Vol 2|Issue 2| 2012 |111-118.

International Journal
of
Pharmacy Review & Research
www.ijprr.com

e-ISSN: 2248 – 9207
Print ISSN: 2248 – 9193

SPECTROPHOTOMETRIC DETERMINATION OF CINACALCET HYDROCHLORIDE IN BULK

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ABSTRACT

Literature survey reveals that few analytical methods were reported for the estimation of cinacalcet hydrochloride by LC, UV methods. Hence the present study aims in developing simple, rapid, precise and validated methods for cinacalcet hydrochloride in bulk. The methods are Colorimetric determination of Cinacalcet Hydrochloride by Visible Spectrophotometric method, Validation of Cinacalcet Hydrochloride by Visible Spectrophotometric method. The suitable solvent selected for performing estimation of Cinacalcet hydrochloride by UV spectroscopic method development and validation and fixed the λ_{max} for the drug cinacalcet hydrochloride. The present study successfully estimated the Cinacalcet hydrochloride from the formulation and performed validation studies of the drug Cinacalcet hydrochloride.

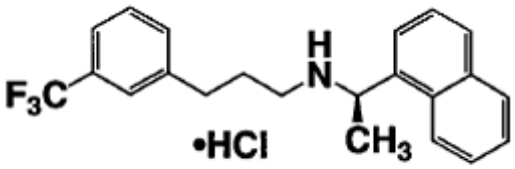
Key words: Cinacalcet hydrochloride, UV spectroscopic method, validation studies.

INTRODUCTION

Spectroscopy is a technique that measures the interaction of molecules with electromagnetic radiation. Light in the near-ultraviolet (UV) and visible range of the electromagnetic spectrum has an energy of about 150–400 kJ mol21. The energy of the light is used to promote electrons from the ground state to an excited state. A spectrum is obtained when the absorption of light is measured as a function of its frequency or wavelength. Molecules with electrons in delocalized aromatic systems often absorb light in the near-UV (150–400 nm) or the visible (400–800 nm) region. Absorption spectroscopy is usually performed with molecules dissolved in a transparent solvent. The absorbance of a solute depends linearly on its concentration and therefore absorption spectroscopy is ideally suited for quantitative measurements. The wavelength of absorption and the strength of absorbance of a molecule depend not only on the chemical nature but also on the molecular environment of its chromophores. Absorption spectroscopy is therefore an excellent technique for following ligand-binding reactions, enzyme catalysis and conformational transitions in proteins and nucleic acids. Spectroscopic measurements are very sensitive and nondestructive, and require only small amounts of material for analysis [1-5].

Drug Profile

CINACALCET HYDROCHLORIDE



Molecular Structure

[(1R)-1-(naphthalen-1-yl)ethyl]({3-[3-(trifluoromethyl)phenyl]propyl})amine

Molecular Formula : $C_{22}H_{22}F_3N$
Molecular Weight **:** 357.412
Indications

For the treatment of secondary hyperparathyroidism in patients with Chronic Kidney Disease who are on hemodialysis or peritoneal dialysis. Also for the treatment of hypercalcemia in patients with parathyroid carcinoma.

Overdose

Overdosage may lead to tachycardia, severe hypotension and Convulsions.

Mechanism of action

The calcium-sensing receptors on the surface of the chief

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cell of the parathyroid gland are the principal regulator of parathyroid hormone secretion (PTH). Cinacalcet directly lowers parathyroid hormone levels by increasing the sensitivity of the calcium sensing receptors to activation by extracellular calcium, resulting in the inhibition of PTH secretion. The reduction in PTH is associated with a concomitant decrease in serum calcium levels.

Absorption: Rapidly absorbed following oral administration

Distribution: Widely distributed.

Plasma protein binding: Approximately 93 to 97% bound to plasma proteins

Metabolism: Metabolized hepatically.

Excretion

Elimination half-life: Terminal half-life is 30 to 40 hours. The mean half-life of cinacalcet is prolonged by 33% and 70% in patients with moderate and severe hepatic impairment, respectively. Mainly excreted in the urine as unchanged drug and metabolites.

