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Spectrophotometric Estimation of Cilnidipine in Tablets

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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Short Research Article

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ABSTRACT

Aim: The paper discusses the Spectrophotometric method developed to determine Cilnidipine in pharmaceutical formulation.
Methodology: The method depends on the reaction of the nitro group of the drug with Potassium Hydroxide in Dimethyl Sulphoxide (DMSO) medium to form a coloured product, which shows maximum absorbance at 425 nm. It also Common excipents used in the formulation bear no effect on the proposed method. The authenticity and performance of the proposed method is approved by point and interval hypothesis tests and through recovery studies.
Results: The linear regression equations obtained by applying least square regression analysis for Cilnidipine were r2= 0.9997 and adheres Beer's Law in the concentration range of 1– 9 µg/ml.
Conclusion: The method was validated for specificity, linearity, accuracy, precision, Limit of detection and limit of quantification are found to be suitable to be employed in Quality Control as per the International Conference on Harmonization guidelines.

Keywords: Cilnidipine; dimethylsulphoxide; potassium hydroxide and visible spectroscopy.

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1. INTRODUCTION

Cilnidipine is a di esterified 1,4-dihydropyridine-3,5-dicarboxylic acid. Its Chemical name is (±)-(E)-cinnamyl 2-methoxyethyl 1,4-dihydro-2,6-dimethyl-4-(m-nitrophenyl)-3,5-pyridinedicarboxylate [1]. It was first synthesized in Fuji & Rebio Pharmaceutical Co., Ltd. (Hino, Japan) (Fig. 1).

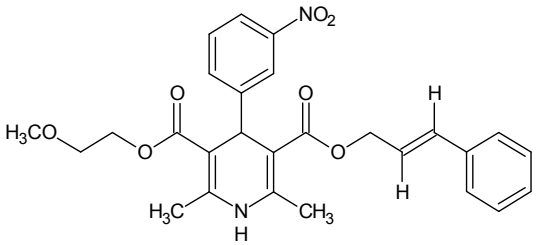


Fig. 1. Structure of cilnidipine - $C_{27}H_{28}N_2O_7$

This drug is a unique 1,4-dydropyridine derivative and calcium antagonist that exerts potent inhibitory actions on L-type and N-type voltage-dependent calcium channels. Blockade of the neural N-type calcium channel supresses the secretion of norepinephrine from peripheral neural terminals and depresses sympathetic nervous system activity [2]. The estimation of the drug as a single molecule by UV Spectrophotometric [3-5], visible spectroscopic [6] HPLC [7] and and in combination with other drugs by UV [8-12], HPLC [13-17] was available. The literature survey reveals the lack of visible Spectrophotometric methods for the estimation of cilnidipine.

The present work describes sensitive spectrophotometric estimation of cilinidipine in tablet. This paper reflects the reaction of nitro group in cilinidipine with potassium hydroxide in presence of solvent dimethyl sulphoxide to produce yellow colored product which shows the maximum absorbance at 425 nm. The developed method was also validated as per International Conference on Harmonization (ICH) guidelines [18] and could be applied directly for the day to day analysis of Cilinidipine.

2. MATERIALS AND METHODS

2.1 Instruments

The following materials have been used to get the targeted product. A JENWAY-UV/VIS Spectrophotometer model-6800and Ultra violet - visible - near infra red -spectrophotometer (Jasco, Japan, Model V-570) with 1 cm matched quartz cells was employed to measure the absorbance.

2.2 Reagents and Standards

Potassium hydroxide, Methanol, Dimethyl-sulphoxide was procured from M/S. Sigma-Aldrich (USA). The standard cilnidipine was provided by J.B. Chemicals and Pharmaceuticals Ltd. (Mumbai, India). Cilacar-20 tablets (J.B. Chemicals) were purchased. The pure stock solution of 0.1% Cilnidipine was prepared in Methanol and preserved in darkness. All chemicals used were of spectroscopic grade.

2.3 Procedure

Stock solution of 100 µg/ml of Cilinidipine was prepared by dissolving 10 mg of cilinidipine in 100 ml. of methanol. From the above stock solution, different volumes of the drug were transferred to a series of 10 ml volumetric flasks. To the flasks 1 ml. of 0.05 M. potassium hydroxide was added and the volume was made up with solvent dimethyl sulphoxide to get different concentrations in a range of 1-9 µg/ml. The spectrum was recorded and measured maximum absorbance at 425 nm. in order to obtain a standard curve.

