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HPTLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF AMLODIPINE BESYLATE AND CELECOXIB IN PURE AND COMBINED DOSAGE FORM

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Keywords:

HPTLC, Amlodipine besylate, Celecoxib, Method development, Validation

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ABSTRACT: HPTLC is simple, reliable, precise and accurate for separation and identification of drug in a combined dosage form which gives satisfactory accuracy in results when applied on amlodipine besylate (Amlo) and celecoxib (Celo). Toluene, ethyl acetate, methanol and conc. ammonia (6:5:1.5:0.3 v/v/v/v) was required as a mobile phase for separation and identification of drugs. These drugs were scanned by densitometry at 310 nm. The developed method was validated for linearity, precision, accuracy, LOD, and LOQ. The linearity range and correlation coefficient for Amlo and Celo were obtained to be 100 to 600 ng/ml and 2 to 12 µg/ml, 0.9971, and 0.9989, respectively. The results of the recovery study for Amlo and Celo have obtained the range of 99.52% to 99.83% and 99.83% to 99.98%, respectively. The method precision was established by repeatability study. LOD and LOQ are 17.88µg/ml and 54.20 µg/ml for Amlo and 0.39µg/ml and 1.20µg/ml for Celo, respectively. The statistical parameters and recovery data indicated that the method may be engaged for efficient, rapid drug analysis from tablet formulation. The percentage label claim present in tablet formulation was obtained to be 100.25% and 99.93% for Amlo and Celo, respectively. The outcomes of analysis clearly specified that with no interference from excipients in the formulation.

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 an antihypertensive agent used for the treatment of hypertension [1, 2]. Celecoxib (Celo) chemically known as 4-[5-(4-methyl phenyl) –3-(trifluoromethyl) pyrazol-1–yl] benzene sulphonamide **Fig. 1B**. Celecoxib is a NSAID (Non-Steroidal Anti-inflammatory Disorder) that exhibits anti-inflammatory, analgesic, and antipyretic activities [3].

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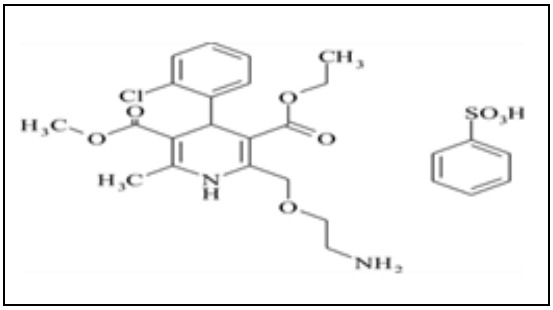

FIG. 1A: CHEMICAL STRUCTURE OF AMLODIPINE BESYLATE

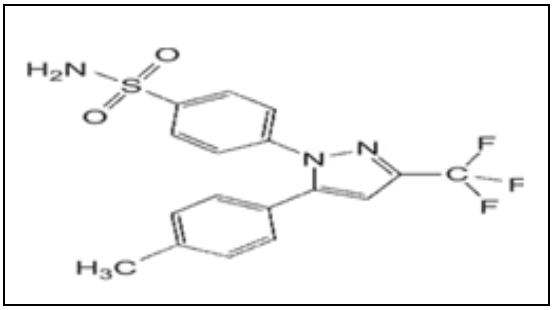

FIG. 1B: CHEMICAL STRUCTURE OF CELECOXIB

It is a purpose for preventing rheumatoid arthritis & osteoarthritis. In some cases, patients have problems, hypertension, and osteoarthritis. In that condition, they used to take both tablets and sometimes they may face the non- compliance for taking two tablets. Such conditions can be overcome in a single tablet, *i.e.*, combination, by administering both drugs. Therefore, Amlo and Celo in a combination used for both conditions. These combinations were prepared to overcome the possibility of osteoarthritis in hypertension patients and *vice versa*.

As per the literature review, for estimating Amlo using HPLC, HPTLC, and UV spectrophotometry methods have been indicated for the analysis of alone or in combination with other drugs [4-11]. Various methods have been stated that the analysis of Celo in a pharmaceutical formulation such as HPLC and UV spectrophotometry [12-14]. Therefore, on HPTLC method developed for the simultaneous estimation of Amlo and Celo. This method shows better mobile phase composition for the determination of drugs. HPTLC method is a modification of TLC for the identification and separation of drug analysis. This technique is widely used in very many fields both for the qualitative & quantitative (identification and estimation) analysis of single and mixture of substance [15].

