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Development and Validation of a specific stability indicating RP-HPLC method for quantitation of Cinitapride hydrogen tartrate in bulk drug

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ABSTRACT: A selective stability-indicating high performance liquid chromatographic method has been developed and validated for analysis of Cinitapride hydrogen tartrate in bulk drug. Reversed phase chromatography was performed on a C18 column using acetonitrile-ammonium acetate buffer (0.01M), 75:25 (%, v/v), as mobile phase at a flow rate of 1.0 ml/min. Detection was performed at 263 nm and a sharp peak was obtained for Cinitapride hydrogen tartrate at retention time of 5.383±0.01 min. The results of analysis have been validated as per ICH guideline. Drug showed linear response in the concentration range of 20-120 µg/ml; the regression coefficient was 0.999 and the linear regression equation was y = 70590x+14599. The detection and quantification limits were 0.679 and 2.058 µg/ml respectively. Mean percentage recovery for Cinitapride hydrogen tartrate was 100.166%. Specificity of the HPLC method was checked by injecting the degradation products. Drug was subjected to acidic, alkaline, neutral and oxidative hydrolysis, dry heat and photolytic degradation. Results of forced degradation studies indicated that the drug undergoes extensive degradation under acidic hydrolysis, to a reasonable extent under oxidative, neutral and alkaline hydrolysis, but it was stable towards dry heat and photolytic conditions. The degradation products were well resolved from the peak of pure drug with significant differences in their retention time values. The method was employed for quantitation of Cinitapride hydrogen tartrate in marketed tablet formulation with mean±standard deviation assay value of 99.3604±0.1318. Since the method could effectively separate the drug from its degradation products, it could be regarded as stability indicating one.

KEYWORDS: Cinitapride Hydrogen Tartrate, Stress studies, RP-HPLC, Validation

I. INTRODUCTION

Cinitapride hydrogen tartrate [1-2] (CNT, Fig.1) is chemically 4-amino-N-[3-(cyclohexan-1-yl-methyl)-4-piperidinyl]-2-ethoxy-5-nitrobenzamide. It has molecular formula of $C_{21}H_{30}N_4O_4$ and molecular weight of 402.49. It is a gastro enteric prokinetic agent and shows synergistic effects on serotonergic 5-HT2 (inhibition), 5-HT4 (stimulation) and dopaminergic D2 (inhibition) receptors in the neuronal synapses of the myentericplexi[3-5]. CNT is indicated for the treatment of gastrointestinal disorders associated with motility disturbances such as gastroesophageal reflux disease, non-ulcer dyspepsia and delayed gastric emptying. The use of CNT is efficient and safe in treatment of patients with disorder in the gastric emptiness related to gastric oesophageal reflux and functional dyspepsia as well as individuals that present irritable bowel syndrome with constipation and abdominal pain [6].

The ICH guideline Q1A (R2) emphasizes that the testing of those features which are susceptible to change during storage and are likely to influence quality, safety and efficacy, must be done by validated stability indicating testing method. The information on the stability of the drug substance is an integral part of the systemic approach to stability evaluation. Stress testing of drug substance can help identify the likely degradation products, which can in turn help establish the degradation pathways and the intrinsic stability of the molecule and validate the stability indicating power of the analytical procedures used [7]. ICH Q3A (R2) and Q3B (R2) address issues relevant to the regulation of impurities in the drug substance and drug product[8-9]. The stability indicating assay method can be specific or selective. Specific stability indicating assay method (SIAM) is developed to measure active ingredient in presence

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of all degradation products, excipients and additives expected to be present in the formulation.

CNT is not official in any of the Pharmacopoeias. Spectrophotometric methods for the determination of CNT as single drug[10-11] and in combination with Omiprazole[12-13] and pentaprazole[14] are reported. One colorimetric [15] and high performance thin layer chromatographic[16] (HPTLC) method is reported for estimation of drug. Literature also suggests reports on quantification of CNT using RP-HPLC[17-20] and in combination with Omeprazole and Pantoprazole[21-26]. A single stability indicating RP-HPLC method has been reported and employs combination of acetonitrile and phosphate buffer as mobile phase[27]. Since phosphate buffer is LC-MS non-compatible, in order to characterize the degradation products using LC-MS, we tried to develop a new stability indicating RP-HPLC assay method using ammonium acetate buffer.

Thus the objective of this study was to develop and validate LC-MS compatible stability indicating assay method which could quantify CNT in presence of degradation products.

