**IJPSR (2016), Vol. 7, Issue 9** **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 25 April, 2016; received in revised form, 02 July, 2016; accepted, 14 July, 2016; published 01 September, 2016

# DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHODS FOR ESTIMATION OF CELIPROLOL HYDROCHLORIDE IN TABLET FORMULATION

Manisha Chavan* and Manisha Sutar

Alard College of Pharmacy, Hinjewadi, Pune - 411057, Maharashtra, India

**Keywords:**

Celiprolol Hydrochloride, UV spectrometry, zero order derivative, area under curve, validation

**Correspondence to Author:**
**Manisha Chavan1**

Assistant professor and HOD (Pharmaceutical Chemistry), Alard College of Pharmacy, S. No. 50, Marunje, Near Hinjewadi, Pune - 411057, India.

**Email:** manishas_18@rediffmail.com

**ABSTRACT:** Simple, specific, accurate and cost economic UV spectrophotometric methods were developed and validated for determination of Celiprolol Hydrochloride. Instead of using organic solvents, mixture of dilute hydrochloric acid and water was used during method development and validation. The drug was extracted from tablet formulation with a mixture of water and hydrochloric acid. Stability of drug solution and formulation was established throughout the analysis time. Zero Order Derivative and Area Under Curve methods were developed for estimation of drug and both the methods were validated as per ICH guidelines. The linearity of drug was established by performing calibration curve. The analytical range of drug was established from linearity study. Precision of the method was established with Intraday and interday analysis. Accuracy was established by conducting recovery study. Robustness of both the methods was confirmed by conducting analysis with different analysts.

**INTRODUCTION:** Celiprolol Hydrochloride (CPHL) is a highly cardio-selective β1 adrenergic blocker with partial β2 agonist activity (Vasodilating) [1]. CPHL is equal to other β-blockers in controlling blood pressure and heart rate increases during exercises. The drug is comparable or superior to other β-blockers and calcium channel blockers in angina [2]. CPHL is primarily indicated in conditions like Angina pectoris, Hypertension[3], mild to moderate hypertension, and can also be given in adjunctive therapy as an alternative drug of choice in Arrhythmias and Supraventricular Arrhythmias [3-9].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.7(9).3781-86

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.7 (9).3781-86

It is official in Indian & British Pharmacopoeia. Chemically it is 3-[-3Acetyl-4-[(2RS)-3-[(1, 1-dimethylethyl)amino]-2-hydroxypropoxy]phenyl]-1,1-diethyl urea hydrochloride[10]. It is a white off, very slightly yellow, crystalline powder, freely soluble in water and in methanol, soluble in ethanol (96%) and very slightly soluble in Methylene Chloride. Analytical methods available for CPHL are Potentiometric method [10] HPLC, and Colorimeteric method [11].

Literature survey revealed that no zero order and Area Under Curve UV spectrometric method was reported for estimation of drug. In the present work, the study was conducted using aqueous solvent so that waste disposal was not a problem. The present paper was aimed to provide simple, accurate, cost economic and precise method for estimation of CPHL in tablet formulation.

## MATERIAL AND METHODS:

CPHL pure drug was obtained as a gift sample from Ranbaxy Goa. All other chemicals and reagents used were of analytical grade. Ethanol was purchased from Research-lab Fine Chemicals, Mumbai. Sodium Hydroxide was purchased from Research Lab, Pune. Con. Hydrochloric acid was purchased from Fine chemicals, Pune. UV spectrophotometer used was Jasco V-630 with UV visible detector. Other apparatus, utilized were digital electronic balance (Shimadzu BL-220H), Ultrasonicator (Equiptron), Micro Syringe (Hamilton micro lab), Glassware (Borosilicate) used were calibrated.

### Selection of suitable solvent:

The solubility of drug was determined in a solvents such as Methanol, Ethanol (IP, BP) as well as in dilute HCl.