Due to its benefits of low operating costs, elevated sample throughput, and essential for minimum sample preparation. TLC today is quickly becoming a routine analytical technique. TLC's main benefit is that multiple samples may be run simultaneously by a small amount of mobile phase, not like HPLC, thus reducing the time and cost of analysis. It is an improved form of TLC. There are some developments to the basic thin-layer chromatography method to computerize the different steps, which increase the resolution and allows for more precise quantitative measurements [16].

The main objective is to develop a rapid, sensitive, and accurate HPTLC method for the simultaneous estimation of amlodipine besylate and celecoxib in pure and combined dosage form.

MATERIALS AND METHODS:

Reagents and Chemical: Standard drug sample of Amlodipine besylate and Celecoxib was provided as a gift sample from Smruthi Organics Limited, Solapur, and Aarti Drugs Limited, Mumbai respectively. Toluene, ethyl acetate, methanol, conc. Ammonia (analytical grade) was procured from Research Lab (Mumbai, India).

Instrumentation: In this HPTLC method, samples were spotted in the form of bands of width 8 mm with 100 µl sample syringe on 10 cm × 10 cm aluminum plates precoated with silica gel 60 F 254 using a Spraylin Aetron sample applicator. Densitometric scanning was performed on a Camag TLC Scanner 3. The source of radiation was a deuterium lamp emitting a continuous UV spectrum between 200 and 400 nm. An evaluation was performed using peak areas with linear regression.

Experimental Work for HPTLC Method:

Selection of Common Solvent: The solubility of drugs was estimated in different solvents by using Indian pharmacopeia standards. Solubility was performed in polar to non-polar solvents. The common solvent was established to be methanol, and it was chosen as a solvent for the HPTLC method, and it was selected on account of its readily available, cost factor, and solubility for the analysis of Amlo and Celo.

Selection of Mobile Phase: Selection of the mobile phase for the further experiment of HPTLC was prepared by using precoated thin layer plates made by silica gel 60 F_{254}. As per the literature survey, a variety of mobile phases were tried for proper identification of R_f values of Amlo and Celo. From trial and error basis combination of mobile phase were selected.

Selection of Chromatographic Condition: After the selection of the mobile phase, the next important part was the selection of chromatographic conditions and selection of proper parameters for the sample application, plate development, documentation, and determination of plate parameters. The following are the parameters selected for the study of Amlo and Celo**.**

Preparation of Standard Solution: Standard drug solution (stock solution) of both drug was prepared by dissolving 10mg Amlo with sufficient to produce 10ml methanol and 200mg of Celo with sufficient 10ml methanol to produce 10ml solution

separately to obtained stock solution having 1000 µg/ml of Amlo and 20,000µg/ml of Celo. All solutions were protected from the light to avoid degradation.

Selection of Analytical Wavelength: The solution of both standard drugs was suitably dilute with methanol, to acquire the concentration of 10µg/ml of Amlo and Celo separately and they can also be scanned separately in a wavelength range of 200-400 nm against blank as methanol to estimate the wavelength of maximum absorption for the drugs. From the overlain spectrum, 310 nm of wavelength was selected for Amlo & Celo detection by densitometry for measured peak area.

Preparation of Calibration Curve: For the calibration curve, dilution of 1, 2, 3, 4, 5, and 6µl of a standard solution of Amlo and Celo were applied to the TLC plate. The range of concentration Amlo was between100 to 600 ng/spot, whereas Celo has a range between 2 to 12 µg/spot. TLC plates were developed, dried and examined photometrically. The calibration curve was plotted between concentration *vs*. respective area for Amlo and Celo separately.

Analysis of Sample Preparation: A total of 20 tablets were correctly weighed and grinding by using a glass mortar pestle. A quantity equivalent to one tablet (containing 10 mg Amlo and 200 mg of Celo) was transferred to 10 ml flask of volumetric. The powder was firstly dissolved in few ml of methanol, and the flask were sonicated for 5 min, the volume was up to 10 ml and then filtered through Whatman filter paper, the resultant clear solution was diluted with methanol to get the concentration of 1000 µg/ml of amlodipine besylate and 20,000 µg/ml of celecoxib. The concentration of Amlo and Celo per tablet was estimated by plotting the value of the area from the calibration curve.

