

## **A concise review of various analytical approaches for quantitative analysis of amlodipine and celecoxib in different matrices**

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### **ABSTRACT**

Osteoarthritis and hypertension are comorbidities that may have a complex and bidirectional relationship that involves both systemic and local factors. Both diseases require long-term pharmacotherapy management by concurrent administration of antihypertensive and anti-inflammatory medications. Recently, a fixed-dose formulation containing both amlodipine (AML), as the besylate, and celecoxib (CXB) emerged to manage this comorbidity. This combination medicine is sold under the brand name Consensi® and is used in adults who have both osteoarthritis and high blood pressure. This systematic review gathers and discusses a variety of analytical techniques employed in the literature to quantify AML and CXB in their fixed-dose pharmaceutical formulation and other matrices, including capillary electrophoresis, UV-visible spectroscopy, spectrofluorimetry, electrochemical methods, and other chromatographic techniques such as LC-MS, UPLC, and GC. The comparative use of various analytical methods for quantifying CXB and AML is discussed in this review. Further analytical research for estimating CXB and AML may be successfully conducted using the information presented in this review paper.

*Keywords:* Analytical approaches, Amlodipine, Celecoxib, Hypertension, osteoarthritis

## 1. Introduction

Clinical research on both osteoarthritis (OA) and cardiovascular manifestations (CVD) has revealed a continuous link between these two diseases. The onset of ischemic heart diseases, cerebrovascular diseases, and hypertension were found related to the earlier onset of OA in some investigated patients (1). OA has been shown in multiple studies to be a contributory factor to CVD illnesses, as evidenced by clinical trials referring to minimal inflammatory events, particularly in instances of knee OA, which may be a precursor to the development of CVD illnesses (2). Hypertension (HTN) is among the most common CVD illnesses associated with OA, accounting for 40% of cases (3), as both disorders are regarded as risk factors for one another (4). HTN is a medical illness characterized by a long-term increase in blood pressure that, if untreated, may result in various organ dysfunction that may lead to death (5). OA is the 11th leading cause of disability globally, and OA patients are more likely to develop CVD disorders such as HTN owing to activity limitations (6). Moreover, HTN patients are more likely to develop OA because chronically high blood pressure damages certain microvessels that feed the fragile cartilage layers enclosing all joints throughout the body, causing degeneration and the deposition of OA (7).

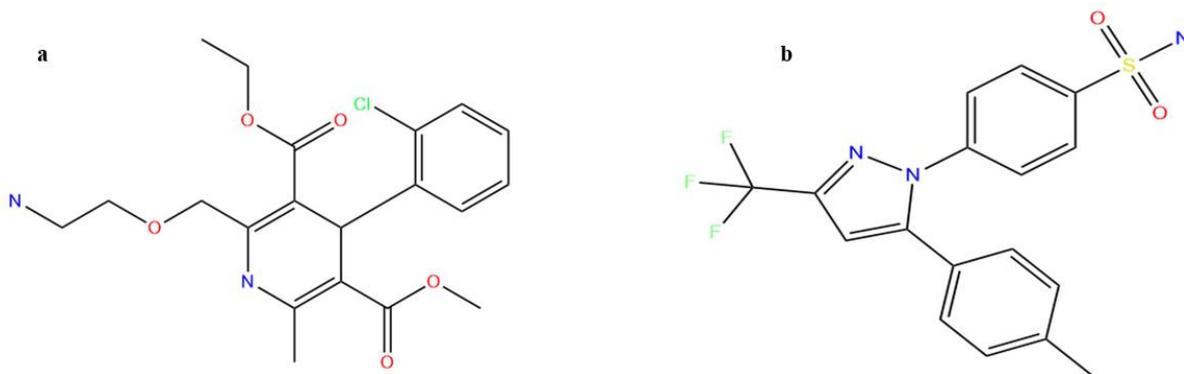
Owing to the rising number of patients with HTN and OA, novel fixed-dose pharmaceutical products of CVD medications are being developed to treat both conditions while maintaining a favorable tolerability profile. This novel fixed-dose pharmaceutical product of Amlodipine (AML) and Celecoxib (CXB), which provides effective therapy for both diseases, was authorized by the Food and Drug Administration (FDA) on May 31, 2018. The novel combination increased effectiveness while reducing the likelihood of adverse events, cutting medical costs, and enhancing patient compliance and adherence.

