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ESTIMATION OF AMLODIPINE BESYLATE AND CELECOXIB IN COMBINED DOSAGE FORM BY RP-HPLC

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ABSTRACT

A simple, precise, accurate, and rapid reverse phase-high performance liquid chromatography (RP-HPLC) based analytical method with UV detection has been developed for the quantification of amlodipine besylate (AML) and celecoxib (CEL) in their fixed dose formulation. The analysis was performed on Thermosil C_{18} analytical column (250 × 4.6 mm, i.d., 5 µm). The mobile phase consisted of a mixture of 70 volumes of methanol and 30 volumes of 0.1 % ortho-phosphoric acid run in isocratic mode at a flow rate of 1 mL/min. Detection of analytes was carried at 252 nm and with linearity obtained at concentration ranges of 3-18 µg/ml and 75-450 µg/ml for AML and CEL respectively. The retention time of AML and CEL were 2.582 and 3.407 min respectively. The recoveries obtained were 99.46–101.36 % for AML, and 99.96–100.87 % for CEL. The analytical method validation was done in accordance with the guidelines of international conference of harmonization for the parameters with accuracy, precision, specificity, robustness, limits of detection and quantitation. The developed HPLC method was successfully applied in the analysis of commercial available dosage forms containing AML and CEL.

Keywords : Amlodipine besylate, Celecoxib, RP-HPLC, Tablet dosage form

INTRODUCTION

Amlodipine Besylate (AML) a synthetic dihydropyridine and chemically, 3-ethyl 5-methyl 2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-carboxylate-benzene sulfonic acid has anti-hypertensive and anti-anginal effects **[1]**. AML prevents the entry of calcium ions (extracellular) into cardiac and vascular smooth muscle cells (peripheral), leads to decrease in the contraction vascular and myocardial cells. This consequence in dilatation of coronary and systemic blood vessels, lowers cardiac tissue contraction, increased blood and oxygen supply to the cardiac tissue, and decreased total peripheral resistance. Through inhibition of the an ATP dependent p-glycoprotein efflux pump AML modulate multi-drug resistance (MDR) activity. Celecoxib (CEL) a nonsteroidal anti-inflammatory drug is chemically 4-[5-(4-Methylphenyl) -3-(trifluoromethyl) pyrazol-1-yl] benzenesulfonamide has selective inhibitory action on cyclo-oxygenase-2 (COX-2) results in cell death **[2-4]**. Both the selected drugs are official in IP **[5]** and BP **[6]**. The hypertension associated with osteoarthritis (OA) is another cause of disability in elderly peoples. Now a days rising number of cases were seen for OA in elderly and obese patients and many studies reported that hypertension is a key factor for the occurrence of knee OA. To control hypertention associated with OA a fixed-dose combination of amlodipine and celecoxib in oral dosage form has been approved . AML **(Figure 1**) a long acting calcium-channel blocker used to control hypertension and angina. Celecoxib (CEL; **Figure 2**) is a selective COX-2 inhibitor used for the management of inflammatory and pain problems related with OA. When compared to other available nonsteroidal anti-inflammatory drugs (NSAIDs), CEL is preferred due to its harmless effects on the gastrointestinal tract and kidney. When compared to other NSAIDs is has less effect on hypertention. Literature survey reveals various analytical methods reported for the estimation AML either alone or in combination with other drugs in API, pharmaceutical formulations and biological fluids includes spectrophotometric **[7-8]** and HPLC **[9-12]**. As per literature survey for CEL the analytical methods reported for its estimation in pharmaceutical formulation either alone or in combination with other drugs which includes spectrophometric **[13]** and HPLC **[14-16]**. For the estimation of AML and CEL in their dosage forms in

combined dosage form according to the literature reports few UV[17-20]and HPLC **[21-23]** methods methods reported. Obviously, HPLC methods are the method of choice when compared to spectro-photometric methods in simultaneous quantification of drugs. But the reported HPLC methods are facing with the drawbacks of long retention times for drugs and use of complex nature mobile phases. The long retention time in the chromatographic analysis consumes a lot of mobile phase. So, there is need of availability of HPLC method , where there is less retention time with the utilization of simple mobile phase composition.