Method Validation: The method was validated as per the guidelines of ICH Q2 (R1), in terms of parameters like linearity, accuracy, precision, and specificity of the sample application. All the results were discussed in the result and discussion.

Linearity: The linearity of the method was investigated by serial dilution of standard solution. Six different concentrations of the standard drugs of Amlo and Celo were prepared for linearity studies and injected into the system. The response was measured as peak areas. The linearity was calculated for two drugs, amlodipine besylate, and celecoxib, separately by plotting a calibration curve for peak area against their respective concentration. From the calibration curve, it was clear that Amlo has better linearity between 100 to 600 ng/spot, whereas Celo has a range between 2 to 12 µg/spot.

Precision: The precision of the analytical method is the degree of arrangement among the individual test results when the method is useful for repeated the multiple aliquots of the same samples. The method precision was tested by determining the percentage relative standard deviation (%RSD) for three determination of peak areas of Amlo (500 ng per band) and Celo (10 µg per band).

Accuracy: In order to ensure the accuracy of the proposed method, recovery studies were performed. The recovery studies were carried out by standard addition method at the level of 80%, 100% & 120%. Known amounts of standard solutions of Amlo (400, 500 & 600 ng/band) and Celo (8, 10 & 12 µg/band) were added to pre-analyzed sample solutions of Amlo (500 ng/band) and Celo (10 µg/band). The concentration of Amlo and Celo was determined by applying obtained values to the regression equation of the calibration curve.

Limit of Detection: It is the lowest concentration of an analyte in a sample that may be detected but not essentially quantitated under the indicated experimental conditions. It can be calculated by using the following equation,

$$LOD = 3.3 \times (\sigma/S)$$

Where, σ = response of Standard deviation, S = calibration curve slope

Limit of Quantitation: It is the lowest concentration of an analyte in a sample that may be quantitated with acceptable precision and accuracy under indicated experimental conditions. It can be determined by using the following equation,

$$LOQ = 10 \times (\sigma /S)$$

Where, σ = response of Standard deviation, S = calibration curve slope.

RESULTS AND DISCUSSION:

Selection of Detection Wavelength: After chromatogram development, bands was scanned at a range of 200-400 nm. It was observed that both the drugs show considerable absorbance at 310 nm. So, 310 nm was selected as the wavelength for detection **Fig. 2**.

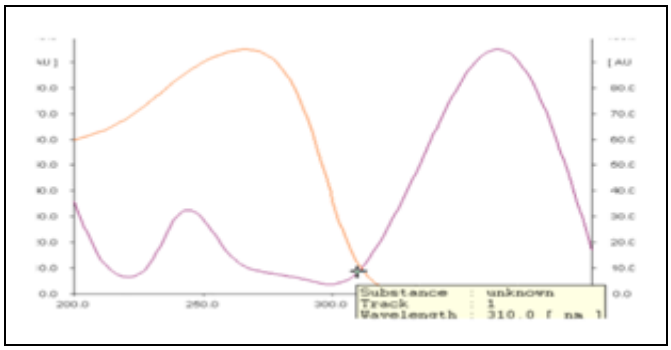

FIG. 2: OVERLAY SPECTRUM OF AMLO AND CELO

TABLE 1: MACHINE PARAMETERS USED FOR HPTLC METHOD

S. no.	Parameters	Chromatographic condition	
		Amlodipine Besylate	Celecoxib
1	Syringe size (µ/l)	100	100
2	Syringe volume (µ/l)	80	80
3	Pre dosage volume (µ/l)	2	2
4	Dead volume (µ/l)	5	5
5	Dosage speed (sec/µl)	5	5

As per trial and error basis, mobile phases were selected in different ratios but only Toluene: Ethyl acetate: Methanol: Glacial acetic acid (6:5:1.5:0.3 v/v) gives good resolution, minimum tailing and values of R_f are 0.28 and 0.73 for Amlo and Celo respectively.