AML, with a chemical name of 3-O-ethyl-5-O-methyl-2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate (**Fig. 1 a**) (8), is a dihydropyridine member of the calcium channel blockers family. AML blocks the calcium ions in flow by binding to the voltage-gated calcium channels (L-type) in the vascular smooth muscle. This blockade causes vasodilation to the arterial blood vessels and a decrement in blood pressure (9). AML is effective in cases of unstable (Prinzmetal's) angina due to the vasodilation caused in the coronary arteries delivering oxygen to the heart itself (10). So, AML is occasionally prescribed for patients suffering from HTN for their blood pressure management in many CVD complex manifestations (11). A pharmacophore modeling study was conducted based on 15

training set compounds that are clinically active as calcium channel blockers revealed the importance of the presence of a hydrogen bond acceptor, donor, and a lipophilic group as essential groups important for binding to the L-type gated calcium channels (12). AML is a solid, white powder that is water and methanol with a molecular weight of 408.9 g/mol.

On the other hand, CXB, with a chemical name of 4-[5-(4-methyl phenyl)-3-(trifluoromethyl) pyrazol-1-yl] benzenesulfonamide (**Fig. 1 b**) (13), is a non-steroidal anti-inflammatory drug (NSAID) prescribed for OA patients for pain and inflammation management which represent the primary manifestation for these patients (14). CXB binds to the human iso-zyme cyclooxygenase-2 (COX-2) selectively which is extensively expressed over tissues suffering inflammations, with lowered selectivity towards the COX-1 variant expressed at the mucosa of the gastrointestinal tract (GIT). So, CXB is considered an NSAID with decreased GIT side effects (15). Regarding CXB structure, it contains an amine substituted group in the form of benzene sulfonamide, where the amino group plays a strategic role in the pharmacophore of the drug, acting as a hydrogen bond acceptor, which is essential for active site binding in the Cox-2 enzyme according to the postulated top-ranked chemical feature-based pharmacophore mode (16). CXB is considered a pale yellow solid powder with a molecular weight of 381.4 g/mol, which is physically freely in methanol, ethanol, and methylene chloride and poorly soluble in water (13,17).

This review article aimed to describe several analytical methods for determining celecoxib and amlodipine, either alone or together. These methods comprised chromatographic, electrochemical, spectrophotometric, and spectrofluorimetric approaches.



**Fig.1:** Chemical structures of (a) Amlodipine, (b) Celecoxib

## 2. Analytical techniques

### 2.1. Official and reported analytical methods for the analysis of amlodipine

#### 2.1.1. Official analytical method

Reversed-phase HPLC method using C18 column (0.25 m x 4.0 mm) using ammonium acetate: methanol (30:70 % v/v) at 1.5 mL/min and UV detection at 237 nm was reported in the European pharmacopeia (8).

#### 2.1.2. Reported analytical methods

##### a. Spectroscopic methods

###### *UV spectrophotometric methods*

Three spectrophotometric methodologies were published earlier for the quantification of AML in its dosage form; a zero-order method at 238 nm using water as solvent (18), a second Zero-order method at 243 nm using a hydrotropic agent (urea) to enhance water solubility (19) and another Zero-order method at 355 nm using methanol as solvent (20).

###### *Colorimetric methods*

A colorimetric methodology was published earlier for quantifying AML in its dosage form by forming a charge transfer complex using 7,7,8,8-tetracyanoquinodimethane and tetracyanoethylene using acetonitrile solvent, and detection was at 745 and 396 nm, respectively (21).

###### *Spectrofluorimetric method*

A method for determining AML in spiked human plasma without derivatization, Using ethanol as a solvent and detection was at 440 nm after excitation at 360 nm (22).

##### b. Electrochemical methods

Two voltammetric methods based on Cyclic waves were reported for estimating AML in its dosage form using working electrodes decorated by diamond-doped boron (23) and multiwall carbon nanotube (24).

## 2.2. Official and reported analytical methods for the analysis of celecoxib

### 2.2.1. Official analytical method

A liquid chromatographic method using a phenyl column (0.25m x 4.6 mm) at 60 °C and the mobile phase was (methanol: acetonitrile: potassium dihydrogen phosphate buffer (pH=3) (30:10:60 % v/v/v) with flow rate 1.5 mL/min at UV detection of 215 nm was reported in British pharmacopeia (13).