Adverse Drug Reaction

GI disturbances, headache, vertigo, syncope, rash, pruritus, paraesthesia, cephalea, light headedness, dizzing, itching, flushing.

Drug Interactions

Erythromycin-may increase the serum concentration and toxicity, itraconazole, ketoconzole, tamoxifen may increase the effect and toxicity of cinacalcet [6-11].

REVIEW OF CINACALCET HYDROCHLORIDE

Al-Hilali N et al, **(**2011), cinacalcet has markedly improved the management of hyperparathyroidism in patients on hemodialysis. However, to the best of our knowledge, there are no specific studies addressing the dose regimen of cinacalcet. The aim of the study was to evaluate the efficacy of cinacalcet on the achievement of targets in the treatment of hyperparathyroidism in two different dosage schedules. Twenty-seven adult patients who were on hemodialysis for more than four months and with severe secondary hyperparathyroidism (intact parathyroid hormone (iPTH) >88 pmol/L) resistant to conventional treatment were included in this prospective study. We used the targets of K/DOQI-clinical guidelines as optimal target of iPTH, calcium and phosphate. Group 1 received a single daily administration of 30 mg of cinacalcet along with the main meal as the starting dose, and the dose was titrated thereafter monthly. There was no noteworthy difference in side effects between both the groups. Our results indicate that cinacalcet twice weekly is reasonably safe and effective in suppressing high PTH levels in hemodialysis patients, with fewer side effects.

Raggi P et al, (2004**)** This prospective, randomized, controlled trial compared the progression of vascular and cardiac valve calcification in 360 prevalent adult hemodialysis patients with secondary hyperparathyroidism treated with either cinacalcet plus low-dose vitamin D sterols or flexible doses of vitamin D sterols alone. In hemodialysis patients with moderate to severe secondary hyperparathyroidism, cinacalcet plus low-dose vitamin D sterols may attenuate vascular and cardiac valve calcification.

Peacock M et al, (2011) primary hyperparathyroidism (phpt) is characterized by elevated serum calcium (ca) and increased pth concentrations. Objective: the objective of the investigation was to establish the efficacy of cinacalcet in reducing serum ca in patients with phpt across a wide spectrum of disease severity. Design and setting: the study was a pooled analysis of data from three multicenter clinical trials of cinacalcet in phpt. Patients: patients were grouped into three disease categories for analysis based on the following: 1) history of failed parathyroidectomy (n = 29); 2) meeting one or more criteria for parathyroidectomy but without prior surgery (n = 37); and 3) mild asymptomatic phpt without meeting criteria for either above category (n = 15). Intervention: the intervention in this study was treatment with cinacalcet for up to 4.5 yr. outcomes: measurements in the study included serum ca, pth, phosphate, and bone-specific alkaline phosphatase, and areal bone mineral density (abmd). Vital signs, safety biochemical and hematological indices, and adverse events were monitored throughout the study period. Cinacalcet is equally effective in the medical management of PHPT patients across a broad spectrum of disease severity, and overall cinacalcet is well tolerated.

Wetmore JB, et al, (2010**)** fibroblast growth factor-23 levels are elevated in esrd and have been associated with adverse outcomes. The effects of various treatments for secondary hyperparathyroidism on levels in esrd have not been examined in a clinical trial. design, setting, participants, & measurements: we assessed intact fgf23 levels in 91 subjects over the course of the achieve trial, which was designed to compare escalating doses of cinacalcet plus fixed low-dose calcitriol analogs (cinaclcet-d) with titration of calcitriol analogs alone (flex-d) to suppress parathyroid hormone. Between-group and within-group changes in log-transformed fgf23 levels were analyzed. Factors associated with change in fgf23 were assessed using a multiple regression model. treatment with cinacalcet plus low-dose calcitriol analogs results in lower levels compared with a treatment regimen using calcitriol analogs alone in ESRD. The mechanisms underlying the differential effects of these treatment regimens on levels and the clinical impact of these changes on fgf23 remain to be defined.