Amlo and Celo has good solubility in methanol as compared to ethanol, acetone, and water. Toluene: Ethyl acetate: Methanol: Conc. Ammonia was finalized as a mobile phase by using various ratios, in which 6:5:1.5:0.3 (v/v) ratio shows comparatively good resolution of a drug. For detection of a drug at 310 nm wavelength was selected **Fig. 3** and **4**.

TABLE 2: PLATE PARAMETERS USED FOR HPTLC METHOD

S. no.	Parameters	Chromatographic condition	
		Amlodipine Besylate	Celecoxib
1	Plate length (mm)	100	100
2	Plate width (mm)	100	100
3	Start –X (mm)	10	10
4	Start –Y (mm)	10	10
5	Band space (mm)	5	5
6	Band length (mm)	8	8
7	No. of tracks	6	6
8	Actual no. of tracks	6	6

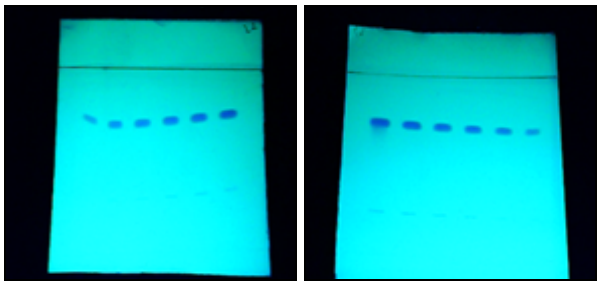

FIG. 3: PHOTOGRAPH OF DEVELOPED HPTLC PLATE

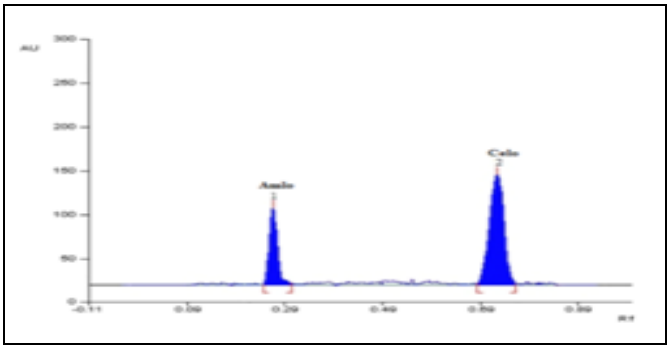

FIG. 4: DENSITOGRAM OF AMLO ANDCELO AT 310 nm

Analysis of Sample Solution: A sample solution containing Amlo 10 mg and Celo 200 mg was used for analysis. The sample solution was analyzed three times, and the concentration of drugs determined by the HPTLC method was is good with the label claim. The results for the sample solution was observed to be 100.02% and 99.93% for Amlo and Celo, respectively. The result for analysis of formulation shows that % RSD values are < 2%, which indicates within limit **Table 4**.

TABLE 3: CHROMATOGRAPHIC CONDITIONS USED HPTC METHOD

S. no.	Parameters	Chromatographic condition	
		Amlodipine Besylate	Celecoxib
1	Stationary phase	Silica gel 60 F_{254}	Silica gel 60 F_{254}
2	Mobile phase	Toluene: Ethyl acetate: Methanol: Conc. Ammonia (6:5:1.5:0.3 v/v)	Toluene: Ethyl acetate Methanol: Conc. Ammonia (6:5:1.5:0.3 v/v)
3	Chamber saturation	10 min	10 min
4	Plate development time	25 min	25 min
5	Migration distance	7 cm	7 cm
6	Radiation source	D2& W	D2&W
7	Scanning wavelength	310nm	310nm

TABLE 4: RESULT OF ANALYSIS OF SAMPLE PREPARATION

Drug	Label claim (mg/tab)	Amount taken (mg)	AUC (Mean area)*	Amount found (mg)	% Drug content	±SD	% RSD
Amlo	10	10	2366.44	10.02	100.25	27.55686	1.16
Celo	10	10	2549.97	199.86	99.93	23.50914	0.92

*n=3 (Three measurement)

Method Validation: As per ICH Q2 (R1) guidelines, the method validation parameters like linearity, precision, accuracy, the limit of detection and limit of quantitation were performed.