Based on the above need, we proposed to develop a simple, rapid, precise, and accurate RP-HPLC method for the simultaneous quantification AML and CEL in the available fixed dose combination.

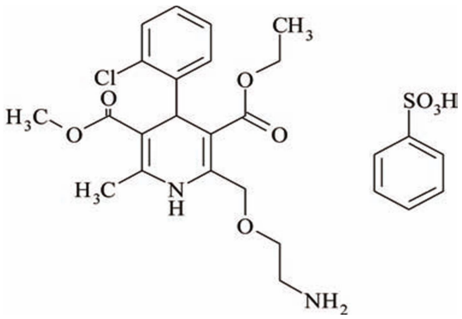

Figure 1: Structure of AML

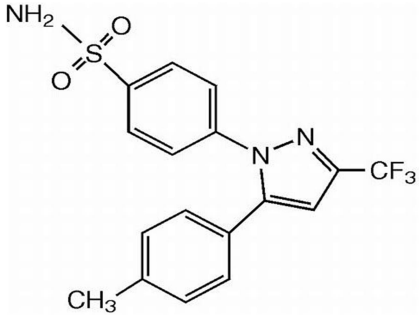

Figure 2: Structure of CEL

MATERIALS AND METHODS

Materials and Reagents

Amlodipine Besylate and Celecoxib API were obtained as a gift sample from RA Chem Ltd, Hyderabad Pvt. Ltd (India). The commercially available tablet formulation "CONSENSI" containing Amlodipine Besylate (5 mg) and Celecoxib (200 mg) were procured from local market. The prepared solutions were well protected from the effect of light and were analyzed on the day of preparations. The glasswares utilized in the methodology were through cleaned with a mixture of chromic acid and sulfuric acid , rinsed thoroughly with double distilled water and dried in hot air oven. The reagents used were of analytical-reagent (AR) grade. Millipore water, HPLC grade methanol, acetonitrile was procured from Merck, India.

Instrumentation

The chromatographic analysis was carried out on a reversed-phase column based high performance liquid chromatography method (Waters Separation module 2695) equipped with a variable wavelength programmable UV detector 2487. The sample injection

system consists of rheodyne injector fitted with a 20 µL loop was and data were recorded and analysed using empower software version 2.0. Weighing was carried out on Digital balance (SHIMADZU AUX 220). Ultra sonicator (Citizen ultra sonicator) was used for sonicating the drug and sample solution.

Chromatographic Conditions

The separation of analytes was carried out on the Thermosil C18 column (250 x 4. 6 mm ID, 5 µm particle) using the mobile phase composition of methanol : 0.1 % ortho-phosphoric acid in the ratio of 70 : 30 v/v at a flow rate 1.0 ml/min. The injection volume was 20 µL and detection of eluents done at 252 nm. The chromatographic analysis carried at a room temperature of 30°C.

Preparation of Stock and Standard Solutions

A quantity of 25 mg each of AML and CEL was weighed transferred to a 25 ml calibrated flask; 15 ml of methanol is added, and sonication done for 15 min to make clear solution. The volume is made up to the volume with methanol to obtain a stock solution of 1000 µg/ml of each AML and CEL. From the above prepared solution, 2.5 ml was pipette out and transferred to a 25 ml calibrated flasks, and the volume is made up to the volume with mobile phase to obtain working standard solutions of 100 µg/ml of each AML and CEL.

Preparation of Sample Solution

The assay for the content of AML and CEL in commercial tablets was performed with the developed chromatographies condition and results have accuracy and reliability. Twenty tablets each tablet claim to contain (5 mg AML/200 mg CEL) were weighed; their mean weight was determined and triturated to powder form. Tablet powder equivalent to 10 mg AML containing relevant quantities of CEL was weighed and transferred to a 100 ml calibrated flask containing little quantity of methanol , sonicated for 15 mins and volume was made up to mark with methanol. From the above solution 0.9 ml was transferred into a 10 ml calibrated flask and volume was made up to volume with mobile phase, and final solution (9 µg AML, 360 µg CEL/ml) was filtered through 0.45 µ millipore filter and it was analyzed by HPLC system.