Muscheites J et al, (2010**)** ,The efficacy and acceptability of cinacalcet for treatment of secondary hyperparathyroidism (SHPT) was assessed in seven pediatric patients suffering from end-stage renal disease (ESRD) presenting with inadequately controlled SHPT despite conventional management. Patients received daily treatment with cinacalcet (dosage 0.25 mg/kg body weight) for a total of 4 weeks. Within 4 h after application of the first dose, median levels of serum parathyroid hormone (PTH) had decreased from 932 pg/ml (range 511-1,938 pg/ml) to 584 pg/ml (88-937 pg/ml), and final pre-dose values after 4 weeks were 199 pg/ml (121-940 pg/ml; each $P < 0.05$ versus baseline). Median concentrations of serum calcium (Ca) decreased within 4 h of the first

administration, from 2.56 mmol/l to 2.38 mmol/l, returning to 2.58 mmol/l at 24 h, and they remained slightly decreased compared to baseline values thereafter (each P < 0.05 versus baseline). Both the median levels of serum phosphorus (P) and the Ca x P ion product decreased significantly during the 4-week period. Cinacalcet was well tolerated and without drug-related adverse effects. Thus, even with approximately half of the dose usually given to adult dialysis patients, PTH and the Ca x P ion product were markedly reduced in pediatric ESRD patients presenting with inadequately controlled SHPT. Therefore, our results support the initiation of a randomized, controlled, long-term trial in children.

Schaefer RM et al, (2010) Cinacalcet, a novel calcimimetic, simultaneously lowers parathyroid hormone (PTH), phosphorus (P), calcium (Ca) and Ca x P in patients who are on dialysis with secondary hyperparathyroidism (sHPT) associated with CKD. Previous studies have required cinacalcet to be administered during the dialysis session and at the same time on non-dialysis days. The aim of the sensor study was to demonstrate that cinacalcet given in a more clinically practical manner with the first major meal after dialysis is noninferior to cinacalcet given with food during the dialysis session. In this open-label study dialysis patients with poorly controlled sHPT (intact PTH (iPTH) (3) 300 pg/ml) were randomized to receive cinacalcet either daily with their post-dialysis meal (n = 337) or with food during the dialysis session (n = 336). The primary endpoint was the proportions of patients with mean iPTH pound 300 pg/ml (pound 31.8 pmol/l) at Weeks 11 and 13 of a 21-week treatment period. Secondary endpoints included the proportion of patients with Ca x P < 55 mg2/dl2 (< 4.44 mmol2/l2) at Weeks 11 and 13 and patients who discontinued the study due to nausea or vomiting. Administering cinacalcet with the first main meal after dialysis was as effective as administration with food during the dialysis session. Cinacalcet was well tolerated. The incidence of gastrointestinal adverse events appeared to be lower when cinacalcet was administered in the evening.

Padhi D et al, (2008) Cinacalcet HCl (cinacalcet), approved for secondary hyperparathyroidism and parathyroid carcinoma-associated hypercalcaemia, may be coadministered with warfarin. The purpose of this study was to determine the pharmacokinetics/pharmacodynamics and tolerability of warfarin during cinacalcetcoadministration. In this phase 1, randomised, double-blind, placebo-controlled, two-treatment, two-period crossover study, 21 healthy subjects received oral cinacalcet (30 mg) or placebo twice a day for 7 days and once on day 8, with a single warfarin dose (25mg) on day 5. After a 3-week washout, subjects received the alternative treatment. Samples for warfarin pharmacokinetics/pharmacodynamics were obtained until 144 hours post-dose. Single-dose administration of warfarin to subjects receiving cinacalcet did not demonstrate altered pharmacodynamics of either the R- or S-enantiomer. Geometric means ratio (90% CIs) for R- and S-warfarin [12-19].