### Determination of λ max of drug:

Accurately weighed quantity of 10 mg drug was dissolved in 30 ml distilled water & 2 ml conc. HCl and was transferred to 100 ml volumetric flask and volume was made with 0.01 N HCl to prepare 100 µg/ml and kept for ultrasonication for 5 min. The resulting standard stock solution of bulk drug was further diluted with Dil. HCl to get 15 µg/ml concentrations. The solution was scanned between 200-400 nm range against Dil. HCl as blank. After observing the UV-Spectrum of drug, 232 nm was selected as λ max for further analysis **(Fig. 1).**

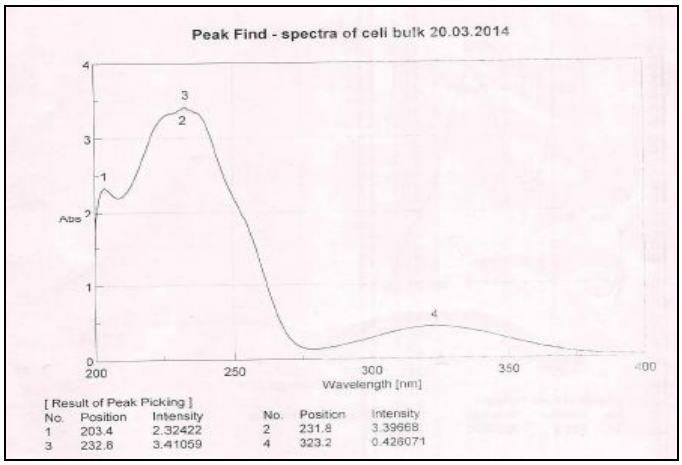


**FIG. 1 : λ MAX OF CPHL**

### Extraction of CPHL from tablet formulation: [11]

Accurately weighed quantity 10mg of drug was transferred to a volumetric flask and dissolved in the mixture of 30 ml water and 2.0 ml of conc. HCl. The sample was centrifuged, filtered and diluted with 0.01 N HCl up to the mark to prepare 100 µg/ml solution. Similarly, tablet powder equivalent to 10 mg of drug was also processed using water and conc. HCl to prepare 100 µg/ml solution. The absorption spectrums of series of dilution of CPHL extracted from tablet were scanned from 200 nm to 400 nm and compared with that of bulk solution **(Fig. 2 & 3).**

