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High Performance Liquid Chromatographic Determination of Carboplatin in Injections

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The present study describes the development and validation of a stability indicating reverse phase HPLC (RP-HPLC) method for the analysis of carboplatin, an anticancer drug, in injection dosage forms. The proposed RP-HPLC method utilizes Inertsil amino, 250 mm × 4.6 mm i.d., 5 µ column (at ambient temperature), isocratic run (using acetonitrile and water as mobile phase), at a flow rate of 2.0 mL min^{-1} and UV detection at 230 nm for analysis of carboplatin. The reported method is linear. Carboplatin was exposed to acidic, alkaline, oxidative, thermal and photolytic stress conditions- and the stressed samples were analyzed by the proposed method. Chromatographic peak purity results indicated the absence of co-eluting peaks with the drug peak, which demonstrated the specificity of assay method for estimation of carboplatin in presence of degradation products. The proposed method can be used for routine analysis stability testing of carboplatin in quality control laboratories.

Key Words: Reverse-phase-HPLC, Carboplatin, Stability-indicating assay, Forced degradation.

INTRODUCTION

Carboplatin is a platinum coordination divalent inorganic compound used as a cancer chemotherapeutic agent[1,2]. The chemical name for carboplatin is platinum, diammine [1,1-cyclobutanedicarboxylato (2-)-0,0']. Carboplatin is a crystalline white powder, (m.f. $C_6H_{12}N_2O_4Pt$, m.w. 371.25[3]) (Fig. 1). It is soluble in water at a rate of *ca.* 14 mg/mL and the pH of a 1 % solution is 5-7[4]. It is virtually insoluble in ethanol, acetone and dimethyl acetamide.

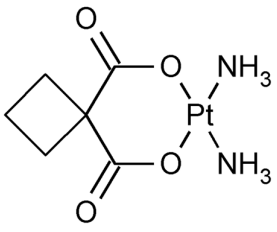

Fig. 1. Structural formula for carboplatin

To date, published analytical methods are all estimation of carboplatin with reversed-phase high performance liquid chromatography (RP-HPLC) but the present study is the first time report on stability indicating assay of carboplatin in presence of degradation products by HPLC. In this method isocratic elution method is selected for the analysis of carboplatin because it gave better base line separation and peak shape, which is suitable for the routine analysis of carboplatin. In view of above in the present study we hereby report the development and validation of a stability indicating isocratic reverse-phase HPLC (RP-HPLC) method for analysis of carboplatin in presence of degradation products as per ICH guidelines[5].

EXPERIMENTAL

Carboplatin standard and injection were provided by Dabur Research Foundation, Ghaziabad, India. Acetonitrile and hydrogen peroxide were from Qualigens, Mumbai, sodium hydroxide and hydrochloric acid were from Merck (Darmstadt, Germany). All chemicals were at least of analytical grade and used as received. Purified HPLC grade water was obtained by reverse osmosis and filtration through a Milli-Q® system (Millipore, Milford, MA, USA) and was used to prepare all solutions.

The HPLC system-Agilent 1100 separation module having maximum pressure of 5000 psi, detector-DAD G1313A, photodiode array detector. To develop a precise, linear, specific and suitable stability indicating RP-HPLC method for analysis of carboplatin, different chromatographic conditions were applied and the results are presented in Table-1 and the following optimized conditions were applied for final analysis. The chromatographic separation was performed using inertsil amino, 250 mm × 4.6 mm i.d. 5 µ particle size column (at ambient temperature), isocratic run, at a flow

TABLE-1
METHOD DEVELOPMENT FOR RP-HPLC ANALYSIS OF CARBOPLAIN

Fig. No.	Column used	Mobile phase isocratic/gradient	Observation result
2[A]	YMC PACK ODS-A (50 mm × 4.0 mm i.d., 3 µ)	$CH_3CN + H_2O$ 85:15 % v/v/isocratic	Peak width was more, hence method rejected Peak shape was poor, hence method rejected
2[B]	Supelco C_{18} (250 mm x 4.6mm i.d, 5 µ)	$CH_3CN + H_2O$ 85:15 % v/v/isocratic	Peak fronting was observed, hence method rejected
2[C]	Inertsil amino (250 mm × 4.6 mm i.d., 5 µ)	$CH_3CN + H_2O$ 90:10 % v/v/isocratic	Peak tailing was there method rejected
2[D]	Inertsil amino (250 mm × 4.6 mm i.d, 5 µ)	$CH_3CN + H_2O$ 80:20 % v/v/isocratic	Peak shape and response was good, hence method accepted
2[E]	Inertsil amino (250 mm × 4.6 mm i.d, 5 µ)	$CH_3CN + H_2O$ 85:15 % v/v/isocratic	