2.4 Procedure for Determining Cilnidipine in Tablets

The Cilacar-20 tablets procured from the market was powdered. From this, the powder equivalent to 100 mg. of cilnidipine was weighed and transferred to separating flask and extracted with 50ml. of chloroform by shaking. The mixture was filtered using Whatmann No. 42 filter paper. The filtrate was dried under vacuum and the residue obtained was dissolved in methanol. It was transferred to 50 ml volumetric flasks and diluted to 50 ml. The sample analysed was performed by following the procedures, was used for the standard sample of cilnidipine.

3. RESULTS AND DISCUSSION

3.1 Discussion

Mono nitro compounds are known to give different colours with alkali in different polar media. Bost and Nicholson have reported that 3-nitro-4-aminotoluene produced orange colour with acetone and sodium hydroxide [19].

Aromatic nitro compounds, when reacted with alkali in acetone, produce red colour due to the formation of nitroquinoid ions. Acetone maybe replaced with *N, N'*-dimethylforamide or dimethyl sulphoxide [20,21]. The nitro group present in Cilnidipine reacts with KOH in methanol and dimethyl sulphoxide solvent to produce a coloured nitroquinoid ion which absorbs maximum at 425 nm. Since it was adsorbed on anion exchange resin beads, the coloured chromophore formed was found to be negatively charged. Finally, the reaction mechanism was proposed and as given in Scheme 1. It is based on the literature background and our experimental findings.

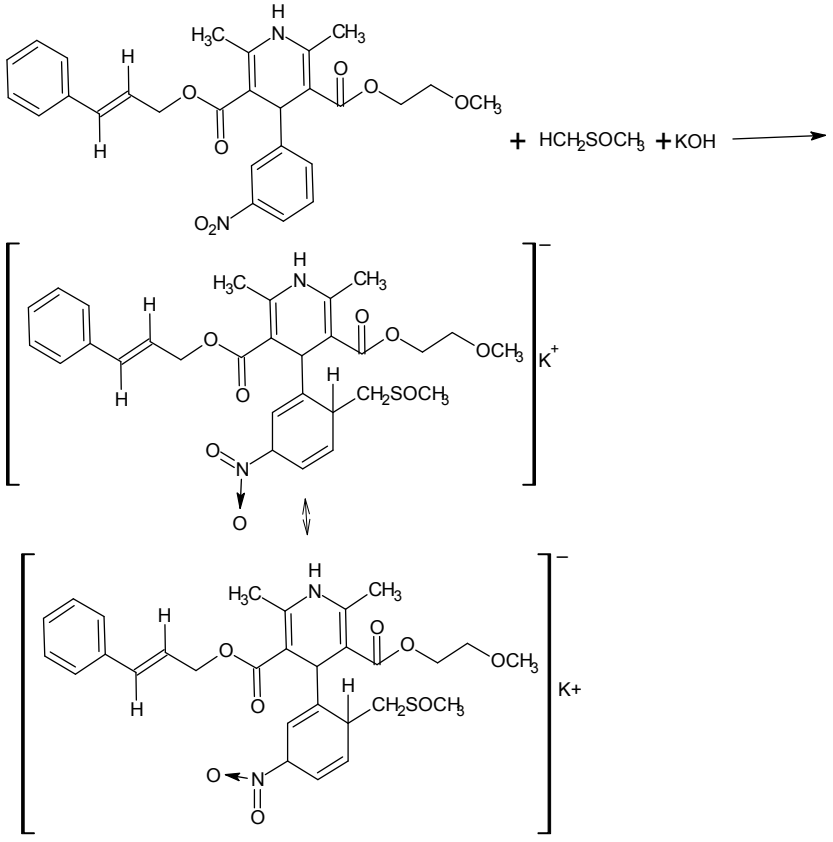
4. Analytical Data

Calibration graphs were constructed under the optimized experimental conditions, plotting the absorbance against the concentration of cilnidipine. It adheres to the Beer's law in the concentration range 1-9 µg/ml. Table 1 summarizes the optical characteristics and the results of statistical analysis of the experimental data such as linear regression equation, correlation coefficient, standard deviation of slope and intercept, and intercept detection limit and quantitation limit. The limit of detection (LOD) and quantitation (LOQ) were calculated using the experimental data by using slope and Standard deviation.