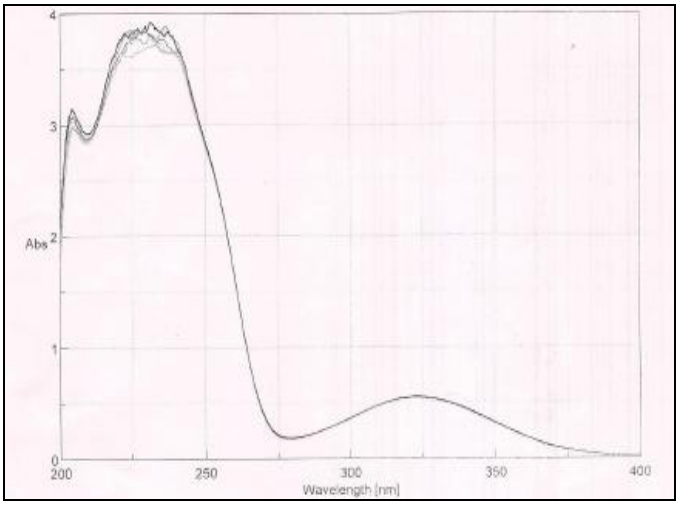


**FIG. 2: OVERLAY SPECTRA OF CPHL BULK**

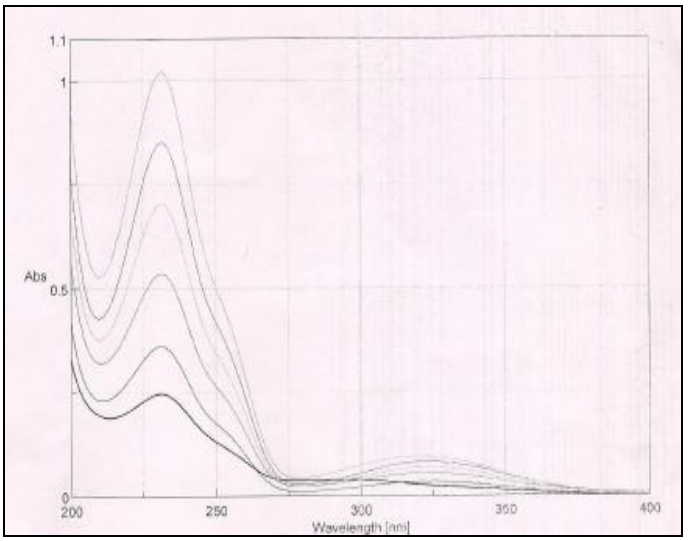


**FIG. 3: OVERLAY OF CPHL TABLET**

### Stability of solution: [12-13]

Series of dilutions of tablet stock solutions were prepared separately to get 3-18 µg/ml of working standards. The stability of these solutions was checked by measuring the solutions at 232 nm at two different time intervals, i.e. initially and after 72 hrs. The overlain spectra of tablet solution,

obtained at two different times were compared to verify the stability of solution. **(Fig.4** and **5)**.

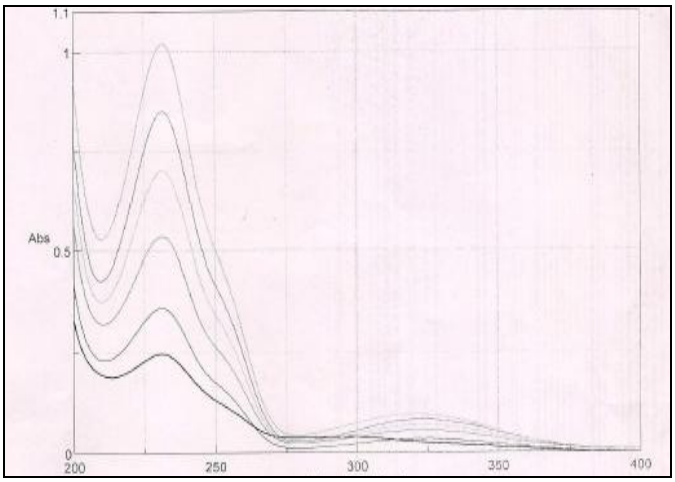


**FIG. 4: SPECTRA OF TABLET FORMULATION AT 0 HOURS**

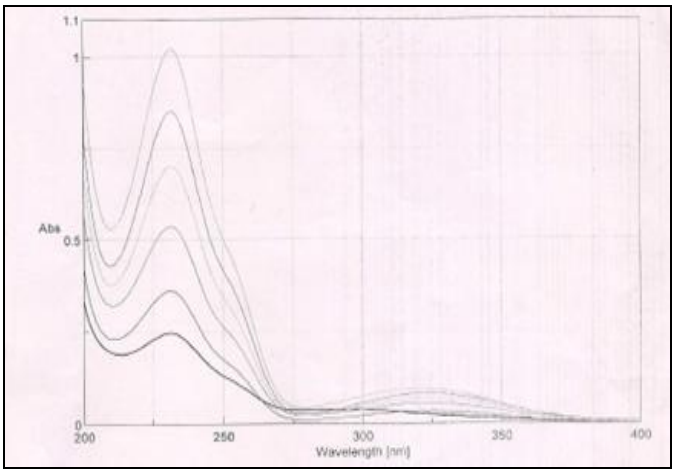


**FIG. 5: SPECTRA OF TABLET FORMULATION AT 72 HOURS**

## Preparation of standard and test solutions of tablet formulation:

The quantity of tablet powder equivalent to 10 mg drug was dissolved drug tablet powder was dissolved in 30ml distilled water & 2 ml conc. HCl and was transferred to 100 ml volumetric flask and volume was made with 0.01 N HCl and kept for ultrasonication for 5 min. It was filtered and volume was made up to the mark to prepare concentration of 100 µg/ml. From the resulting standard stock solution, series of dilutions were prepared to get 9, 12, and 15 µg/ml of laboratory samples.