MATERIALS AND METHODS

Chemicals and solvents

- Methanol AR grade,
- Distilled Water
- Hydrochloric acid
- 1,4 Naphthoquinonesulphonate reagent
- Tris buffer

Instruments Used

- SHIMADZU UV Pharmspec Spectrophotometer 1700
- SHIMADZU (ELB 300) Electronic balance
- SHIMADZU (BL 22OH)Electronic balance
- TOSHIBHA (India) Ultra sonicator

METHOD DEVELOPMENT OF CINACALCET BY VISIBLE SPECTOSCOPY

Selection of Various Parameters

1. **Selection of solvent**
   Solubility of the drug in different solvents were tried. Cinacalcet Hydrochloride was soluble in Methanol, Ethanol, acetonitrile, and 0.01N HCl. As the drug showed good spectrum and was stable in methanol, it was selected as a solvent of choice.
2. **Preparation of standard stock solution**
   Stock solution of Cinacalcet Hydrochloride was prepared by dissolving 10mg of pure drug in 100ml methanol to obtain a concentration of 100 μg/ml.
3. **Selection of wavelength**
   The stock solution was suitably diluted with methanol:water in the ratio of 1:1 to obtain a concentration of 10μg/ml of Cinacalcet Hydrochloride. The solution was scanned in the UV-region from 200 to 400 nm and found that Cinacalcet Hydrochloride exhibited maximum absorbance at about 271nm. Hence 271 nm was selected for the proposed study.
4. **Preparation of standard curve**
   Adequate dilutions were made from the stock solution to get concentration ranging from 1-10μg/ml of CinacalcetHydrochloride using Methanol:Water in the ratio of 1:1. Absorbance of these solutions were measured at 271nm. The measured absorbanceswere plotted against concentration. From the graph it was found that the beer's law concentration for CinacalcetHydrochloride lies between 1-10 μg/ml (Table 1)

VALIDATION OF THE METHOD

The developed method was validated in terms of linearity and stability studies.

1. **Linearity**
   Cinacalcet Hydrochloride was found to be linear in a concentration range of 1 to 10 μg/ml. The absorbance of thesesolution measured at 271nm and calibration graph was plotted using absorbance Vs concentration. The slope, intercept, and correlation coefficient were found to be 0.0689, 0.0091 and 0.9994 respectively (table 3).

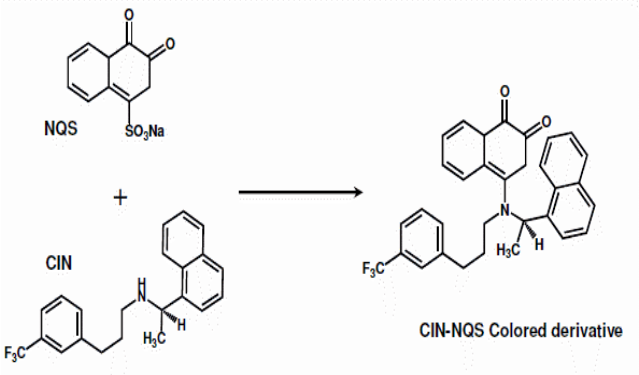
2. Stability

The drug solution was found to be stable for 24hrs about at room temperature and refrigeration conditions (table 2) [20-24].

COLORIMETRIC DETRIMINATION OF CINACALCET HYDROCHLORIDE BY VISIBLE SPECTROSCOPIC METHOD

This method is based on the reaction of cinacalcet Hydrochloride with 1,4naphthoquinonesulphonate in presence of tris buffer to form orange yellow coloured chromogen. The 1,4naphthoquinonesulphonate combines with hydrazine sulphate and forms 2,4 dinitrophenylhydrazine. Treating this complex with cinacalcet Hydrochloride will give orange yellow colour. The orange yellow colour chromogen has absorbance maxima in the visible region.

Reaction



Preparation of Solutions

Standard CIN Solution

An accurately weighed amount (50 mg) of CIN was quantitatively transferred into a 25-ml calibrated flask, dissolved in 20 ml distilled water, completed to volume with the same solvent to obtain a stock solution of 2 mg/ml. The stock solution was found to be stable for at least two weeks when kept in a refrigerator. The stock solution was further diluted with water to obtain working solutions in the range of 1 – 10µg/ml.