### 2.2.2. Reported analytical methods

#### a. Spectroscopic methods

##### *UV spectrophotometric methods*

Two zero-order methods were published earlier for the determination of CXB in its capsule dosage form based on acetylation of CXB using acetyl chloride in methanol, and acetyl derivative of CXB was determined at 270 nm (25) and using (acetonitrile: sodium phosphate buffer pH 5.6) (50:50 % v/v), and determination was at 251 nm (26). Stability indicating the first derivative method using methanol as solvent and determination was determined at 269 nm (27). Another First derivative method using methanol and detection was reported at 252 nm (28).

##### *Spectrofluorimetric method*

This method was applied utilizing methanol as solvent, with the assistance of  $\beta$ -cyclodextrin as fluorescence enhancer, where the emission and excitation were at 390 nm and 290 nm, respectively (29).

##### *Chemiluminescence method*

The method is an application to pharmaceutical and biological samples. The method depends on the cerium (Ce) sulfite system in the presence of terbium (Tb III) ions, and detection was at 490 and 545 nm (30).

#### b. Electrochemical methods

Cyclic voltammetry was reported based on a molecularly imprinted poly(vinylidene fluoride) membrane doped by gold nanoparticles (31). Adsorptive stripping voltammetry using a mercury electrode as a working electrode was also reported for CXB determination (32).

### c. Chromatographic methods

#### *High-performance liquid chromatography*

Four liquid chromatographic methods have been reported for the determination of CXB in its pharmaceutical dosage form, as presented in **Table 1**

**Table 1:** High-performance liquid chromatographic methods on CXB.

Chromatographic Column	Mobile phase	Detector type	Sample matrix	Reference
L <sub>11</sub> column (250 mm x 4.6 mm, 5 µm), Based on molecularly imprinted polyvinylidene fluoride membrane as a solid phase extracting medium	Methanol: acetonitrile: phosphate buffer (pH=3) (3:1:6 % v/v/v) with flow of 1.5 ml/min	UV detector, 249 nm	Pharmaceutical dosage form	(35)
Inertsil® C8 column (250 mm, 4.6mm, 5 µm)	Acetonitrile: water (65:35 % v/v) with flow of 1.25 mL/min	UV detector, 230 nm	Pharmaceutical dosage form	(26)
Kromasil C18 column (150 mm x 4.6 mm, 5 µm)	Isocratic proportion (70:30 % v/v) tri ethyl amine and acetonitrile.	UV detector, 235 nm	Pharmaceutical dosage form	(36)
Agilent SB-C18 column (250 mm x 4.6 mm, 5 µm)	Acetonitrile: water with 0.1% formic acid buffer (70:30 % v/v)	MS/MS	Rat plasma	(37)

#### *Capillary electrophoresis*

The electrophoretic setup consisted of a Beckman P/ACE MDQ system with a mobile phase of Tris buffer (10 mM; pH 11) with 60 mM sodium octane- sulfonate, and 20% ACN and UV detection was done at 200 nm (33).

*High-performance thin-layer chromatography*

Determination of five coxibes based on the mobile phase of toluene : acetone: chloroform (2:5:12) % v/v/v was reported, and densitometric determination was at 254 nm (34).

### 2.3. Recently reported analytical methodologies for the determination of AML and CXB in binary mixtures

#### a. Spectroscopic methods

*UV spectrophotometric methods*

Several spectrophotometric methodologies were published for the concurrent estimation of AML and CXB in their combined tablets, as shown in **Table 2**.

**Table 2:** Several spectrophotometric methods were reported for the simultaneous determination of AML and CXB in their combined dosage form

Method	Sample Matrix	Solvent	Working wavelength		Reference
			AML	CXB	
First order derivative	Pharmaceutical dosage form	Methanol	290 nm	250 nm	(39)
Ratio difference	Pharmaceutical dosage form	Methanol	363.4 and 345.6 nm	268.8 and 236.5 nm	(40)
Ratio derivative	Pharmaceutical dosage form	Methanol	334.2 nm	254.2 nm	(40)
First derivative	Pharmaceutical dosage form	Methanol	252 nm	289.4 nm	(41)
Fourier self-deconvoluted method	Pharmaceutical dosage form	Methanol	360 nm	269 nm	(42)
Absorption correction method	Pharmaceutical dosage form	Methanol	361	253 nm	(42)
Induced dual wavelength	Pharmaceutical dosage form	Methanol	-	251 and 270 nm	(42)