rate of 2.0 mL/min and UV detection at 230 nm for analysis of carboplatin. The mobile phase consisted of acetonitrile-water (85:15) filtered through 0.45 µm nylon filter and degassed in ultrasonic bath prior to use. Wavelength was selected by scanning standard solution of drug over 200-400 nm using Perkin Elmer double beam UV-visible spectrophotometer (model Lambda 35). Measurements made with an injection volume of 20 µL and ultraviolet (UV) detection at 230 nm, showed reasonably good response.

Preparation of solution

Standard solution: About 50 mg of carboplatin reference/working standard was accurately weighed and transferred into a clean 50 mL of volumetric flask, 25 mL of water was added and sonicated for 5 min to dissolve the carboplatin, volume was made up to the mark with water. This gave 1 mg/mL solution of carboplatin.

Sample solution: 5 mL of carboplatin injection (10 mg/mL) was accurately pipetted out and transferred into a clean 50 mL volumetric flask. 25 mL water was added and mixed well, volume was made upto the mark.

Blank solution: Water was used as a blank solution (diluent).

Placebo solution: Water was used as a Placebo solution.

Optimization of chromatographic conditions: The chromatographic conditions were optimized by different means. [Using different column, different buffer and different mode of HPLC run, Table-1].

Specificity (forced degradation studies): Carboplatin was allowed to hydrolyze in base (0.1N NaOH), acid (0.1N HCl) and hydrogen peroxide (10 % v/v). Carboplatin was also studied for its thermal degradation at 80 ºC for 2 days and photolytic degradation for 10 days, exposed to white fluorescent light (1.2 million lux) near UV fluorescent light (200 w/m^2). 10 mL of carboplatin injection was diluted with 20 mL of base (NaOH), acid (HCl) or hydrogen peroxide 10 % v/v (H_2O_2) with 20 mL of water and kept at room temperature to study the degradation, 5 mL of sample was taken at various time interval (time schedule is given in Table-2) and it was neutralized with 0.1N HCl or 0.1 NaOH solution and diluted with water to get the final concentration of 1 mg/mL of carboplatin. Similarly placebo solution was prepared. Sample and placebo solutions were analyzed as per methodology, calculated the percentage degradation. The results of stability studies are presented in Table-3.

Validation: Linearity was determined by injecting different concentration of sample solutions (700-1300 µg/mL, Fig. 3). For system precision, standard solution (1 mg/mL) was injected in six replicates to check % RSD (relative standard deviation) and for method precision six samples were prepared and each of these were injected in duplicate. Mean of all of these values gives rise to assay value. To establish the within-day (intra-assay) and between-day (inter-assay) accuracy and precision of the method, carboplatin was assayed on one day and three separate days. Intra-assay and inter-assay were calculated and the data are presented in Tables 3 and 4, respectively. Robustness of method was investigated by varying the chromatographic conditions such as change of flow rate (10 %), organic content in mobile phase (2 %), wavelength of detection ((± 2 nm) different batch of column and column oven temperature (± 2 °C). Robustness of the developed method was indicated by the overall % RSD between the data at each variable condition (Table-6).

Stability in analytical solution: The stability of the standard and sample solution were demonstrated by analyzing the standard and sample solutions at regular time intervals (*i.e.*, 4 h) till 24 h, keeping the solution at room temperature and

TABLE-2
SAMPLING PLAN

Time point	Base stress	Acid stress	Peroxide stress	Heat stress	Light stress
Initial	√	√	√	√	√
4 h	√	√	√	–	–
8 h	√	√	√	–	–
24 h	√	√	√	√	–
2 days	–	–	–	√	–
5 days	–	–	–	–	√
10 days	–	–	–	–	√

