UV spectroscopic method for simultaneous estimation of Celecoxib and Amlodipine

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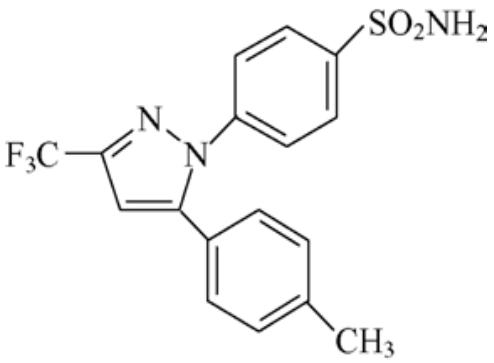
Abstract

A simple, precise and accurate First order derivative UV spectroscopic method has been developed for estimation of Celecoxib (CEL) and Amodipine (AML) simultaneously. The linearity was established over the concentration range of 15–40 µg/mL and 3–8 µg/mL for CEL and AML respectively. The mean % recoveries were found to be 99.78%for CEL and 100.36% for AML. The proposed method was validated as per ICH guidelines and successfully applied for assay of CEL and AML in their synthetic mixture.

Keywords: *Celecoxib, Amlodipine, UV-spectrophotometry*

INTRODUCTION

Celecoxib (CEL) is selective COX-2 inhibitor. CEL is chemically, 4-[5-(4-Methylphenyl)-3-(trifluoromethyl) pyrazol-1-yl] benzene sulphonamide (Fig. 1).



Celecoxib

Fig 1: Structure of CEL

Celecoxib is believed to be prostaglandin synthesis inhibitor. Most NSAIDs inhibit both types of cyclooxygenases (COX-1 and COX-2), celecoxib is a selective non-competitive inhibitor of cyclooxygenase-2 (COX-2) enzyme. CEL applied as anti-inflammatory, analgesic and antipyretic actions with low ulcerogenic potential so indicated as to relieve the signs and symptoms of Rheumatoid arthritis (RA) and Osteoarthritis (OA) [1-3].

Amlodipine besylate is chemically 3-Ethyl 5-methyl (4RS)-2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate benzene sulphonate (Fig. 2). Amlodipine reduces contractility of arterial smooth muscle and following vasoconstriction by inhibiting calcium ions influx through L-type calcium channels. The overall decrease in blood pressure is due to vasodilatory effects of amlodipine. Amlodipine, long-acting CCB commonly used for management of hypertension1 and coronary artery disease [1-3].

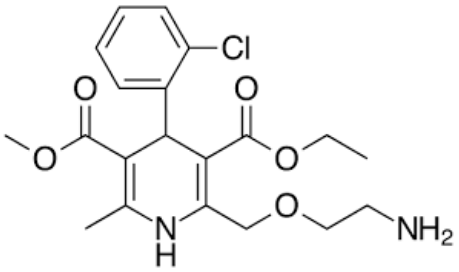


Fig 2: Structure of AML

KIT-302 (CEL and AML) is a NSAID and Calcium channel blocker drug combination in development for both, the treatment of hypertension and pain associated with osteoarthritis [4].

Several analytical methods for quantifying CEL has been reported; such as UV-spectrophotometry [6,7], HPLC [8-10] and LC-MS/MS [11]. Several analytical methods for quantifying AML have been reported; such as UV-spectrophotometry [12,13], HPLC [14-16], UPLC [17], HPTLC [18,19] and LC-MS/MS [20]. From the literature survey it revealed that none of the methods were reported for simultaneous estimation of CEL and AML.

EXPERIMENTAL

A. Chemicals and Reagents

Analytically pure CEL and AML was procured as gratis samples from Prudence Pharma Chem. Ankleshwar, Gujarat, India. Tablet of CEL and AML were prepared synthetically in lab.

B. Instruments

A LAB-INDIA 3600^{+} double beam spectrophotometer with wavelength accuracy 0.5 nm, 1cm matched quartz cells and UV-Win 5 software was used. Calibrated analytical balance Shimadzu was used for weighing purpose. All statistical calculations were carried out using MS-Excel-2010 analytical tool.

C. Preparation of Sample and Solutions

1) Preparation of Tablet

Immediate release tablets of total weight 350 mg each, containing 200 mg of CEL and 10 mg of AML were prepared.

2) Preparation of Standard Primary Stock Solutions

Accurately weighed 100 mg of CEL and AML standard was transferred to a separate 100 mL volumetric flask and dissolved in 25 mL of Methanol. The flasks were shaken and volume was made up to the mark with Methanol having strength of 1000 µg/mL CEL and 1000 µg/mL AML.