## Estimation of drug concentration in test solutions:

**Method A-** Zero order derivative spectrometry [14-18]

In this method, calibration curve was obtained by plotting absorbance of working standards (3-18 µg/ml) against concentrations at 232 nm to get zero order spectra of CPHL**(Fig. 6** and **Table 1)**.

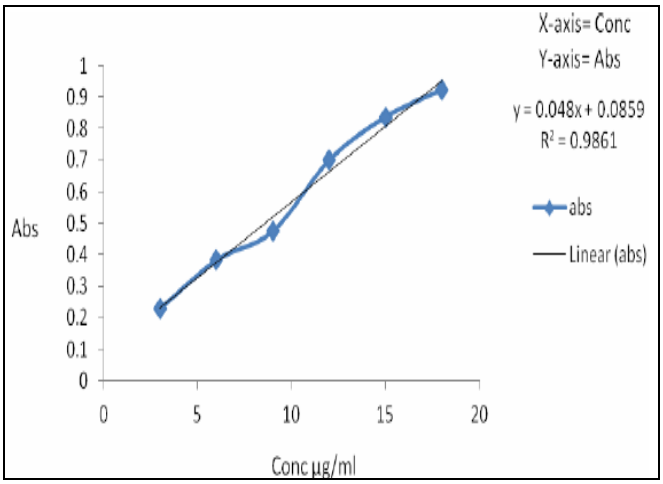


**FIG. 6: CALIBRATION CURVE OF CPHL**

**TABLE 1: CALIBRATION CURVE OF CPHL**

| Concentration(µg/ml) | Absorbnace |
|---|---|
| 3 | 0.2293 |
| 6 | 0.3829 |
| 9 | 0.4738 |
| 12 | 0.6989 |
| 15 | 0.8362 |
| 18 | 0.9213 |

The regression equation and correlation coefficient was obtained from the calibration curve. The absorbance of laboratory solutions of tablet (9, 12, and 15 µg/ml) was measured separately against 0.01 N HCl blank. From the regression equation, the concentrations of laboratory solution of tablet formulation were estimated. **(Table 2)**

**TABLE 2: RESULT FOR LABORATORY ANALYSIS OF TABLET FOR METHOD A & B**

| Conc (µg/ml) | Conc. Found Mean ±SD | | Content %w/v | | RSD % | |
|---|---|---|---|---|---|---|
| | Method A | Method B | Method A | Method B | Mtd A | Mtd B |
| 9 | 9.0919 ± 0.0271 | 9.0827±0.0048 | 100.6 | 100.9 | 0.2980 | 0.0531 |
| 12 | 11.999 ± 0.0181 | 12.081± 0.0128 | 99.99 | 100.5 | 0.1508 | 0.1059 |
| 15 | 15.0231± 0.0100 | 15.084± 0.0073 | 100.1 | 100.5 | 0.0665 | 0.0483 |

**Method B-Area under curve (AUC): [19-24]**

**Selection of analytical wavelength:**

For the selections of analytical wavelength, 15 µg/ml solution of drug was prepared and scanned in the spectra measurement mode across 200-400 nm range. From the spectra of drug, area under the curve in the range of 231-241 nm was selected for further analysis **(Fig. 1).**

**Estimation of drug concentration in laboratory samples:**

The calibration curve was obtained by plotting AUC of working standards in the range of 3-18 µg/ml against concentration **(Fig. 7).** The laboratory solutions of tablet (9, 12, and 15µg/ml) were measured separately against 0.01 N HCl blank. From the regression equation, the concentration of drug in the laboratory solutions of tablet was estimated **(Table 2).**

**Validation of method A and Method B for bulk and tablet formulation:**

The analytical methods were validated as per ICH guidelines [12] for the parameters linearity, range, accuracy, precision, limit of detection, limit of quantitation, and robustness.

