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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE DETERMINATION OF CINITAPRIDE BY USING UV-DETECTION METHOD

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ABSTRACT

A sensitive and specific Reverse Phase High Pressure liquid chromatographic method for the determination of Cinitapride by using shimadzu HPLC Class VP Series, Phenomenex C_{18} (150x4.6mm i.d.., Particle size5µm) column in isocratic mode with mobile phase containing PH-4 buffer and Acetonitrile in the ratio of (65:35) the flow rate was 1.0ml.min and the eluent was monitored at 262nm. The selected chromatographic conditions were found to be effectively separate Cinitapride at 4.7mins. The assay of Cinitapride was linear, Calibration curve over the range 25-150 µg/ml. The method shows precision value at 0.90 in tablets. The proposed method was statistically evaluated and validated for linearity accuracy, precision and selectivity follows ICH recommendations due to its simplicity, Accuracy and uniqueness as it offers a significant advantage in determining the Cinitapride and this method can be used for routine quality control analysis.

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INTRODUCTION

Cinitapride is chemically known as *(RS)*-4-amino-*N*-[1-(1-cyclohex-3-enylmethyl)-4-piperidyl]-2-ethoxy-5-nitro-benzamide.Cinitapride is a substituted benzamide gastroentric prokinetic agent acting via Complex,but synergistic effects on serotonergic 5-HT_2(inhibtion) and 5 HT_4(Stimulation) receptors and dopaminergic D_2(inhibition) receptors in the neuronal synapse of the myenteric plexus.

However the present literature survey shows that there are very few method are present for the determination of cintapride in the both bulk and formulations .The aim of this present work is to develop an accurate , specific, repeatable and validated method for the determination of cintapride in formulations.

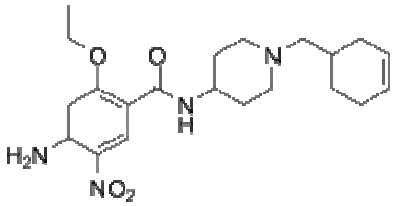


Fig: 1

MATERIALS AND METHODS:

Cinitapride Hydrogen tartarate tablets containing 1mg were obtained from cipla therapeutic index. Acetonitrile is of HPLC grade, potassium di hydrogen phosphate and Ortho- Phosphoric acid is of GR grade were obtained from Merck.

HPLC METHOD AND CHROMATOGRAPHIC CONDITIONS:

The chromatography estimation was performed by using following conditions, Shimadzu HPLC, Phenomenex C_{18} (150x4.6mm i.d.., Particle size5µm) column and mobile phase containing PH-4 buffer (Prepared by1.36g of potassium di hydrogen phosphate dissolve it in 1000ml of miliQ-water , add 1.5ml of Triethylamine and Adjust the PH to 3 ± 0.5 with dil.Orthophosphoric acid) and Acetonitrile in the ratio of (65:35) were passed through 0.45µm nylon membrane filter and degas .The Flow rate is monitored at1.0ml/min the column temperature is maintained at ambient temperature and detection was carried out at 262nm.

PROCEDURE:

CNP Standard stock Preparation

Weigh and transfer accurately about 10.0 mg of CNP standard into a 100 ml clean dry volumetric flask, add about 60 ml of diluent, sonicate for 5 minutes, and dilute to volume with diluent.

Preparation of Sample solution:

5 tablets of Cinitapride were powdered and an amount of the powder equivalent to 1000µg of drug was accurately weighed and transferred to the 100ml volumetric flask made up to the volume with diluents. The solution was sonicated for 20mins and filter through 0.45µm membrane filter to get 10µg/ml solution

Assay of Cinitapride standard solution:

Pipette out 10 µl of the diluted Cinitapride standard stock solution was injected each time into the column at flow rate of 1.0ml/min. Evaluation of the drug was performed with PDA detector at 262nm. Peak area was recorded for all peaks. A plot for peak area versus the respective concentration over the peak area was computed. The retention time and average peak areas were recorded. Calibration graph was plotted by taking concentration of CNP on X-axis and peak areas on Y-axis. The regression equation was used to estimate the amount of Cinitapride in tablets.