3) Preparation of Standard Secondary Stock Solutions

Appropriate volume of stock solution was withdrawn from primary standard stock solution of CEL and AML to produce secondary stock solution having strength of 500 µg/mL and 100 µg/mL of CEL and AML respectively.

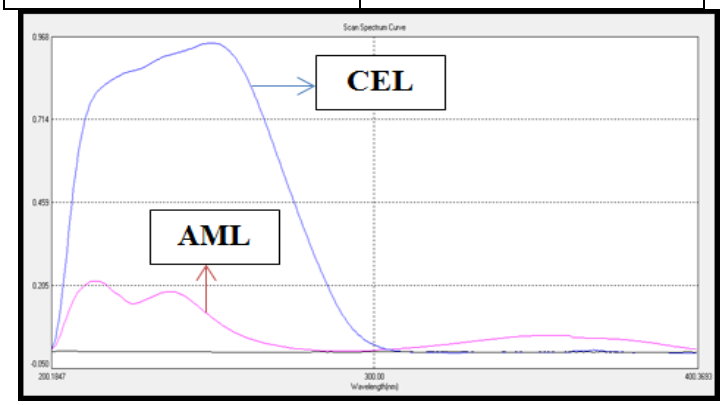
D. Selection of Analytical Wavelength

Appropriate volume of aliquot from CEL and AML secondary standard stock solution was transferred to volumetric flask of 10mL capacity. The volume was adjusted to the mark with Methanol to give working standard solutions containing 25μg/mL of CEL and 5 μg/mL of AML respectively. The spectrum was recorded between 200-400 nm and all the zero-order spectrum (D^0) (Fig 3a) were converted to first derivative spectrum (D^1) using co-efficient value 100 and no. of points 21. The overlain 1^{st} derivative spectrums of CEL and AML was recorded. The zero-crossing point (ZCP) of CEL was found to be 250 nm and ZCP of AML was found to be 290 nm (Fig 3b).

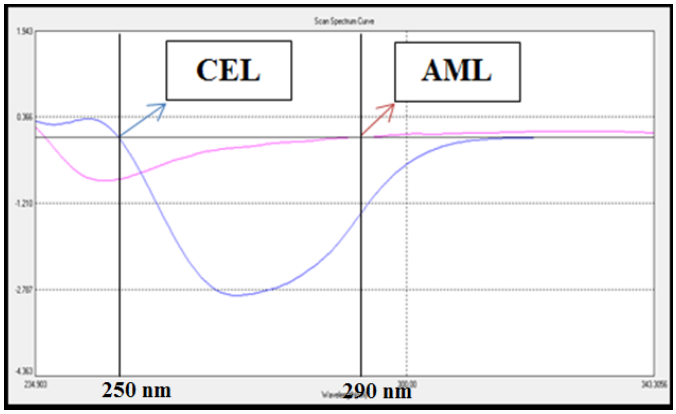
Similarly, all test concentration of CEL (15-40 µg/mL) and AML (3-8 µg/mL) were prepared and scanned in the range of 200-400 nm and converted to D^1 spectra. The optimized condition for the method validation was mentioned in Table 1.

Table 1: Optimised conditions

Parameters	Optimized conditions
Solvent	Methanol
Slit width	0.5 nm
No. of points	21
Co-efficient value	100
Range for CEL	15-40 µg/mL
Range of AML	3-8 µg/mL
ZCP of CEL	250 nm
ZCP of AML	290 nm



(a)



(b)

Fig 3: (a) Overlain D0 spectra of CEL (25 µg/mL) and AML (5 µg/mL) (b) Overlain D1 spectra CEL (25 µg/mL) and AML (5 µg/mL)

RESULTS AND DISCUSSION

A. Method Validation [5]

The proposed method was validated in terms terms of linearity, accuracy, precision, limits of detection (LOD) and quantification (LOQ). The accuracy was expressed in terms of percent recovery of the known amount of the standard drugs added to the known amount of the synthetic formulation. The precision (% relative standard deviation—% RSD) was expressed with respect to the repeatability, intraday, and interday variation in the expected drug concentrations. After validation, the developed methods have been applied to synthetic dosage form.