**Range and Linearity study:**

Drug solutions of concentration from 3-18 µg/ml were measured at 232 nm for reading absorbance. The calibration curve was prepared. The correlation coefficient and regression equation was obtained. The range was established from linearity study. **(Table 3, Fig. 6)**

**TABLE 3: RESULT FOR VALIDATION PARAMETERS FOR BOTH METHODS**

| Parameters | | Method A | Method B |
|---|---|---|---|
| Analytical Wavelength (nm) | | 232 | 231-241 |
| Beer's law limit (µg/ml) | | 3-18 | 3-18 |
| Regression equation | | Y=0.048X + 0.0859 | Y=0.1007X + 0.203 |
| | | $R^2$= 0.9861 | $R^2$= 0.997 |
| Robustness (n=3) | Mean conc.±SD | 9.974± 0.0228 | 10.061± 0.0092 |
| | %RSD | 0.2273 | 0.0914 |

**Accuracy:**

Accuracy of tablet solution was determined by performing recovery studies by spiking pure drug in pre-analyzed sample solution at different level i.e. 80%, 100% and 120%. The resulting solutions were analyzed by proposed methods to estimate the concentrations. The percent recovery, SD and %RSD were calculated to establish the accuracy of the method. **(Table 4)**

**TABLE 4: RESULT FOR ACCURACY STUDY**

| Level % | Conc. (µg/ml) | | Total conc. µg/ml | Conc. found (µg/ml) ±SD (n=3) | | % Recovery Methods | | RSD % Methods | |
|---|---|---|---|---|---|---|---|---|---|
| | Taken | Standard added | | Method A | Method B | A | B | A | B |
| 80 | 8 | 6.4 | 14.4 | 14.17±0.02 | 14.12±0.01 | 98.42 | 98.12 | 0.14 | 0.070 |
| 100 | 8 | 8 | 16 | 16.06±0.01 | 16.07±0.01 | 100.3 | 100.4 | 0.062 | 0.070 |
| 120 | 8 | 9.6 | 17.6 | 17.05±0.01 | 17.54±0.01 | 100.9 | 99.67 | 0.057 | 0.057 |

**Precision:**

Precision of the method was studied as intra-day and inter-day variations. Intra-day precision was established by analyzing three concentrations (8, 10, and 18 µg/ml) of tablet formulation in triplicate, three times a day by the proposed methods, to estimate the concentration of drug in solutions **(Table 5).**

**TABLE 5: RESULT FOR PRECISION STUDY**

| Precision | Conc. (µg/ml) | Conc. Found Mean ±SD (n=3) | | Content % w/v Methods | | RSD % Methods | |
|---|---|---|---|---|---|---|---|
| | | Method A | Method B | A | B | A | B |
| Interday | 8 | 8.087 ±0.009 | 7.99±0.008 | 101 | 99.96 | 0.1112 | 0.1001 |
| | 10 | 10.08±0.003 | 10.07±0.004 | 100.8 | 100.7 | 0.0382 | 0.0397 |
| | 18 | 18.09 ±0.006 | 18.08±0.004 | 100.5 | 100.5 | 0.0331 | 0.0221 |
| Intraday | 8 | 7.978 ±0.010 | 7.99± 0.012 | 99.72 | 99.93 | 0.1253 | 0.1501 |
| | 10 | 10.07 ±0.012 | 10.04± 0.025 | 100.6 | 100.4 | 0.1121 | 0.2490 |
| | 18 | 18.08 ±0.089 | 18.06± 0.008 | 100.3 | 100.3 | 0.4922 | 0.0442 |

Inter-day precision was determined by analyzing three concentrations 8, 10, and 18 µg/ml of tablet formulation, in triplicate and on three consecutive days by the proposed methods to estimate the concentration of drug in solutions. For both inter and intraday precision, SD and % RSD were calculated, to ascertain the precision **(Table 5)**