Linearity: Linearity was determined by using different concentrations of amlodipine besylate and celecoxib. The stock solution of Amlo (1000 µg/ml) and Celo (20,000 µg/ml) was prepared in flask of volumetric and applied on the pre-coated TLC plate in a range of concentration 100-600 ng/spot for Amlo and 2-12 µg/spot for Celo. After plate development, the peak area was determined for each concentration, and a calibration graph was plotted between peak area *vs.* drug concentrations.

From the graph, the value of R^2 was observed to be 0.9971 for Amlo, and the value of R^2 was observed to be 0.9989 **Table 5-8, Fig. 5-9**.

TABLE 5: CALIBRATION CURVE OF AMLO

S. no.	Sample volume in µl	Conc. of Amlo (ng)	AUC (Mean area)*	±SD	% RSD
1	0.1	100	569.8867	9.544927	0.208
2	0.2	200	1110.547	18.27891	0.509
3	0.3	300	1499.953	25.69189	0.462
4	0.4	400	1950.627	30.14439	0.458
5	0.5	500	2360.507	40.40886	0.449
6	0.6	600	2927.61	24.51116	0.439

*n=3 (Three measurements)

TABLE 6: CALIBRATION CURVE OF CELO

S. no.	Sample volume in µl	Conc. of Celo (ng)	AUC (Mean area)*	±SD	% RSD
1	0.1	2	841.36	9.905004	1.177
2	0.2	4	1225.817	13.38723	1.092
3	0.3	6	1721.647	15.89962	0.923
4	0.4	8	2144.78	33.82947	1.577
5	0.5	10	2551.73	39.91856	1.564
6	0.6	12	3053.633	46.89966	1.535

*n=3 (Three measurements)

TABLE 7: PRECISION STUDIES FOR AMLO AND CELO

Drug	Concentration	*Mean AUC	±SD	%RSD
Amlo	500ng/band	2360.50	40.40886	1.71
Celo	10µg/band	2551.73	39.918557	1.56

*n=3 (Three measurements)

TABLE 8: RECOVERY STUDY OF AMLO AND CELO

Drug name	Initial Amount (ng/band)	Added Amount (ng/band)	*Mean AUC	±SD	%RSD	%Recovery
	500	400	4254.483	8.596705	0.202	99.52
Amlo	500	500	4728.063	10.05794	0.212	99.61
	500	600	5206.613	20.00885	0.384	99.83
	10	8	4647.383	33.31653	0.716	99.98
Celo	10	10	5162.413	34.17049	0.661	99.9
	10	12	5676.037	28.48506	0.501	99.83

*n=3 (Three measurements)