Preparation of 0.5%w/v 1,4naphthoquinonesulphonate Reagent

500mg of 1,4 NQS reagent was taken in25 ml flask and 100 ml of Tris buffer was prepared by mixing 100 ml 0.1 M tris(hydroxymethyl) amino methane with29.4 ml of 0.1 M HCl was added and mixed well.

Optimization of Reaction Conditions

Effect of NQS Concentration

Studying the effect of NQS concentration on its reaction with CIN revealed that the reaction was dependent on the NQS concentration as the readings increased with the increase in the reagent concentration. The highest readings were attained at a concentration range of 0.4 – 0.6% (w/v) beyond which the readings slightly decreased. A concentration of 0.5% (w/v) was used in all the subsequent experiments.

Volume of Tris buffer

Keeping the standard drug solution 1ml and the volume of 1,4NQS reagent as fixed 3 ml, various volume of tris buffer solution was added and noted for the chromogen formation and absorbances noted shown in table 5.

Order of addition of reagent

Effect of order of addition of reagents on chromogen formation was studied by changing the order as shown in table 6

- Drug +Tris buffer + 1,4 naphthoquinonesulphonate reagent
- Drug + 1,4naphthoquinone sulphonate reagent + Tris buffer

Even by changing the order of addition blue coloured chromogen was formed and λmax was546 nm. Whereas good absorption and linearity was observed only when the order is kept as Drug + 1,4 NQS +Tris buffer.

Effect of Temperature and Time

The effect of temperature on the reaction was studied by carrying out the reaction at room temperature (25 ± 2 °C) and at varying elevated temperatures (30 – 60 °C). The results (Figure 3) revealed that there was no significant difference between the readings that have been obtained at room temperature and those at elevated temperatures up to 50 °C, beyond which the readings significantly decreased. In order to establish simple analytical procedures with no need for extra equipment (water bath), further experiments were carried out at room temperature. In order to determine the optimum time that is required for completion the reaction, it was allowed to proceed at room temperature for varying periods of time. It was found that the reaction goes to almost completion within 5 min (Figure 3), however for higher precision readings, the reaction was allowed to proceed for quite longer time; reactions in all the subsequent experiments were carried out for 10 min [25-31].

VALIDATION PARAMETERS

Linearity

Cinacalcet Hydrochloride was found to be linear in a concentration range of 1-10 µg/ml. The absorbance of this solution was measured at 546 nm and calibration graph was plotted using absorbance V_S Concentration. The slope intercept and correlation coefficient values were found to be 0.9997 respectively table7.

Robustness and Ruggedness

Robustness was examined by evaluating the influence of small variation in the method variables on its analytical performance. In these experiments, one parameter was changed whereas the others were kept unchanged, and the recovery percentage was calculated each time. It was found that small variation in the method variables did not significantly affect the procedures. This indicated the reliability of the proposed method during its routine application for the analysis of cinacalcet hydrochloride.

Ruggedness

Ruggedness was also tested by applying the proposed methods to the assay of CIN using the same operational conditions but using two different instruments at two different laboratories and different elapsed time. Results obtained from lab-to-lab and day-to-day variations were reproducible, as the relative standard deviations (RSD) did not exceed 2%. The proposed method gave satisfactory results with CIN in bulk [31-36].

RESULTS

The proposed colorimetric method for of Cinacalcet hydrochloride in bulk form is simple, rapid, precise and can be employed for routine analysis. Once the absorbance of the samples is determined, it required only simple calculations. This method can be applied for substances obeying Beer's law.