II. MATERIALS AND METHODS

CNT (assigned purity 99.8%) was obtained as gift from Symed Labs, Hyderabad, India and used without further purification. Cintapro tablets® (Label claim: 1 mg CNT, ZydusAlidac, Batch No: LL 1370) were purchased from the local market. Acetonitrile (HPLC grade) was purchased from Merck Specialities Pvt. Ltd. (Mumbai, India). Ultra-pure water was obtained from an ELGA Purelab UHQ-II (Bucks, UK) water purification unit. Analytical reagent (AR) grade Sodium hydroxide and hydrochloric acid (HCl) were purchased from Loba Chemie Pvt. Ltd. (Mumbai, India) and S. D. Fine-Chem Ltd. (Mumbai, India) respectively. Hydrogen peroxide (AR) was purchased from Loba Chemie Pvt. Ltd. (Mumbai, India).

High performance liquid chromatographic system (Shimadzu, LC-2010CHT) with a UV-VIS detector was employed to acquire the chromatographic data. The HPLC system consisted of a quaternary pump, an auto injector, and a column oven. The data were acquired and processed by the use of LC solution software (SP1, Ver. 1.25) on hp computer. The chromatographic separations were carried out on a HiQsil C18 HS column (250 mm × 4 .6 mm, 5µm). In addition, an electronic balance (Shimadzu, AX200), a sonicator (Spectra Lab, USB 40) and a hot air oven (Bio Technics India, BTI Sr. No. 1040) were used in this study.

The chromatographic separation was achieved with low pressure gradient mode using a mobile phase consisting of acetonitrile and 0.01M ammonium acetate buffer in the ratio of 75:25 (v/v) with flow rate of 1 ml/min at 263 nm. Column temperature was maintained at 25^0 and injection volume was 20 µl for all injections. A mixture of acetonitrile and 0.01M ammonium acetate buffer in the ratio of 75:25 (v/v) was employed as diluent.

III EXPERIMENTATION

Preparation of standard solution:

CNT (100 mg) was accurately weighed and dissolved in 100 ml of methanol (1000 µg/ml). It was further diluted to obtain solution of concentration of 100 µg/ml using diluent and was used for chromatographic studies.

Preparation of buffer and mobile phase:

Ammonium acetate buffer (0.01M) was prepared by dissolving 0.778 g of ammonium acetate in 1000 ml of HPLC grade water. Mobile phase was prepared by mixing acetonitrile with buffer solution in 75:25 proportions at a flow rate of 1 ml/min using gradient mode. Mobile phase was filtered through 0.22 µm nylon filters (Pall India Pvt. Ltd., Mumbai, India) and sonicated before use.

Forced degradation studies

CNT was exposed under different stress conditions as recommended by international conference on harmonisation guideline Q1A R2[7].

Acidic, alkaline and oxidative induced degradation:

CNT (20 mg) was mixed with 20 ml each of 0.1M HCl, 0.1M NaOH and 3% hydrogen peroxide. The mixtures were refluxed for 15min, 5h and 3h respectively at 80^o in water bath. Samples treated with acid were neutralised using equal strength of base and vice versa.

Neutral degradation:

CNT (20 mg) was mixed with 20 ml of HPLC grade water and mixture was refluxed for 2 h at 80° in water bath.

Dry heat degradation:

Solid drug was heated in an oven in a sealed glass vial at 100° for 12h. Similarly a control sample was maintained at room temperature.

Photolytic degradation:

Solid CNT was left in sunlight for 8 h to check the stability towards photolytic degradation. Summary of Forced degradation study is shown in Table 1.

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Preparation of samples for HPLC analysis:

Degraded samples were withdrawn after specified time interval and suitably diluted with diluent in case of acid, base, neutral and oxidative stress to produce a predetermined concentration of 100 µg/ ml of CNT. In case of thermal and photolytic stress, solid drug was weighed appropriately and diluted with suitable quantity of diluent to produce 100 µg/ml of drug. A solution of 100 µg/ml concentration of drug was separately prepared and was treated as control.

IV RESULTS AND DISSCUTION

Development of Stability-indicating assay method:

The HPLC procedure was optimized to develope a stability indicating assay method. Pure drug and its degradation products were injected and chromatographed using different mobile phases. A mobile phase consisting of acetonitrile-to-ammonium acetate buffer (0.01M) in ratio of 75:25 (v/v), at a flow rate of 1.0 ml/min resulted in an acceptable retention time, (t_R = 5.383 min), tailing factor (1.39), theoretical plates (2865) and symmetrical peak shape (Fig.2a).