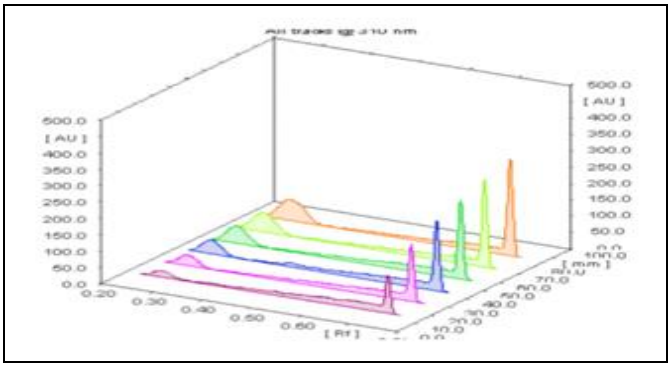

FIG. 5: 3D CHROMATOGRAM OF AMLO AND CELO SPOT FOR EACH CONCENTRATION DETECTED AT 310 nm

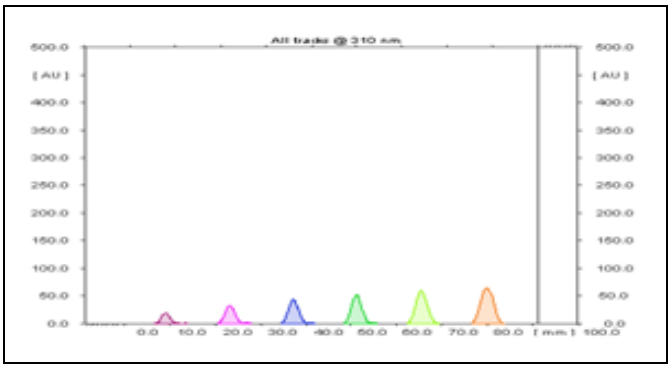

FIG. 6: 3D CHROMATOGRAM OF AMLODIPINE BESYLATE

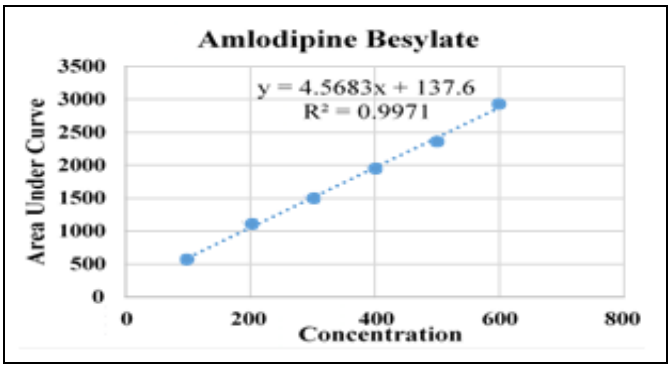

FIG. 7: CALIBERATION CURVE AT 310 nm OF AMLO

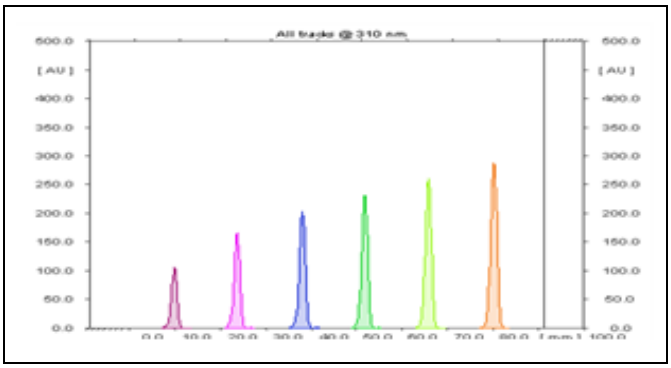

FIG. 8: 3D CHROMATOGRAM OF CELO AT 310 nm

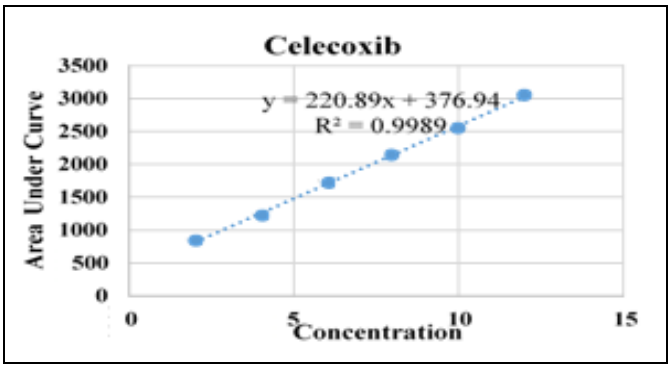

FIG. 9: CALIBERATION CURVE OF CELECOXIB

Precision: The precision study of Amlo and Celo shows that the developed method is precise with %RSD within limit *i.e.*, <2%.

Accuracy: The recovery of Amlo and Celo was described in terms of ±SD and %RSD. Percentage recovery values were observed to be 99.52% to 99.83% for Amlo and 99.83% to 99.98% for Celo in a range with low values of %RSD of drug content.

Limit of Detection and Limit of Quantitation (LOD and LOQ): It is determined by the slope (s) of calibration curve and means of SD (σ) of response. These were observed to be 17.88 µg/ml and 54.20 µg/ml for Amlo and 0.39 µg/ml and 1.20 µg/ml for Celo, respectively.

CONCLUSION: It is concluded that the developed HPTLC method is simple, new, fast reproducible and sensitive methods for the simultaneous estimation of Amlo and Celo in the pure and combined dosage form. This method is successfully validated using ICH Q2 (R1) guidelines for analytical method validation. This method help for routine drug analysis in pharmaceutical dosage form with no interference of excipient with good sensitivity.

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CONFLICTS OF INTEREST: Nil

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