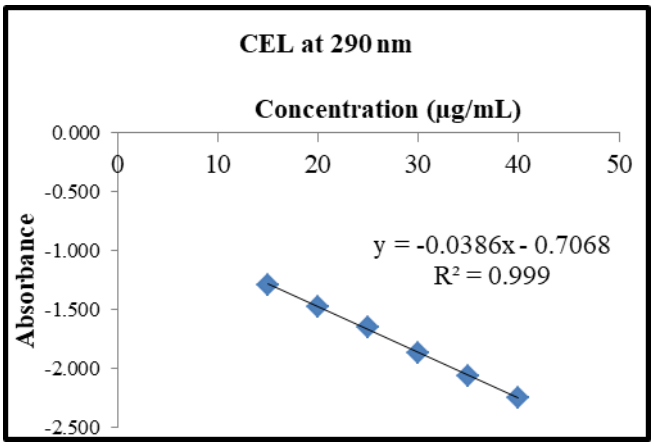
1) Linearity

CEL and AML showed linearity in the range of 15-40 µg/mL and 3-8 µg/mL, respectively. Linear regression equation and correlation coefficient (R^2) are: Y_{CEL}= -0.0386x – 0.7068 (R^2 = 0.9992) and Y_{AML} = -0.1467x – 0.0316 (R^2= 0.9991) (Table 2, Figure 4 and 5).

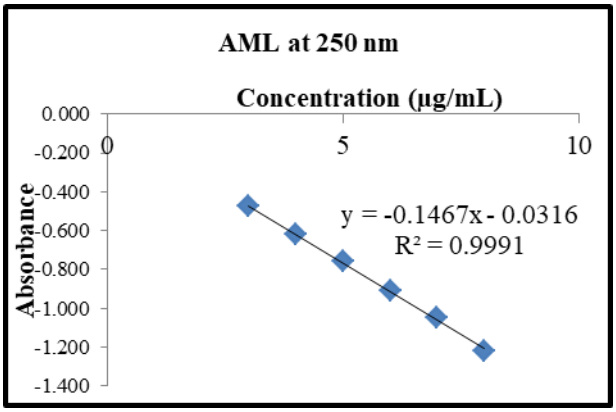
Table 2: Statistical data of CEL and AML

Parameters	CEL	AML
Linear range	15-40 µg/mL	3-8 µg/mL
Slope	0.0386	0.1467
Intercept	0.7068	0.0316
SD of	0.00273	0.00697

intercept		
Regression co-efficient (R^2)	0.9992	0.9991

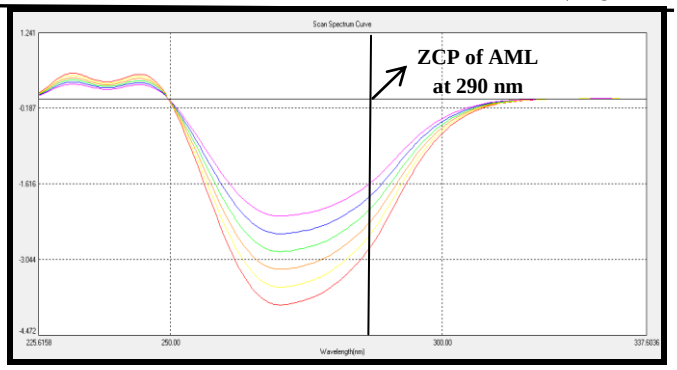


(a)

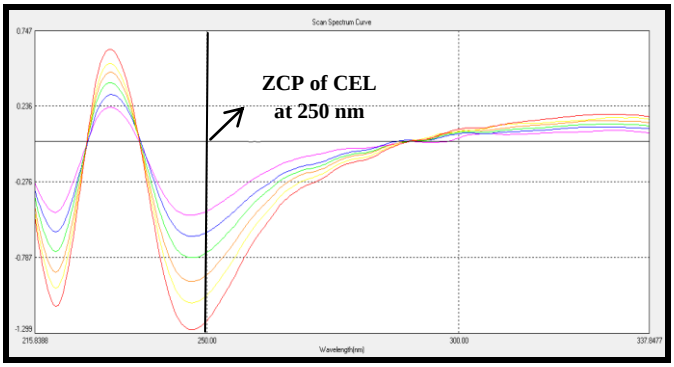


(b)

Fig4: Calibration curve of (a) CEL at 290 nm and (b) AML at 250 nm



(a)



(b)

Fig 5: Overlain D1 spectra of (a) CEL (15-40 µg/mL) and (b) AML (3-8 µg/mL) in Methanol

2) Precision

The precision of the method was checked by Repeatability and Intermediate precision (Intraday and Interday). The Relative Standard Deviations (R.S.D.) for CEL and AML were found to be within the acceptable limit i.e. 2%. (Table 3,4 and 5)