**Robustness:**

For ascertaining robustness three different analysts analyzed the tablet solutions for estimation of drug concentration by proposed methods. Mean, SD, %RSD were calculated and reported to signify the robustness of the method **(Table 6).**

**TABLE 6: RESULT FOR ROBUSTNESS**

| Analyst | Concentration (µg/ml) | Mean (µg/ml) ±SD % | | %Content % w/w | | RSD % | |
|---|---|---|---|---|---|---|---|
| | | A | B | A | B | A | B |
| 1 | 10 | 10.045±0.007 | 10.073± 0.008 | 100.4 | 100.7 | 0.0696 | 0.0754 |
| 2 | 10 | 9.974±0.022 | 10.06±0.009 | 99.74 | 100.6 | 0.2205 | 0.0894 |
| 3 | 10 | 10.04±0.011 | 10.06± 0.012 | 100.4 | 100.6 | 0.1184 | 0.1192 |

## RESULT AND DISSCUSSION:

The present work was aimed to provide simple, accurate, cost economic and precise analytical methods for estimation of CPHL in tablet formulation. Solvent used for analysis of tablet was mixture of hydrochloric acid and water. The analytical wavelength for method A was determined and was observed 232 nm **(Fig. 1)**, and for that of method B was 231-241 nm. The drug was extracted from tablet formulation by using the mixture of water and dilute hydrochloric acid. Scans of working standard of tablet solution was compared with that of bulk solution of drug to verify that drug has properly extracted from tablet formulation (**Fig. 2** and **3**). The patterns of overlain spectra of tablet solutions obtained at two different time intervals, i.e. initially and after 72 hrs were found identical which confirmed that the analytical solutions were stable throughout of analysis time **(Fig. 4 & 5).**

The estimation of drug concentration in laboratory samples of tablet formulation by Method A (Zero order derivative spectrometry) at 232 nm was performed with three concentrations **(Table 2)**. The results of analysis were found with standard deviation from 0.0100-0.0271 and the % content was found in the range of 99.99-100.6%w/w. For method B (AUC method), the results of analysis were found with standard deviation from 0.0128-0.0073. and the % content was found in the range of 100.5-100.9%w/w.

Study of validation parameters revealed that Beer's law limit of the drug was 3-18 µg/ml. The regression equation for method A was Y=0.048X + 0.0859 with $R^2$= 0.9861 and for that of method B was Y=0.1007X + 0.203 with $R^2$= 0.997 (**Table 3**). Accuracy study (Table 4) revealed % Recovery for both the methods in the range of 98.12-100.9. The Precision **(Table 5)** was established with inter-day and intraday precision study. Both the methods were found robust (Table 6) with the standared deviation of 0.0228 and 0.0092 respectively.

**CONCLUSION:** The study of validation parameters revealed that both the methods were simple, accurate, precise, robost, and can be applied for routine analysis of tablet fromulation. Study conducted using aqueous solvent which made anlaytical methods cost-effective and ecofriendly.

**ACKNOWLEDGMENT:** This work was supported by Alard Charitble Trust's Alard College of Pharmac, Pune, Maharashtra, India.

**CONFLICT OF INTEREST**: No conflict of interest

## REFERENCES:

1. Detlev Ganten, Patrick J. Mulrow: Pharmacology of antihypertensive therapeutics, Springer Science & Business Media, 2012, 162
2. Milne RJ, Buckley MM-T: Celiprolol: An updated review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy in cardiovascular disease, Drugs 1991; 41:941–69.
3. https://pubchem.ncbi.nlm.nih.gov/compound/celiprolol_hydrochloride#section=TopRiddell,2016.