TABLE-3
RESULTS OF FORCE DEGRADATION STUDIES OF CARBOPLATIN

Stress condition/duration/solution	Degradation (%)
Alkaline degradation (0.1N NaOH, 4 h)	17.9
Alkaline degradation (0.1N NaOH, 8 h)	17.9
Alkaline degradation (0.1N NaOH, 24 h)	19.7
Oxidative degradation (10 % H_2O_2, initial)	83.2
Oxidative degradation (2 % H_2O_2, initial)	46.8
Oxidative degradation (2 % H_2O_2, 1 h)	74.1
Acidic degradation (0.1N HCl, 4 h)	16.0
Acidic degradation (0.1N HCl, 8 h)	19.5
Acidic degradation (0.1N HCl, 24 h)	44.9
Thermal degradation (liquid sample, 24 h)	5.4
Thermal degradation (liquid sample, 48 h)	10.5
Photolytic degradation (UV fluorescent light 200 w/m^2, 5 days)	6.1
Photolytic degradation (UV fluorescent light 200 w/m^2, 10 days)	8.4

TABLE-4
INTRA-ASSAY PRECISION DATA OF PROPOSED RP-HPLC METHOD (METHOD RUGGEDNESS)

	Mean (% w/w)	SD (n = 6)	RSD (%)
Assay-1	100.3	0.252	0.25
Assay-2	100.2	0.115	0.12
Intra assay	100.2	0.183	0.18

TABLE-5
INTER-ASSAY PRECISION DATA OF PROPOSED RP-HPLC METHOD

	Mean (% w/w)	SD (n = 6)	RSD (%)
Analyst-1	100.9	0.022	0.22
Analyst-2	100.05	0.259	0.25
Inter assay	100.5	0.052	0.51

TABLE-6
ACCURACY DATA

Interpolated concentration [mean ± SD (n = 3)]		RSD (%)
Carboplatin concentration (mg/mL)		
0.8	0.804 ± 0.115	0.12
1.0	0.998 ± 0.252	0.25
1.2	1.194 ± 0.208	0.21

refrigerator (2-8 °C). The area counts of the carboplatin peak in the standard and sample solutions were calculated, the cumulative RSD for area counts were calculated.

RESULTS AND DISCUSSION

Chromatographic conditions: To develop a precise, linear, specific and suitable stability indicating RP-HPLC method for analysis of carboplatin, different chromatographic conditions were applied and the results observed are presented in Table-1. Isocratic elution is simple, requires only one pump and flat baseline separation for easy and reproducible results. In case of RP-HPLC various columns are available, but here Inertsil amino, 250 mm × 4.6 mm i.d. 5 µ column was preferred because using this column peak shape, resolution and absorbance were good and acetonitrile was selected as mobile phase, because of its favourable UV transmittance. Among the different mobile phases composition employed the mobile phase consisted of water-acetonitrile (15:85) was found to be suitable for the analysis of carboplatin. Further, a flow rate of 2.0 mL/min, an injection volume of 20 µL and UV detection at 230 nm was found to be best for analysis.

Forced degradation studies: In the present study even though the conditions used for forced degradation are in the range of producing 20-80 % target degradation, degradation of carboplatin could not be achieved even after prolonged duration. During the study it was observed that upon treatment of carboplatin with base (0.1N NaOH), acid (0.1N HCl) and hydrogen peroxide 10 % v/v (H_2O_2) the degradation was observed. In case of heat after 2 days was found 10 % degradation and in case of light after 10 days 7.0 % degradation was found. Table-3 shows the extent of degradation of carboplatin under various stress conditions. The peak of carboplatin is unaffected (Fig. 2), which indicates that these degraded peaks are due to the presence of impurities (amine or polar groups) and the carboplatin is stable under the applied stress conditions like heat, light, acid, alkaline hydrolysis and oxidative degradation states.

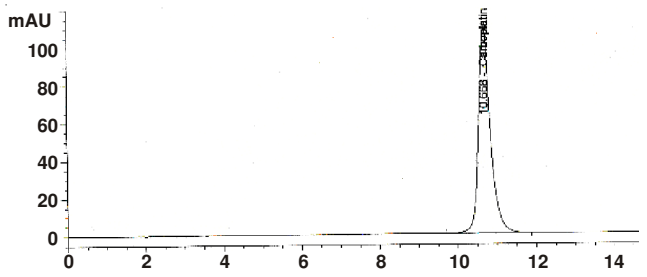

Fig. 2. Chromatogram of carboplatin

Linearity: The calibration curve showed good linearity in the range of 700-1300 µg/mL, for carboplatin with correlation co-efficient (r^2) of 0.999 (Fig. 3). A typical calibration curve has the regression equation of y = 2.255x - 30.433 for carboplatin.