Assay method for tablets:

5 tablets of Cinitapride were powdered and an amount of the powder equivalent to 1000µg of drug was accurately weighed and transferred to the 100ml volumetric flask made up to the volume with diluents. The solution was sonicated for 20mins and filter through 0.45µm membrane filter to get 10µg/ml solution. Separately inject 20µl of the blank, Standard (five injections) and sample solution in duplicate into the liquid chromatograph, record the chromatographs and measure the peak areas. Precision of the method is expressed in terms of % RSD.

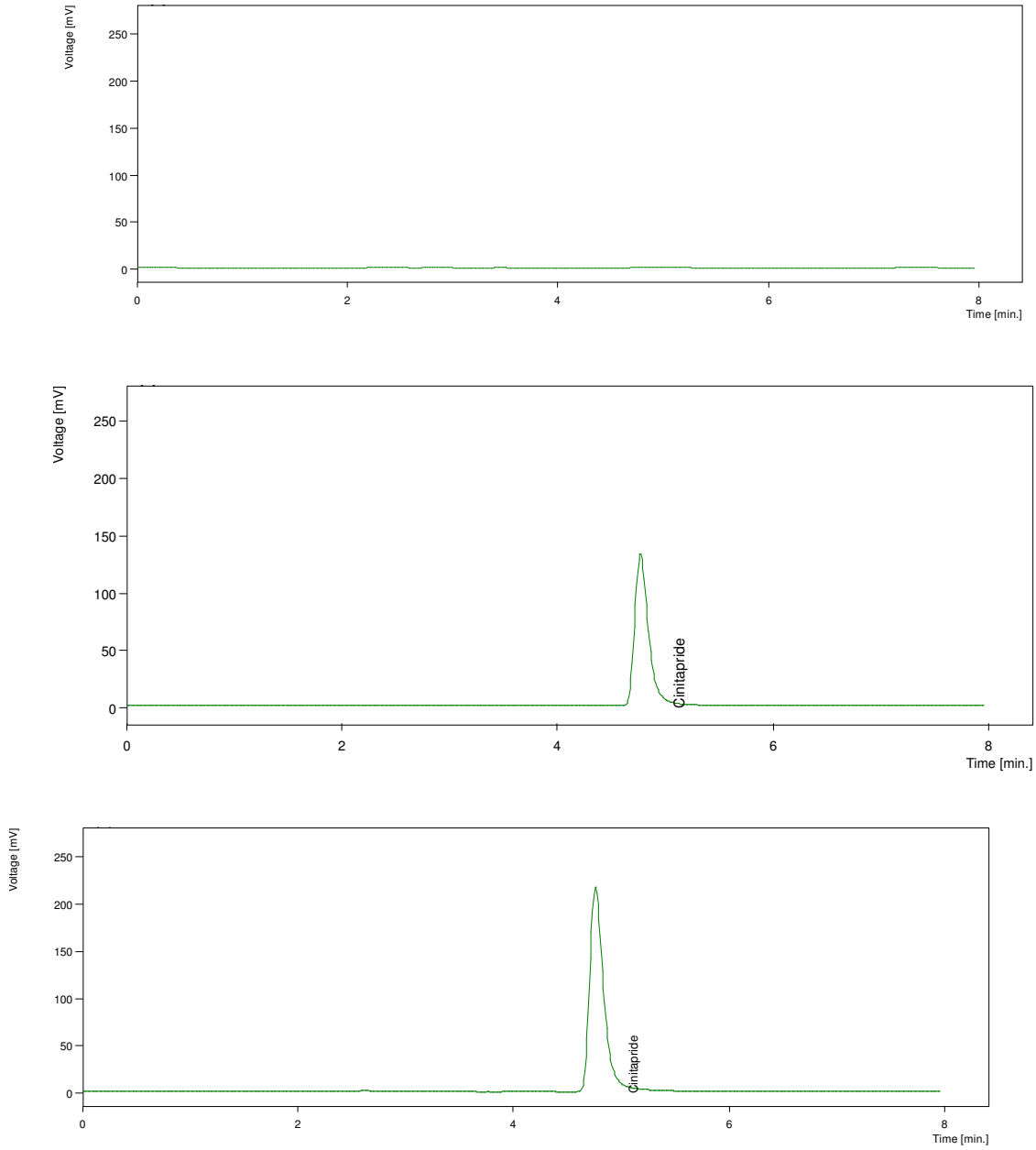
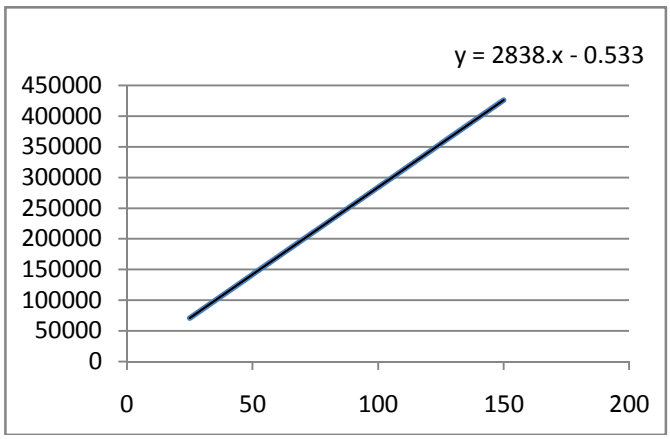