Table 1. Calibration Graph of cinacalcet Hydrochloride

Concentration (μg/ml)	Absorbance(275nm)
1	0.092
3	0.215
5	0.345
7	0.498
10	0.698

Table 2. Stability studies

Time (hrs)	Drug	Absorbance at (271 nm)	
		Room temperature	Refrigerator
0	CinacalcetHydrochloride 5 μg/ml	0	0
1		0.173	0.184
4		0.167	0.154
8		0.151	0.141
12		0.144	0.132
18		0.121	0.102

Table 3. Validation parameters

Parameters	Values
λ_{max}(nm)	271
Beer's law limit (μg/ml)	1-10
Slope (b)	0.0689
Intercept (a)	0.0091
Correlation co-efficient	0.9994

Table 4. Volume of fix reagent

Volume of 1,4 naphthoquinonesulphonate(ml)	Absorbance (546 nm)
0.5	0.002
1	0.094
1.5*	0.103
2	0.213
2.5	0.308
3	0.450

Table 5. Selection of volume of Tris buffer (0.5% w/v)

Volume (ml)	Colour	Absorbance(546nm)
0.5*	**Orange yellow colour**	**0.361**
1 ml	OrangeYellow colour	0.139
1.5 ml	Orange yellow colour	0.115
2ml	OrangeYellow colour	0.087

Table 6. Stability for order of addition

Time (mins)	Absorbance (546 nm)	
	(i)	(ii)
10	0.145	0.152
15	0.157	0.143
20	0.148	0.137
30	0.135	0.126
1 hr	0.156	0.118
2hr	0.144	0.109

Table 7. Validation parameters

Parameters	Values
Colour formed	OrangeYellow
λ_{max} (nm)	546
Linearity range (μg/ml)	1-10
Colour stability (hr)	3hr
Intercept (a)	0.0114
Slope (b)	0.0866
Correlation coefficient	0.9997