*Spectrofluorimetric methods*

AML and CXB were determined in the presence of each other in pharmaceutical dosage form and human plasma by applying the first derivative synchronous spectrofluorimetric method at  $\Delta\lambda = 100$  nm, using methanol as solvent where excitation wavelengths were at 367 nm and 264 nm and emissions were at 455 nm and 368 nm, respectively (38).

**b. Chromatographic methods***High thin layer liquid chromatography*

AML and CXB binary mixtures were determined using TLC with densitometric detection in their pharmaceutical dosage form. The chromatographic separation was done using TLC aluminum plates precoated with silica gel 60F-254 as the stationary phase. The solvent system consisted of toluene: ammonia: methanol: acetonitrile in the ratio of (6.6:0.12:1.5:2 % v/v/v/v) with UV detection at 240 nm (43).

*High-performance liquid chromatography*

Six liquid chromatographic methods have been reported for the simultaneous determination of AML and CXB in different matrices, as presented in **Table 3**

**Table 3:** High-performance liquid chromatographic methods on AML and CXB

<b>Chromatographic Column</b>	<b>Mobile phase</b>	<b>Detector type</b>	<b>Sample matrix</b>	<b>Reference</b>
Nucleosil C8 column (125 x 4.6 mm, 5 $\mu$ m)	Sodium dihydrogen phosphate buffer and acetonitrile (63:37 % v/v), pH 3.5 with a flow rate of 1.5 mL/min	UV detection, 239 nm	Human plasma	(44)

Zorbax Eclipse XDB-C18 column (150 mm x 4.6 mm, 5 $\mu$ m)	Triethylamine buffer: methanol (40:60 % v/v), pH 3 with a flow rate of 1 mL/min	UV detection, 239 nm	Pharmaceutical dosage form	(45)
C18 column (150 mm x 6 mm, 5 $\mu$ m)	Phosphate buffer: methanol with a flow rate of 2.8 mL/min	fluorescence detection with excitation at 470 nm and emission of 537 nm using 4-Chloro-7-nitrobenzofurazan as fluorogen	Human serum	(46)
kinetex C 18 (100 mm x 4.6 mm, 5 $\mu$ m) column	potassium dihydrogen phosphate: acetonitrile (65:35 % v/v) with a flow rate of 1.2 mL/min	UV detection, 240 nm	Pharmaceutical dosage form	(47)
Waters C18 column (250 mm x 4.6 mm, 5 $\mu$ m)	Acetonitrile: potassium dihydrogen orthophosphate buffer: methanol (15:30:55 % v/v/v), PH 3 at a flow rate of 1 mL/min	UV detection, 240 nm	Pharmaceutical dosage form	(48)
Zorbax C18 RP-HPLC column (150 mm x 4.6 mm, 5 $\mu$ m)	Sodium dihydrogen; pH 5.6 acetonitrile, and methanol (30:55:15 % v/v/v) at flow rate of 1.2 mL/min	UV detection, 239nm	Combined pharmaceutical dosage form (AML + CXB)	(49)

### 3. Conclusion

The current study presents a synopsis of the numerous analytical techniques published in the literature for the detection of AML and CXB in different matrices, including pharmaceutical formulations and serum and plasma samples. AML and CXB were quantified in bulk pharmaceutical dosage form using analytical techniques, including spectroscopy, chromatography, and electrochemical methods. The compilation of review's primary goal is to gather as much information as possible on the AML and CXB analytical techniques and thoroughly examine it. According to the results of this study, just a few analytical techniques based on UV-Vis spectrophotometry and HPLC are accessible, and only a few papers based on hyphenated methods are available. Also, according to the given data for AML, and CXB analysis, HPLC with UV detection is the method most often used to measure both drugs in the pharmaceutical matrix and other biological matrices because it produces exact results with little effort. Furthermore, the study of CXB and its metabolites in biological samples was made possible using TLC methods, which provided exceptional selectivity, sensitivity, and a selection of methods.

### Conflict of Interest

The Authors declare no conflict of interest.

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