

Review Article

**ANALYTICAL METHODOLOGIES FOR DETERMINATION OF CILNIDIPINE: AN OVERVIEW**

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**ABSTRACT**

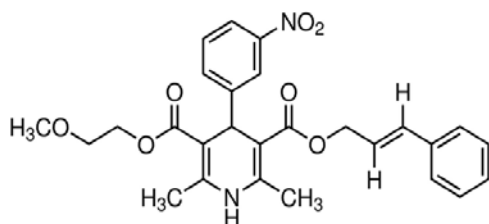
Cilnidipine is a dual blocker of L-type voltage-gated calcium channels in vascular smooth muscle and N-type calcium channels in sympathetic nerve terminals that supply blood vessels. CIL is used in combination with other drugs like Telmisartan, Olmesartan Medoxomil. CIL has been determined in formulations and biological fluids by a variety of methods such as spectrophotometry, High-performance liquid Chromatography with ultraviolet detection, liquid chromatography coupled with tandem mass spectrometry, densitometry. The overview includes the most relevant analytical methodologies used in its determination since the origin still today.

**Keywords:** Cilnidipine, Review, Pharmaceutical formulations.

**INTRODUCTION**

Cilnidipine (CIL) 1,4- Dihydro- 2,6- dimethyl- 4-(3-nitrophenyl)-3,5-pyridinecarboxylic acid 2-methoxyethyl(2E)-3-phenyl-propenyl ester is a novel and unique dihydropyridine calcium channel blocker that possesses a slow-onset, long-lasting vasodilating effect. CIL is used in the treatment of hypertension [1]. CIL shows first pass mechanism. CIL is used in combination with others drugs like Telmisartan (TEL), Olmesartan (OLME). CIL and its formulations are not official in any pharmacopoeias.

The structural formula for CIL is shown in Fig 1 [2].



Introduction of new methods, enabling determinations with maximum accuracy, contributes to increased interest in analytical methods as such. They should enable to simultaneously determine the individual components in multi-component preparations and in biological material. Range of guidelines, standardizing requirements concerning the quality of drugs has been issued. These are numerical parameters that validate reliability of the results and enable comparing efficiency of the methods used. The process that is used to determine the above parameters is the so-called method validation [3].

Several methods have been employed for the determination of CIL in formulation and in combination with other drugs.

The main objective of this review is classified, summarized and also it discusses the different proposed methods for the determination of CIL in formulations and in mixtures.

**SPECTROPHOTOMETRIC METHODS**

Spectrophotometric method for the determination of drugs can be used in laboratories where modern and expensive apparatuses such as that required for GLC or HPLC are not available. However, Spectrophotometric method is versatile and economical particularly for developing countries. Spectrophotometric method has several advantages such as being easy, less expensive and less time consuming compared with most of the other methods. Spectrophotometric method is simple and rapid; so this method can be successfully used for pharmaceutical analysis, involving quality

control of commercialized product and pharmacodynamic studies. The Spectrophotometric methods are simple and rapid but less sensitive. The following are some methodologies for the estimation of CIL in pharmaceutical dosage form [4,5]. Few methods have been proposed for the estimation of CIL in combinations.

**Mixtures**

Two validated UV Spectrophotometric methods for the simultaneous estimation of CIL and Telmisartan in pure powder and in tablet dosage forms has been proposed using simultaneous equation and absorbance ratio method. The method is based on the measurement of absorbance of CIL and Telmisartan at their respective  $\lambda_{max}$  of 241nm and 297nm and at the isoabsorptive wavelength of 264 nm in co-solvent mobile phases i.e. Methanol: Water (80:20 v/v). CIL and Telmisartan at their respective  $\lambda_{max}$  241nm and 297nm obeyed Beer's law in the concentration range 1-10  $\mu\text{g/mL}$  and 2-18  $\mu\text{g/mL}$  respectively with correlation coefficient 0.999 and 0.999 for CIL and 0.999 and 0.999 for Telmisartan for both the methods [6].

Two validated uv spectrophotometric methods for the simultaneous estimation of CIL and Telmisartan in pure powder and in two component dosage forms has been developed, utilizing simultaneous equation and absorbance ratio method. The method is based on the measurement of absorbance of CIL and Telmisartan at their respective wavelengths of 240 nm and 297nm and at the isoabsorptive wavelength of 270 nm in methanol. CIL and Telmisartan at their respective  $\lambda_{max}$  240 nm and 297 nm obeyed Beer's law in the concentration range 4-10  $\mu\text{g/mL}$  and 6-18  $\mu\text{g/mL}$  respectively with correlation coefficient 0.9998 and 0.9992 for CIL and 0.9998 and 0.9991 for Telmisartan [7].