Table: 1 Calibration of proposed method

Drug conc.(µg/ml)	Peak Area
25	70971
50	141943
75	212915
100	283887
125	354858
150	425830



Regression equation from 25-150µg/ml Y=2838 x-0.533 (R^2=0.999)

Table:2 Recovery studies

Label Claimed	Amt of Drug	Recovery from drug		Recovery from tablets	
		Mean amt found	Mean % recovery	Mean amt found	Mean % recovery
100 µg	25	24.93	99.72	24.98	99.92
	50	49.95	99.90	49.97	99.94
	75	74.94	99.91	74.96	99.95
	100	99.90	99.90	99.98	99.98

Table: 3 Assay of Cinitapride in tablets

S.No	Label Amt of drug (µg)	mean±(S.D) Amt found by proposed method	Mean of % labeled amt
Tablet-I	1 mg	0.98 ± 0.01	98.83 ± 0.90
Tablet-II	1mg	0.99 ± 0.004	99.13 ± 0.40

Table: 4 Precision of the proposed method

Conc. Cinitapride (µg/ml)	Observed conc. Of Cinitapride			
	Intra-Day		Inter-day	
	Mean	CV	Mean	CV
25	24.92	0.132	24.59	0.057
50	49.94	0.068	49.33	0.33
75	74.93	0.049	74.65	0.315
100	99.95	0.015	99.96	0.015
125	124.95	0.020	124.95	0.034

SUMMARY TABLE:

Parameters	Results
Retention time	4.9mins
Theoretical plates	12593
Linearity range (µg/ml)	25-150
LOD (µg/ml)	0.054
LOQ (µg/ml)	0.164
Regression equation	Y=2838 x-0.533
Precision (%RSD) Intra-Day Inter- Day	 0.0568 0.1502

RESULTS AND DISCUSSION:

The present study was carried out to develop a sensitive, Precise and accurate HPLC method for the analysis of CNP in bulk samples and its formulations. The retention time for CNP is 4.9mins each sample was injected 5times and the same retention times were observed in all cases. The peak area of different conc. set up as above was calculated. The peak area for the solution was reproducible as indicated by low co-efficient of variation 0.9. A good linear relationship was observed between the concentration of CNP and respective peak areas. The calibration graph was found to be Y=2838 x-0.533 where y is the peak area and x is concentration of CNP in the range of 25-150 µg/ml when we analyzed the proposed Rp-HPLC method for finding out intra-day and inter-day variations a low co-efficient of vriation was observed(table:4) this shows that the present HPLC method is highly precise. The drug content in tablets was quantified using the proposed analytical method .The mean content of CNP in tablets is shown in table:3. The amt of CNP fro mthe pre-analyzed samples containing known amount of drug is shown in table:2 about 99.13 % CNP could be recovered from pre-analyzed samplesindicating the high accuracy of proposed HPLC method

The proposed method is validated accordimg to ICh guidelines.The proposed RP-HPLC method was found to be simple, precise, highly accurate, Specific and less time consuming .It can be concluded that the proposed method was suitable for the estimation of CNP and in routine quality control analysis.

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