Degradation behaviour:

Acid induceddegradation:

CNT was found to be very labile in acid. Complete degradation of the drug was observed in 0.1 M HCl at 80° within 10-15 min. Drug produced two degradants which are designated as Deg. A [Retention time (t_R) = 2.835 min and Relative Retention (RR) = 0.527] and Deg. B. [t_R= 4.323 min. and RR = 0.803](Fig. 2b).

Alkaline induced degradation:

Almost 14% degradation was observed in 0.1 M NaOH at 80° within 5 h. Only one degradant (Deg. C) was formed with retention time 2.992 (RR = 0.585) (Fig. 2c).

Oxidative induced degradation:

Almost 46% of drug was degraded when refluxed with 3% H_2O_2 for 3h at 80°. CNT was found to be susceptible towards oxidative stress. The major degradant which is designated as Deg. D was eluted at retention time of 3.401 min (RR = 0.579) (Fig. 2d).

Neutral degradation:

Under neutral degradation condition, almost 32% degradation of CNT was observed when refluxed for 2 h at 80°. Degradation products which are designated as [Deg. E and F] with retention times of 2.562 min (RR = 0.489) and 3.117 min (RR = 0.595) respectively, were generated (Fig. 2e).

Negligible degradation was seen on subjecting the drug to dry heat and photolytic stressed conditions. Further, it was observed that, under acidic, alkaline, oxidative and neutral degradation studies, the % assay of CNT was found to be decreased as the degradation time was increased.

Validation of the method:

Linearity:

The response for the drug was found to be linear in the concentration range of 20-120 µg/ml. The regression equation of the line obtained was found to be y = 70590x+14599. The correlation coefficient was 0.999 for the drug which proved that the method was linear.

Precision:

The intra-day and inter-day precision of the method was determined at three different concentrations (20, 60 and 100 µg/ml). The % R.S.D. values for intra-day and inter-day precision were found to be ≤2%, confirming the specificity of the method. The data is represented in Table 2.

Accuracy:

Accuracy of the method was determined by conducting recovery studies of the pure drug from degradation sample. Stressed drug sample when spiked with the pure drug at three given concentration levels showed satisfactory recovery as indicated in Table 3.

Robustness:

During robustness check, the % RSD (0.045-1.281%) was well within the acceptance criteria. The method was thus found to be robust since the monitored parameters i.e. flow rate, mobile phase composition and column temperature were not significantly affected.

LOD and LOQ:

The LOD and LOQ values were found to be 0.679 and 2.057µg/mlrespectively, which represents that sensitivity of the method is high.

Specificity:

The specificity of the method was established through study of resolution factors of the drug peak from the nearest resolving peak, and also among all other peaks. The resolution between the peaks was found to be greater than 2.

Analysis of marketed formulation:

The proposed method was applied for the determination of CNT in tablet dosage form. Twenty tablets were powdered and three samples

were analyzed. Mean drug content±S. D. value for the assay was found to be 99.36±0.13. The result of the assay indicated that the method was selective for the assay of CNT without interference from the excipients used in these tablets.

V. CONCLUSION:

In the present study a specific stability indicating RP-HPLC method has been developed and validated as per ICH guideline. The method enabled accurate quantitative analysis of CNT in pharmaceutical tablets without interference from excipients. From the degradation studies it was concluded that CNT was extremely susceptible towards acidic, oxidative and neutral hydrolysis, reasonably susceptible towards alkaline hydrolysis, but is stable toward dry heat and photolytic degradation. This method can be used for characterization of degradation products by using LC-MS/MS.