Method Validation

With the optimized chromatographic conditions the method was validated as per the guidelines framed by ICH[12]. The validation was done for the parameter system suitability test, stability, specificity, linearity, accuracy, precision and robustness.

RESULTS

Optimization of chromatographic condition

It was observed from the UV spectra of AML and CEL that have a considerable absorbance seen at 252 nm which was selected as analytical wavelength. Various combinations with different compositions of acetonitrile, methanol and water as mobile phase were tried. Preliminary experiments were carried out with different combinations of acetonitrile or methanol with 0.1 % ortho-phosphoric acid, to resolve AML and CEL and to obtain suitable retention times and peak symmetry. Finally, a mobile phase consisting of methanol and 0.1 % ortho-phosphoric acid in the ratio of 70 : 30,v/v and Thermosil C18 column (250 x 4.6 mm ; 5 µm) were selected to achieve acceptable system suitability parameters. Flow rates were tried in between 0.5 and 1.2 ml/min. A flow rate of 1.0 ml/min was proved to be better as with both drugs have retention time less than 10 min. The optimized chromatogram **Figure 3**. The retention time of AML and CEL were found to be 2.566 and 3.417 min respectively.

Method Validation

System Suitability Test

From the optimized chromatographic conditions, system suitability parameters for the developed method were determined and compared with recommended limits. System suitability parameters of the method were demonstrated in **Table 1**. According to the results, all of the system suitability parameters were within the recommended limits and the method was found to be suitable for the analysis.

Linearity

The calibration curve for AML and CEL were found to be in concentration range of 3-18 µg/ml and 75-450 µg/ml respectively. The data for AML and CEL was shown in **Table 2**. The calibration plot of AML and CEL were shown in Figure 4. Linear regression data from the calibration plot indicated a good linear response over the concentration range of both drugs indicates the applicability of it in the determination of AML and CEL in pharmaceutical formulation.

Sensitivity

The evaluation of the sensitivity of the method was based on the detection limits (LOD) and quantitation limits (LOQ) values. The values of LOD and LOQ for AML and CEL are presented in **Table 2**. The low values of detection limits (LOD) and quantitation limits (LOQ) proves the sensitivity of the method.

Precision

The precison of the method was evaluated from intra-day and inter-day precision results. The % RSD was computed for intra-day and inter-day precision studies from three different concentrations (6, 9 and 12 µg/ml for AML and 150, 300 and 450 µg/ml for CEL) within linearity range of the calibration curve. The % RSD values for intra-day and inter-day precision were < 2 %, indicated that the method was sufficiently precise, as shown in **Table 3**.

Accuracy

Accuracy studies were performed by recovery values employing the standard addition method. Standard drugs in the range of 80, 100 and 120 % of the sample concentration were added into the sample solution. Each concentration was analyzed in triplicate. Results of recovery studies were found to be in between 98 to 102 % for both AML and CEL, as shown in **Table 4**.

Robustness

Robustness of the method was assed by deliberately giving small variations in method parameters to study whether the response is influenced by variations made. The method parameters investigated in this study are changes in the flow rate (± 0.2 ml/min), the organic composition of the mobile phase (± 5 % v/v) and analytical wavelength (± 2 nm**).** The recovery values of CEL and AML were calculated and evaluated. The variations in flow rate , change in composition of the mobile phase and analytical wavelength does made a significant effect on the analyte response indicates the robustness of the method The robustness data was shown in **Table 5**.

Specificity

For analytical methods specificity means the ability of the method to accurately and specifically determine the analyte of interest without interference from blank or placebo. The peak purities of AML and CEL were assessed by comparing the retention times of standard and the sample of AML and CEL, and there obtained a good agreement between the retention time of the standard and sample of AML and CEL. Placebo and blank were injected and there were no peaks found at the retention time of AML and CEL indicates the specificity of the method.

Analysis of commercial formulation

For the quantification of AML and CEL in marketed formulations(CONSENSI TABLETS) the developed method was applied. The percentage recovery **(Table 6)** was found to be 100.08 ± 0.18 and 100.25±0.45 for AML and CEL respectively.