Four simple, sensitive, rapid, accurate and precise simultaneous UV-spectrophotometric methods have been developed and validated for the estimation of CIL and Telmisartan in combined tablet dosage form. All methods obeyed Beer's law [8].

The spectrophotometric conditions for determination of CIL are presented in Table 1.

**CHROMATOGRAPHIC METHODS**

The simultaneous determination of the active ingredients in multicomponent pharmaceutical products normally requires the use of a separation technique, such as high performance liquid chromatography or gas chromatography followed by their quantitation.

**High Performance Liquid Chromatography**

Among various separating analytical techniques, HPLC constitutes the most popular chromatographic method for separating the mixtures of drugs.

A stability indicating reversed-phase HPLC method has been developed for estimation of CIL in Pharmaceutical Formulation. HPLC analysis was performed on a C18 column using a 80:20 (v/v) mixture of Methanol and 0.05 M Phosphate Buffer at pH 3.0 as isocratic mobile phase at a flow rate of 1 ml min<sup>-1</sup>. UV detection was carried at 254 nm. Good linear correlation coefficients ( $r^2 > 0.9999$ ) were obtained for calibration plots in the ranges tested. Limit of detection was 0.179 µg/mL and limit of quantification was 0.544 µg/mL. Intra and inter-day RSD of retention times and peak areas were less than 1.729% and recovery was between 99.95% and 101.09% [12].

Another paper describes a rapid, sensitive and reliable high performance liquid chromatographic method coupled with tandem mass spectrometry (HPLC-MS/MS) for the determination of CIL. The reversed-phase chromatographic system was interfaced with a TurbolonSpray (TIS) source. Nimodipine was employed as the internal standard (IS). Sample extracts following protein precipitation were injected into the HPLC-MS/MS system. The analyte and IS were eluted isocratically on a C18 column, with a mobile phase consisting of

CH<sub>3</sub>OH and NH<sub>4</sub>Ac (96:4, v/v). The ions were detected by a triple quadruple mass spectrometric detector in the negative mode. Quantification was performed using multiple reaction monitoring (MRM) of the transitions m/z 491.2 → 122.1 and m/z 417.1 → 122.1 for CIL and for the IS, respectively. The analysis time for each run was 3.0 min. The calibration curve fitted well over the concentration range of 0.1–10 ng mL<sup>-1</sup>, with the regression equation  $Y = (0.103 \pm 0.002) X + (0.014 \pm 0.003)$  ( $n = 5$ ),  $r = 0.9994$  [13].

An RP-HPLC method was established for the determination of CIL and its related substances. The separation was performed on a Hypersil C 18 column (5 µm, 4.6 mm×250 mm). The mobile phase was composed of acetonitrile-water (7:3), and the flow rate was 1.0 ml/min. The detection was carried out with UV detector at 240 nm [14].

#### Mixtures

CIL has been analyzed in combinations by HPLC method. Table 2 represents the chromatographic conditions for estimation of CIL in combinations.

**Table 1: Spectrophotometric analysis of Cilnidipine**

Compound	Sample matrix	Detection λ (nm)	Linearity range	Ref.
CIL	Bulk, Tablet dosage form	240	2 – 30 µg/mL	[4]
CIL	Bulk, Tablet dosage form	240	3 – 18 µg/mL	[5]
CIL + OLME	Tablet dosage form	CIL 352.92 OLME 282.99	CIL 10 – 60 µg/mL OLME 20 – 120 µg/mL	[9]
CIL + TEL	Tablet dosage form	CIL 264, 297.4 TEL 229, 246.8	CIL 2 – 6 µg/mL TEL 3 – 15 µg/mL	[10]
CIL + TEL	Tablet dosage form	350, 294	CIL 1 – 25 µg/mL TEL 1 – 24 µg/mL	[11]

**Table 2: LC methods used for the analysis of Cilnidipine**

Compound	Sample matrix	Chromatographic conditions			Ref.
		Column	Mobile phase (v/v)	Flow rate (mL min <sup>-1</sup> )	
CIL + OLME	Tablet dosage form	Shimadzu LC 20AT C18 (250 mm x 4.6mm, 5µm)	ACN+buffer (75+25), pH 6.5 adjusted by TEA	1.0	265 nm [15]
CIL + TEL	Tablet dosage form	HiQ Sil C18 (250 mm x 4.6 mm)	Methanol + 40 mM KH <sub>2</sub> PO <sub>4</sub> (pH 3) (90+10)	1.0	245 nm [16]
TEL + CIL	Tablet dosage form	Waters C18 (250 mm x 4.6 mm, 5µm)	ACN+buffer pH 3 with ortho phosphoric acid(68+32)	1.0	245 nm [17]

