%, i.e. 0.02, 0.004,respectively , hence repeatability concluded.

drug	Concentration (mg/ml)	Mean area	SD	%RSD
Amlodipine	3	350.42	0.08	0.02
Celecoxib	60	4747.23	0.23	0.004

b. Intraday and Inter day Precision

Precision was performed by injecting three replicates of each injection of working standard solution and %RSD was less than 2 in interday and intraday precision so the proposed method is more precise

Drug	Conc. (mg/ml)	Intraday Precision			Interday Precision		
		Mean	SD	%RSD	Mean	SD	%RSD
Amlo	2	237.61	0.96	0.89	232.26	0.96	0.41
	3	351.38	0.86	0.24	349.52	0.42	0.12
	4	453.39	1.59	0.35	452.07	1.48	0.33
Cele.	40	3080.11	1.09	0.04	3053.23	0.39	0.01
	60	4752.35	12.84	0.27	4742.81	13.49	0.28
	80	5964.93	2.40	0.04	5965.37	0.62	0.01

vi. Robustness Study of Milbemycine and Praziquintel-

The changes were did flow rate (±1 ml/ min^{-1}), mobile phase composition (±1 ml/ min^{-1}),and Wavelength (±1 ml/ min^{-1}) .%RSD for peak area was calculated which should be less than 2%.hence analytical method that concluded

Parameters	Amlodipine				Celecoxib			
	Conc. (µg/ml)	area (mean)	SD	%RSD	Conc. (µg/ml)	area (mean)	SD	% RSD
Flow rate change 0.9ml	4	508.92	1.86	0.37	80	6684.41	4.02	0.06
Flow rate change 1.1 ml	4	415.90	1.34	0.32	80	5454.55	7.69	0.14
Mobile phase comp change 71 MeOH+29 water	4	460.7	1.08	0.23	80	6005.5	6.92	0.12
Mobile phase comp change 69 MEOH+ 31 water	4	455.28	3.10	0.68	80	6006.75	5.11	0.08
wavelength change 236nm	4	485.5	1.99	0.41	80	5727.4	4.45	0.08
wavelength change 234nm	4	428.25	1.61	0.38	80	6387.73	7.30	0.11

F. Analysis of marketed formulation

Brand name – CONSENS

std .amlodipine 10 mg and celecoxib 200 mg in 100 ml MEOH = 100 µg/ml amlodipine & 2000 µg/ml celecxib----------stock-1

total 20 tab weight= 12.2 gms

avrage powder weight = 0.610 gms

eq.weight 200 mg= 200 x avg.wt./label claim

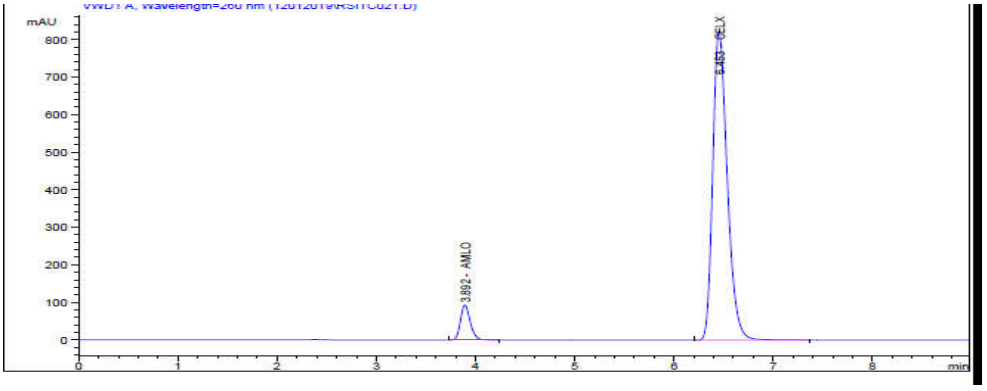
= 200 x 610/200=610 mg

610 mg in 10 ml water sonicate 10 min

take 610 mg tablet powder wt. in 100 ml volumetric flask= 100 µg/ml amlodipine & 2000 µg/ml celecxib ---------- stock-II

tab assay :-

take 5+100 µg/ml for assay (0.5ml from stock II and makeup volume 10ml with mobile phase)



Drug	Conc.	Amt. found	%label Claim	SD	%RSD
Amlo	5	4.98	99.77	0.04	0.04
Cel.	100	99.26	99.26	0.08	0.09

Analysis of marketed formulation % Lable Claim was found to be 99.77 & 99.26 Satisfactory are concluded

G. Forced degradation studies The % drug recovery was calculated based on how much degradation of the standard drug occurred after degradation. It was determined using the peak area of standard drug and the drug after degradation. The generally recommended degradation varies between 2-20% degradation. Very mild degradation was observed during Acid, Base, Hydrogen peroxide & Thermal degradation for Milbemycine and praziquentel.

Stress conditions	Amlodipine		Celecoxib	
	Area of sample	(%) Degradation	Area of sample	(%) Degradation
Acidic hydrolysis	235.20	8.4	3100.04	8.5
Alkaline hydrolysis	240.32	6.4	3180.46	6.1
Peroxide Degradation (H_2O_2)	238.34	7.2	3211.24	5.2
Thermal Degradation (6Hr)	244.27	4.93	3290.48	2.8

4. Conclusion

Straightforward, fast, rapid, precise and exact RP-HPLC techniques are created and approved for the normal investigation of common in API and tablet measurement structures. Strategies are reasonable for the concurrent assurance of those API in multi -part definitions without obstruction of each other. Techniques are sensible for the simultaneous confirmation of those API in various part planning denied of the check of each another. The made techniques are proposed for standard and inside quality control assessment of the investigated calm s in two-section remedial courses of action. the amount saw after extended methodologies has been adequate simultaneousness through the label claim of planning. Likewise the value of standard deviation and coefficient of variety determined were agreeably low, sh owing the reasonableness of the proposed techniques for the normal assessment of tablet measurements structures. Study is to choose and deliver securing reli ability data for drug through constrained corruption thinks under ICH rules and make get stability indicating assay.

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