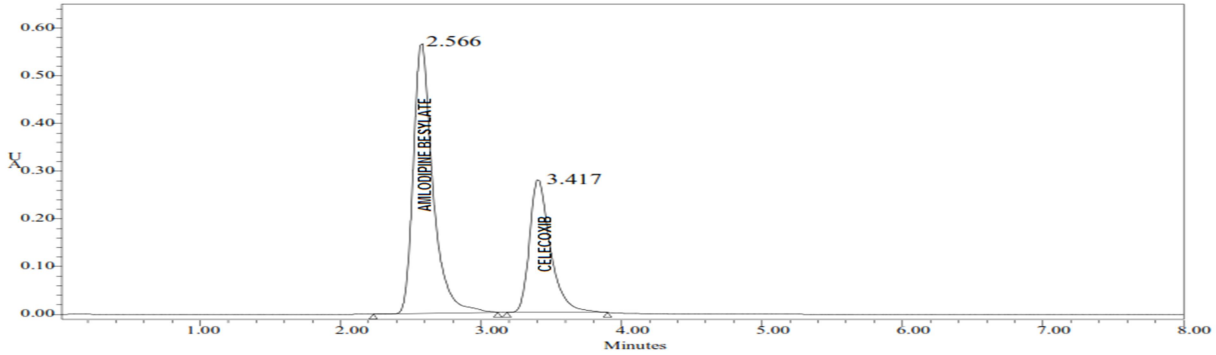

Figure 3: Chromatogram of standard solution AML and CEL from binary mixture

Table 1: Results of system suitability test (n = 6)

Parameter	Criteria	AML	CEL
Capacity factor(k')	$k' > 2$	4.566	5.321
Tailing factor (T)	T < 2	1.34	1.21
Theoretical plates (N)	N> 2000	8248	6481
Resolution (Rs)	$Rs > 2$	-	3.02
% RSD (peak area)	% RSD ≤ 1	0.71	0.89

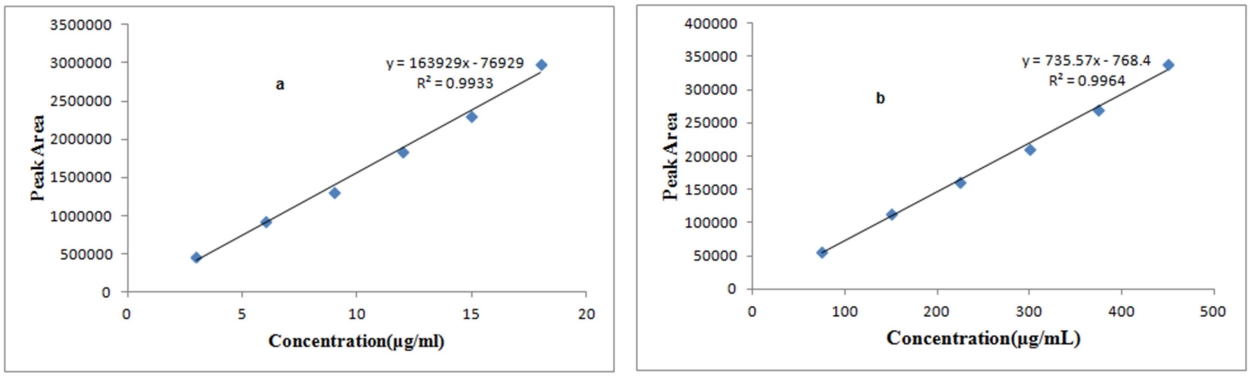

Figure 4: Calibration curve for Amlodipine Besylate(a) and Celecoxib(b)

Table 2: Spectral and statistical data for determination of Amlodipine Besylate and Celecoxib by proposed RP-HPLC method

Analyte			
AML		CEL	
Detection wavelength (nm)	252	Detection wavelength (nm)	252
Linearity range (µg/ml)	3-18	Linearity range (µg/ml)	75-450
Coefficient of determination (r^2)	0.9933	Coefficient of determination (r^2)	0.9964
Regression equation (Y^a)	Y= 163929x – 76929	Regression equation (Y^a)	Y= 735.57 x- 768.4
Limit of detection, LOD (µg/ml)	0.09	Limit of detection, LOD (µg/ml)	0.24
Limit of quantitation, LOQ (µg/ml)	0.29	Limit of quantitation, LOQ (µg/ml)	0.75