**Table 3: Validation data for Cilnidipine**

Compound	Method	Linearity range (µg/mL)	LOD	LOQ	Precision (R. S. D %)		Recovery (%)	Ref.
CIL	UV	2-30	0.0255	0.0772	0.5745	0.5745	99.86-100.67	[4]
CIL	UV	3-18	-	-	0.2010	0.1490	98.0-102.0	[5]
CIL+OLME	UV	CIL 10-60 OLME	CIL 0.2828 OLME 0.4554	CIL 0.8571 OLME 1.3800	CIL 0.11-0.45 OLME 0.13-0.79	CIL 0.12-0.66 OLME 0.13-0.75	CIL 100.34-101.80 OLME 100.72-101.80	[9]
CIL+TEL	UV	CIL 2-6 TEL 3-15	CIL 0.05 TEL 0.088	CIL 0.16 TEL 0.266	CIL 0.17-0.53 TEL 0.12-0.44	CIL 0.77-0.89 TEL 0.12-1.18	CIL 99.7-101.80 TEL 99.3-101.30	[10]
CIL+TEL	UV	CIL 1-25 TEL 1-24	CIL 0.02 TEL 0.087	CIL 0.062 TEL 0.265	- - -	- - -	CIL 98.7-99.20 TEL 98.3-99.00	[11]
CIL+OLME	LC	CIL 10-90 OLME 20-180	CIL 0.130 OLME 0.790	CIL 0.395 OLME 2.397	CIL 0.267 OLME 0.434	CIL 0.932 OLME 0.862	CIL 99.05-99.46 OLME 99.14-99.76	[15]
CIL+TEL	LC	CIL 1-10 TEL 5-30	CIL 0.28 TEL 0.60	CIL 0.86 TEL 1.81	CIL 0.25-0.93 TEL 0.38-0.82	CIL 0.24-0.89 TEL 0.12-0.89	CIL 99.94-100.39 TEL 99.60-99.83	[16]

### Thin Layer Chromatography

Deshpande Padmanabh et al., has developed a high performance thin layer chromatographic method for the simultaneous estimation of CIL and Telmisartan in combined dosage form. The separation was achieved on a Merck aluminium plates precoated with silica gel 60 F254 using Toluene: Methanol: Ethyl acetate (8: 2: 1 v/v/v) as the mobile phase with detection at 250 nm. The results were linear in the range of 200 – 1200 ng/band for CIL and 800 – 4800 ng/band for Telmisartan [18].

### Liquid Chromatography – Mass Spectrometry

Suk-Jae Chung et al has developed a liquid chromatography-mass spectrometry (LC-MS) assay for the quantification of CIL, in human plasma. Plasma samples were processed by liquid-liquid extraction and the analyte, along with nimodipine (an internal standard) were analyzed using selected ion monitoring (SIM) for detection. The detector response was specific and linear for CIL in the range of 0.5–50 ng/mL. CIL levels were readily measured in plasma samples up to 10 hr after an oral administration of 10 mg of a CIL formulation in humans, suggesting that the assay can be used in routine analyses [19].

### VALIDATION OF THE METHODS

Validation of a method is the planned and documented procedure to establish its performance characteristics. Typical parameters that characterize each analytical method include selectivity, specificity, range, linearity, accuracy (recovery), detection limit (LOD), Quantitation limit (LOQ), precision, robustness and ruggedness [3].

The analytical procedures presented in this review have been validated in terms of basic parameters, i.e. linearity, LOD, LOQ, precision and recovery. The validation parameters of the methods used are presented in **Table 3**. The data obtained makes it possible to choose the proper analytical procedure, adopted to the kind of sample (bulk, pharmaceutical preparations, and biological matrices), method of the determination or detection. Comparing validation parameters of already researched methods, it can be concluded which one of them is more sensitive (low LOD and LOQ values), accurate (precision and recovery) and allows markings in a broad linearity scope.

### CONCLUSION

The first and the principle conclusion is that there are different UV spectrophotometric and HPLC methods for the estimation of CIL alone and in combination which has been successfully used on a routine basis and allows the quantification of the drug in pharmaceutical formulations in a short analytical time. These methods are sensitive, simple, fast and possess excellent linearity and precision characteristics. These observations make it possible to anticipate the use of these methods as an official procedure.

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### CONFLICT OF INTERESTS

Declared None

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