4. Kendall MJ, Rajman I: A risk-benefit assessment of Celiprolol in the treatment of cardiovascular disease, Drug Safety, 1994; 10:220–32.
5. Riddell J. Drugs in focus 18, Celiprolol. Prescriber, 1996; 36, 165–8.
6. http://www.mhra.gov.uk/home/groups/par/documents/websiteresources/con2033922.pdf
7. http://druginfosys.com/Drug.aspx/drugCode=1440&drugName=&type=0
8. www.drugfuture.com/mt/celiprolol-hydrochloride.pdf
9. http://druginfosys.com/Drug.aspx/drugCode=1440&drugName=&type=0
10. Indian Pharmacopoeia, Volume II, Indian Pharmacopoeia Commission, Gaziabad, 2014, 1330-1331
11. Klaus Florey, Harry G. Brittain, David J. Mazzo, Timothy J. Wozniak, Gerald S. Brenner, George A. Forcier, Abdullah A. Al-Badr: Analytical Profiles of Drug Substances and Excipients, Academic Press, 20, 1992, no 261.
12. ICH Harmonized tripartite guideline, validation of analytical Procedures: Text and Methodology, Q2 (R1), 2005
13. Taghreed M. Ibrahim, Validated UV-spectrophotometric estimation of diclazurilin API and drug product, Der Pharma Chemica, 2014; 6 (4), 140-146.
14. A. H. Becket, J.B. Stanlake: Practical Phamraceutical chemistry, 4th edition, part-2, CBS publishers & distributors, 2005; 279-280.
15. Jadhav Santosh, Kharat Rekha1, Pirjade Mujawar Farhat, and Tamboli Ashpak: Zero order and area under curve spectrophotometric methods for determination of Omeprazole capsules in pharmaceutical formulation, International Journal of Advances in Scientific Research, Research, 2015; (02), 102-107.
16. Joanna Karpinska: Basic Principles and Analytical Application of derivative Spectrophotometry, Macro to Nano Spectroscopy, In Tech, 2012; 244-269.
17. Ajit K. Nangare, Karan K. Pawa and Abhishek K. Shinde: Zero and first order derivative UV spectrophotometric methods for determination of Efavirenz in pharmaceutical formulation, Der Pharmacia Letter, 2014;6 (4), 143-150.
18. M Himaja , J Kalpana C Anbarasu: Validated Zero Order And First Order Derivative Spectrophotometric Methods For In vitro Analysis Of Tenofovir Disoproxil Fumarate Tablets Using Zeotropic Mixture, International Journal of Pharmacy and Pharmaceutical Sciences, Sciences, 2014; 6, (6), 302-304.
19. Rajan V. Rele: Simultaneous UV-Spectrophotometric estimation of amoxicillin and carbocisteine by area under curve method in combined dosage form, Der Pharmacia Letter, 2014; 6 (5), 1-7.
20. Aparna B. Chauhan, Dipti B. Patel: Area Under the Curve spectrophotometricmethod for determination of Tigecycline in Pharmaceutical Formulation., Journal of Pharmaceutical Science and Bio-scientific Research, 2012; 2 (2), 1, 88-91.
21. Harry G:, Profiles of Drug Substances, Excipients and Related Methodology, Britain, Academic Press, 2012; 37.
22. P.G. Bhange, A.P. Mehere, P.P. Katolka:, Development of UV spectrophotometric method of Cefixim in bulk and Pharmaceutical tablet dosage formulation, International Journal of Research and Development in Pharmacy and Life Sciences, 2013; 2,3, 460-463.
23. Rajan V. Rele and Prathamesh P. Tiwatane: UV spectrophotometric estimation of Carvedilol Hydrochloride by first order derivative and area under curve methods in bulk and pharmaceutical dosage form, Der Pharmacia Sinica, 2014; 5 (6), 29-35.
24. Jain Pritam S et al, Area Under Curve Method Development And Validation For Estimation Of Dronedarone In Bulk And Tablet Dosage Form, Journal of Pharmaceutical BioSciences , 2013; 2, 44-47.


**How to cite this article:**
Chavan M and Sutar M: Development and Validation of UV Spectrophotometric Methods for Estimation of Celiprolol Hydrochloride in Tablet Formulation. Int J Pharm Sci Res 2016; 7(9): 3781-86.doi: 10.13040/IJPSR.0975-8232.7(9).3781-86.