[a] $Y = mx + c$, where x is the concentration (µg/ml)

Table 3: Precision Studies

Drug	Amount(µg/mL	Intra-day(n=3)		Inter-day(n=3)	
		Amount found Mean±SD	%RSD	Amount found Mean±SD	%RSD
AML	6	5.98± 0.074	1.23	6.06 ± 0.084	1.40
	9	9.02 ± 0.082	0.89	9.09 ± 0.148	1.63
	12	11.98 ± 0.119	0.94	11.94 ± 0.155	1.14
CEL	150	149.12 ± 0.58	0.39	149.74 ± 1.45	0.97
	300	299.86 ± 3.381	1.13	300.79 ± 2.19	0.73
	450	449.77 ± 6.497	1.30	449.63 ± 3.82	0.85

Table 4: Recovery studies (n = 3)

Recovery level	AML			CEL		
	Amount Added (µg/ml)	Amount Recovered (µg/ml)	% Recovery	Amount Added (µg/ml)	Amount Recovered (µg/ml)	% Recovery
80 %	7.2	7.17	99.62	288	288.63	100.22
100 %	9	8.98	99.80	360	358.12	99.48
120 %	10.8	10.76	99.66	432	430.92	99.75

Table 5: Chromatographic parameter setting applied in the robustness investigation

Parameter	Modification	% Recovery		% RSD	
		AML	CEL	AML	CEL
Flow Rate(ml/min)	0.9	100.20	99.64	0.67	0.88
	1.0	99.92	99.38	1.21	0.94
	1.2	99.45	99.82	0.82	0.55
Mobile Phase (Methanol:0.1% orthophosphoric acid)	65:35 v/v	99.74	99.34	0.84	0.71
	70:30 v/v	99.49	99.66	0.91	0.86
	75:25 v/v	99.68	99.49	0.72	0.75
Wavelength(nm)	250	99.84	99.63	0.87	1.32
	252	99.92	99.71	1.25	1.01
	254	99.89	99.85	1.41	0.46

Table 6: Analysis of Amlodipine Besylate and Celecoxib in commercial formulation

Formulation	Labelled claim(mg)		Amount found*(mg)		%Recovery*±%RSD	
	AML	CEL	AML	CEL	AML	CEL
CONSENSI TABLETS	5	200	5.04	200.50	100.08 ± 0.18	100.25 ±0.45

*Average of three determinations

DISCUSSION

The present work is based on RP-HPLC technique with UV detection was developed and validated for quantification of amlodipine besylate and celecoxib in the fixed dose combination. The analytical parameters chosen was based on chemical and physical properties amlodipine besylate and celecoxib. The stationary phase selection based on the system suitability parameter vaues of amlodipine besylate and celecoxib. Thermosil C18 column (250 x 4.6 mm i.d, 5µ) was selected for separation of analyte based on evaluation parameters. Mobile phase optimization was done from the preliminary trials carried out w mobile phases composed of mixture of solvents like water, methanol and actonitrile and with 0.1% orthophosphoric acid in water in different combinations under isocratic

conditions. A mixture of methanol and 0.1 % orthophosphoric acid in the ratio of 70:30 % v/v was found to be ideal combination based on satisfactory system suitability parameters. The flow rate of mobile phase was optimized based on resolution between chromatographic peaks and minimal solvent consumption. The flow rate of mobile phase varied from 0.5-2 mL/min. It was found from trials that 1 mL/min flow rate was ideal for successful elution of both drugs. For selection of analytical wavelength standard solutions of both drugs were scanned in wavelength range of 200-350 nm. A detection wavelength of 252 nm was selected. The developed method was validated in accordance with the guidelines of ICH guidelines was found to be simple, specific and reliable. Moreover the RP-HPLC method was successfully applied for the quantification of amlodipine besylate and celecoxib fixed dosage pharmaceutical formulations without any interference from the excipient.

CONCLUSION

The proposed method was validated as per the guidelines framed by ICH and was successfully applied for the quantification of AML and CEL in the fixed dose formulation and it is simple, specific and economical, without any interference from the excipient.

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