Table 3: Repeatability of CEL and AML

Concentration (µg/mL)		Mean (ABS) ± SD (n=6)		% RSD	
CEL	AML **ZCP of CEL at 250 nm**	CEL	AML	CEL	AML
30	6	-1.870 ± 0.0075	-0.909 ± 0.0018	0.40	0.20

Table 4: Intraday Precision of CEL and AML

Drug	Conc. (µg/mL)	Mean (ABS) ± SD (n=3)	% RSD
CEL	15	-1.293 ± 0.0076	0.59
	30	-1.872 ± 0.0091	0.48
	40	-2.246 ± 0.0074	0.33
AML	3	-0.478 ± 0.0040	0.84
	6	-0.908 ± 0.0066	0.72
	8	-1.218 ± 0.0025	0.21

Table 5: Interday Precision of CEL and AML

Drug	Conc. (µg/mL)	Mean (ABS) ± SD (n=3)	% RSD
CEL	15	-1.293 ± 0.013	1.00
	30	-1.869 ± 0.008	0.43
	40	-2.248 ± 0.007	0.31
AML	3	-0.476 ± 0.005	1.05
	6	-0.901 ± 0.010	1.11
	8	-1.215 ± 0.004	0.33

3) Accuracy

Accuracy of the method is to check the closeness of the true value with the obtained result. Accuracy of the method was performed by standard addition method. The recovery study was performed by calculating the spiked concentration of standards at 80 %, 100 % and 120 % of CEL and AML to pre-analyzed mixture containing CEL and AML. The experiment was performed in triplicates. The result was evaluated in terms of % Recovery, which are well within the acceptable limit of 98-102 %. The results of the accuracy studies are summarized in Table 6.

Table 6: Recovery study of CEL and AML

Drug	Level (%)	Amt. taken (total) (µg/mL)	Amt. added (µg/mL)	Amt. recovered (µg/mL)	%Recovery ± SD
CEL	80	27	12	11.92	99.43 ± 0.76
	100	30	15	14.89	99.32 ± 0.78
	120	33	18	18.10	100.59 ± 0.84

AML	80	5.4	2.4	2.415	100.67 ± 0.57
	100	6	3	2.976	99.24 ± 0.47
	120	6.6	3.6	3.642	101.19 ± 0.56

4) Detection Limit and Quantitation Limit

In the present study, the LOD and LOQ were based on standard deviation of the response and the slope of the calibration curve and were calculated according to the 3.3σ/S and 10 σ/S criterions, respectively, where σ is the standard deviation of the y-intercepts of the regression lines and S is the slope of the calibration curve. (Table 7)

Table 7: LOD and LOQ of CEL and AML

Parameters	CEL	AML
LOD	0.686	0.156
LOQ	2.080	0.475

5) Determination of CEL and AML in their combined synthetic tablet dosage form (Assay)

20 Tablets were (Prepared in Lab scale with a Label Claim of 200 mg CEL and 10 mg AML) weighed and triturated. Powder Equivalent to 100mg CEL and 5mg AML (*i.e.* 175.5 mg) was weighed accurately and transferred to 100 mL volumetric flask. 25 mL methanol was transferred to volumetric flask and sonicated for 10 minutes. Volume was made up to mark with methanol after addition of 15 mg of standard AML powder. This solution was used as 1^0 stock solution (1000 µg/mL of CEL and 200 µg/mL of AML). Appropriate volume was pipetted out accurately from 1^0 stock solution, and was diluted up to 10 mL with Methanol, to produce 2^0 stock solution (300 µg/mL solution of CEL and 60 µg/mL solution of AML). Appropriate volume was pipetted out from above stock solution and diluted with methanol up to 10 mL to prepare test concentration (30 µg/mL solution for CEL and 6 µg/mL solution for AML). The test solution was scanned in range of 200-400 nm to obtain the zero-order spectrum. Which was later on transformed to D^1 spectra and the absorbance were measured at respective wavelengths as per the developed method *i.e.* 250 nm & 290 nm for AML & CEL respectively. From the recorded absorbencies, concentrations were found out and %purity was calculated for both CEL and AML. (Table 8)

Table 8: Assay results of synthetic formulation

Drug	Labelled claim (mg)	Amount found per tablet (mg)	% Label Claim ± SD
CEL	200	198.58	99.29 ± 0.35
AML	10	9.93	99.33 ± 0.39

CONCLUSION

The proposed first-order derivative method was found to be simple, specific, precise, and accurate for quantitative estimation of CEL and AML simultaneously in their combined synthetic tablet dosage form. The method was validated as per ICH guidelines and the validation result substantiates that the proposed method can be useful for routine analysis and quality control assay of CEL and AML in their synthetic mixture.

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