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TABLE 1: SUMMERY OF FORCED DEGRADATION STUDIES

Stress condition	Amount drug degraded (%)	Amount drug recovered (%)	$t_R$of degradation product (min.)	Relative Retention Time(RR) of Degradants
0.1M HCl,15 min at 80^o	100	0	Deg. A = 2.8, Deg. B = 4.3	Deg. A = 0.527, Deg. B = 0.803
0.1 M NaOH, 5 h at 80^o	14	86	Deg. C = 2.9	Deg. C = 0.585
3% H_2O_2, 3 h	74.42	25.58	Deg. D = 3.4	Deg. D = 2.564
H_2O, 2 h	42.33	57.67	Deg. E = 2.5, Deg. F = 3.1	Deg. E = 0.489 Deg. F = 0.595
Dry heat 12 h, 100^o	None	0	-	-
Sunlight, 8 h	None	0	-	-

RR is calculated with respect to retention time of CNT., Deg: Degradation product

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TABLE 2: RESULTS FOR PRECISION STUDIES

Precision	Conc. (µg/ml)	Avg. peak area±S.D.	%R.S.D.
Repeatability (n=6)	100	6840346.167±107189.510	1.567
Intra-day (n=3)	20	1508537.333±582.438	0.038
	60	4187688±6443.043	0.153
	100	7032651.667±6214.931	0.088
Inter-day (n=3)	20	1508540±503.363	0.033
	60	4187696.667±6445.158	0.153
	100	7032644.333±6205.749	0.088

TABLE 3: RESULTS FOR ACCURACY STUDIES

Spike level	Amount added (µg/ml)	Amount recovered (µg/ml)	% Recovery	Mean % recovery±S.D.	% R.S.D.*
75%	70	70.51 69.57 70.24	100.74	100.16±0.69	0.692
100%	80	78.10 80.39 80.24	99.39 100.34 98.88	99.894±0.88	0.882
125%	90	90.45 89.87 90.40	100.49 100.3 100.96 100.5 99.86	100.44±0.55	0.553

*n= 3 determinations

Table and figure titles and legends:

TABLE 1: SUMMERY OF FORCED DEGRADATION STUDIES

HCl is Hydrochloric acid, NaOH is Sodium hydroxide, H_2O_2 is Hydrogen peroxide, t_R is Retention time, -- is no significant degradation

TABLE 2:RESULTS FOR PRECISION STUDIES

S.D. is standard deviation, R.S.D. is Relative standard deviation

TABLE 3: RESULTS FOR ACCURACY STUDIES

S.D. is standard deviation for* n = 3 observations, R.S.D. is Relative standard deviation

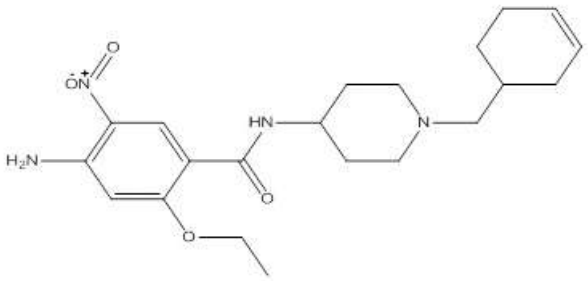

Fig **No 1**: Chemical Structure of Cinitapride

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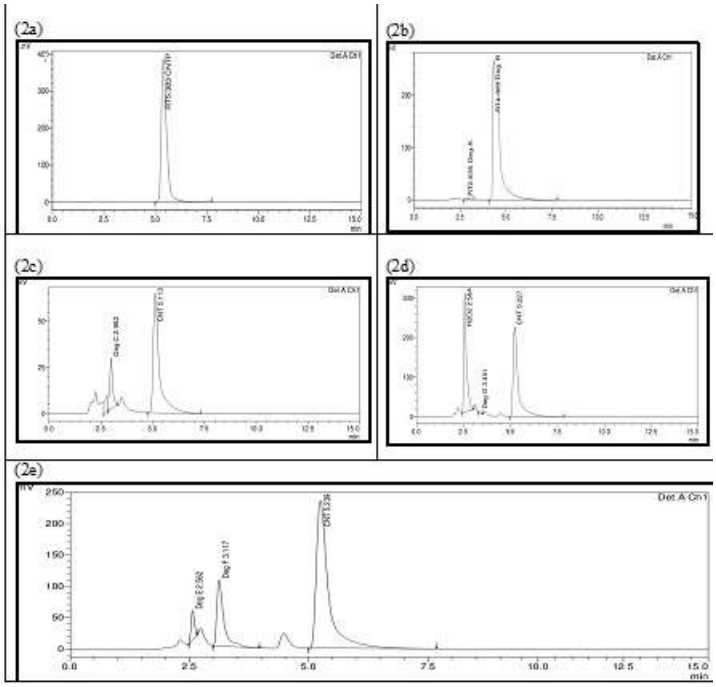

Fig 2 HPLC chromatogram of standard CNT and degradation products of CNT at different degradation conditions