Table 1. Optical and regression characteristics of the proposed methods

Sl. no	Parameters	Results
1	λ max. Nm	430
2	Beer's law range (µgm/ml)	1-9
3	LOD (µgm/ml)	0.7122
4	LOQ (µgm/ml)	2.1582
5	Correlation coefficient – R^2	0.9997
6	Slope	0.01668
7	Y- intercept	0.00775

With respect to A = a + bC, where C is the concentration (µg/ ml) and A is absorbance



Scheme 1. Reaction mechanism of color formation

4.1 Optimization of Variables and Method Development

The concentration of different reagent used for method development was optimized by performing a series of experiments.

4.1.1 Effect of KOH

The effect of KOH was analysed on the formation of the colour. The formation of the colour was examined by using different volume of 0.05 M KOH ranging from 0.1 to 1 ml. The concentration used for the colour developed showed maximum absorbance at 1 ml of 0.05 KOH with 9 µg/ml of Cilnidipine. So 1 ml. of 0.05 M. KOH was used for all the measurements. The maximum absorbance was achieved with 1 ml. of KOH and above this volume absorption remained unchanged.

4.1.2 Specificity

Specificity was evaluated by mixing the standard solution of Cilnidipine with excipients like starch, talk etc and a spectrum was recorded. Spectrum of cilnidipine was overlaid on the spectrum obtained with dosage form. From the spectrum, it was concluded that there was no interference from the excipients.

4.1.3 Solution stability

The solutions of the drug and tablet was kept at room temperature (25±1°C) under darkness for 2 hours and then recorded the absorption spectra using TLC analysis was done to check any degradation products formed during the storage. No change in the absorption spectra of the solutions even after 2 h was observed.

4.1.4 Accuracy

The accuracy of the method was determined by analysing cilinidipine in pure forms at three different concentrations (5.6,7 µg/ml) by short term (intraday) precisions (Table 2) five times. The relative standard deviations and results obtained in the intraday were found to be acceptable. Thus the method is effective for the determination of cilnidipine. The accuracy was also confirmed by performing recovery experiments through standard addition technique. To achieve this, a known amount of pure cilnidipine was added to pre-analyzed dosage forms and then determined by the recommended procedures. The results (Table 3) show that the mean recovery and relative standard deviation were in the range of 99.722-99.816% and S.D 0.376–1.641%. No interference from the common excipients was observed. It is clear from the table that the proposed method is sensitive with acceptable values of relative standard deviations.

4.1.5 Linearity and range

The linearity of the proposed method were determined by analyzing different concentrations prepared from the stock solution and applying least square regression analysis on the data obtained. The Beer's law was obeyed in the concentration range of 1– 9 µg/ml and regression coefficient found to be 0.9997.

Table 2. Evaluation of the accuracy and precision of the proposed methods by intra day assay

Amount taken µg/ml	Found ±S.D	Recovery (%) ±RSD	Confidence limit at 95%
5	5.009±0.0033	100.00±0.0678	0.0028
6	6.002±0.0040	100.01±0.0687	0.0035
7	7.003±0.0038	100.00±0.0540	0.0033

Table 3. Determination of Nisoldipine in pharmaceutical formulations by standard addition technique

Name of commercial product	Amount taken µg/ml	Amount added µg/ml	Found ± SD	Recovery% +SD	Confidence Limit@ 95%
CILACAR-20	4	3.2	7.200±0.008	99.816±0.376	0.329
CILACAR-20	4	4	7.977±0.048	99.722±0.602	0.528
CILACAR-20	4	4.8	8.781±0.144	99.786±1.641	1.438

4.1.6 Robustness

The operational parameter was closely analysed to test the robustness of the method. A sample solution containing 9 µg/ml of active drug was assayed five times. The results showed a mean recovery ± relative standard deviation of 100.11±0.16%. Therefore, the result indicates that the proposed methods to be very robust.

5. CONCLUSIONS

The method is compared with other spectrophotometric methods and found to be more sensitive with low values of relative standard deviations. The proposed method needs no pre-treatment of the drug to its analysis. The newly developed methods are sensitive enough to enable quantitation of the drug at low concentrations. These advantages promote the application of the proposed methods in routine quality control analysis of cilnidipine in pharmaceutical formulations.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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