Table 8. Calibration graph of Cinacalcet Hydrochloride with reagent

Concentration (μg/ml)	Absorbance(546nm)
1	0.1
3	0.282
5	0.45
7	0.622
10	0.873

Fig 1. Calibrationgraph of Cinacalcethydrochloride

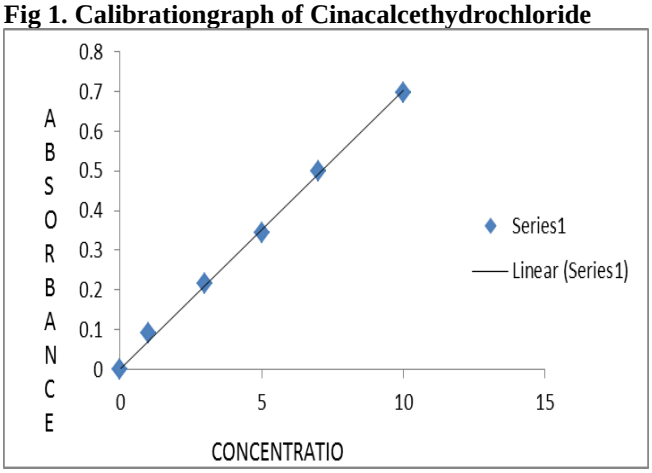


Fig 2. Calibration of cinacalcet hydrochloride with reagent

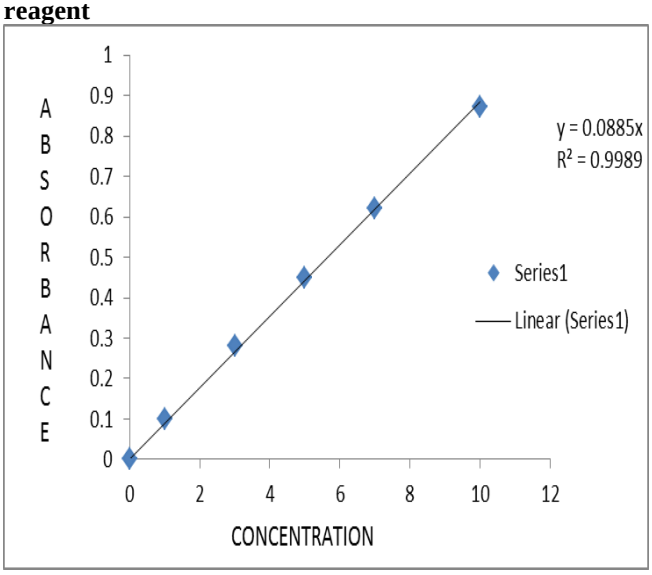


Fig 3. Absorbance Spectrum of Cinacalcet Hydrochloride

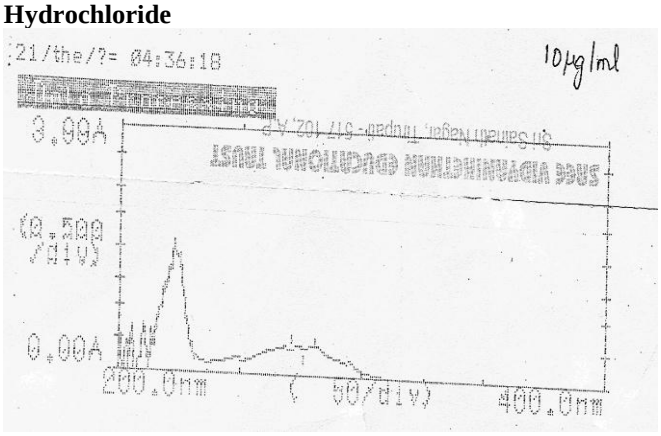
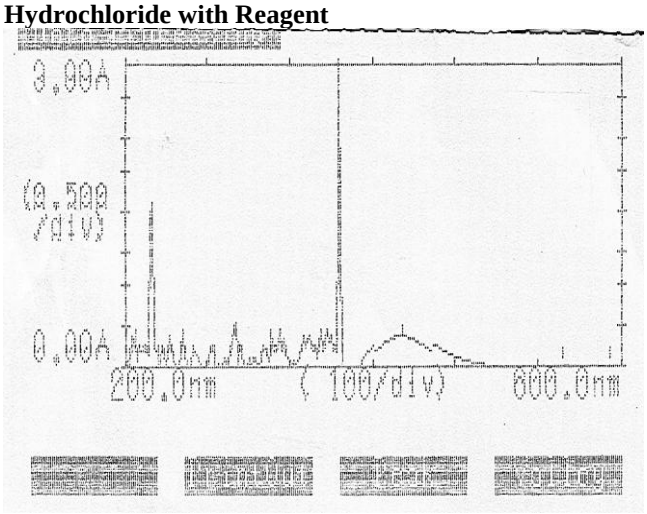


Fig 4. Absorbance Spectrum of Cinacalcet Hydrochloride with Reagent



DISCUSSION AND CONCLUSION

Cinacalcet hydrochloride is used to treat hyperparathyroidism drug. The present work is on method development of cinacalcet by visible spectroscopy. First appropriate reagent is selected for this method. 1,4 NQS is selected as the reagent and the solubility of the reagent was checked. It was found that reagent was soluble in distilled water. Then the concentration and the volume of reagent were optimised. 3ml of reagent was used. In presence of Tris buffer the colour was developed and volume and strength of tris buffer was optimised i.e, 0.5 ml of 5%w/v. The λmax was found at 546 nm and calibration graph was plotted and the linearity was obtained in the range of 1 to 10µg/ml. The slope, intercept and the correlation factor ofcinacalcet hydrochloride at 546 nm was found to be 0.00141, 0.00868, 0.9997. The method was validated and the precisionlinearity, LOD, LOQ were calculated. Interday and intraday precision were performed at 546 nm and the %RSD was found to be 1.22 which is <2. Stability studies were performed the reagent along with the drug was stable for 2 hrs. Beer's limit was found in the concentration range of 1-10 µg/ml. Within this range the drug shows, linearity, precision, ruggedness, robustness.

The present study described the successful evaluation of NQS as an analytical reagent in the development of simple and rapid spectrophotometric method for the accurate. The proposed method used inexpensive reagents with excellent shelf life, and is available in any analytical laboratory. Therefore, the method is practical and valuable for its routine application in quality control laboratories for analysis of cinacalcet hydrochloride.

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