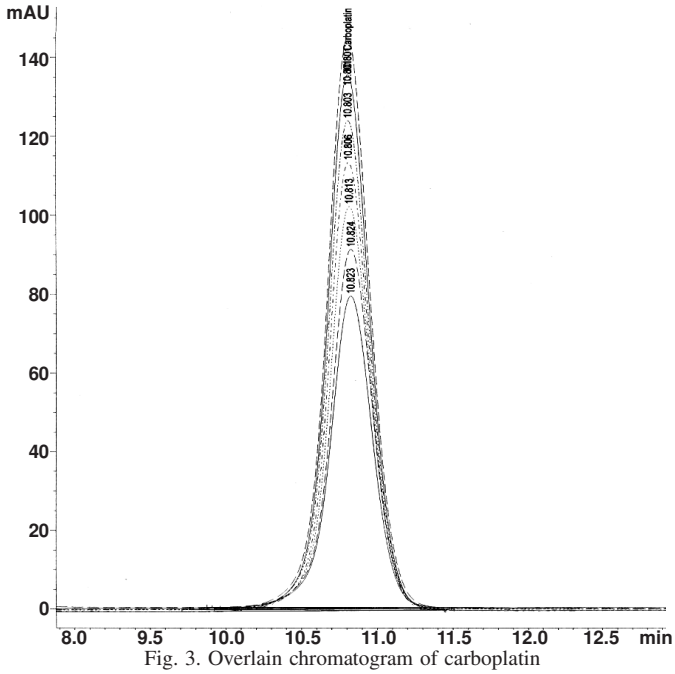

Fig. 3. Overlain chromatogram of carboplatin

Precision: The results of system precision [RSD (%) 0.23] and method precision [RSD (%) 0.22] are found within the prescribed limit of ICH guidelines [RSD (%) < 1 % and RSD % < 2 %], respectively in case of system precision and method precision.

Intra-assay and inter-assay: The intra- and inter-day variation of the method was carried out and the high values of mean assay and low values of standard deviation and RSD (%) (RSD % < 2 %) within a day and day to day variations for carboplatin revealed that the proposed method is precise (Tables 4 and 5).

Accuracy as recovery: Accuracy of the method was carried out by recovery studies. A preanalyzed sample of carboplatin was spiked with standard drug at three different levels (80, 100 and 120 %). Each solution were prepared in triplicate and analyzed in triplicate after suitable dilution. The recovery data (RSD %) obtained at each level was < 2 %. The average recovery yield at three different levels of 80, 100 and 120 % were found to be 100.2, 100.3 and 100.4, respectively. Since the results obtained were within the acceptable range

98.0-102.0 %, the method was deemed to be accurate. The accuracy results are summarized in Table-6.

Method robustness: Influence of small changes in chromatographic conditions such as change in flow rate ((± 10 %)), organic content in mobile phase (± 2 %), wavelength of detection (± 2 nm), different batch of column and column oven temperature (± 2 ºC) studied to determine the robustness of the method are also in favour (Table-7, RSD % < 2 %) of the developed RP-HPLC method for the analysis of carboplatin.

TABLE-7
INFLUENCE OF CHANGES IN
CHROMATOGRAPHIC PARAMETERS ON RP-HPLC

Analysis of carboplatin (method robustness)	
Change in parameter	RSD % (n = 6)
Flow (2.2 mL/min)	0.08
Flow (1.8 mL/min)	0.28
Wave length (232 nm)	0.36
Wave length (228 nm)	0.39
Column oven temperature (23 ºC)	0.48
Column oven temperature (27 ºC)	0.19
Column inertsil amino (S. No. 4GI50080)	0.24
Column inertsil amino (S. No. 6FI50191)	0.18
Organic phase composition (+2 %)	0.32
Organic phase composition (-2 %)	0.48

Specificity and stability in analytical solution: The results of specificity indicated that the peak was pure in presence of degraded sample. It is important to mention here that the carboplatin standard and sample solutions were stable at regular time intervals (*i.e.*, 4 h) till 24 h, keeping the solution at room temperature and refrigerator (2-8 ºC). Carboplatin was stable in solution form upto 24 h at 25 ºC. The results of linearity, precision, inter and intra-day assays, method robustness and specificity and stability in analytical solution established the validation of the developed RP-HPLC assay for the analysis of carboplatin.

Conclusion

A sensitive and selective stability indicating RP-HPLC method has been developed and validated for the analysis of carboplatin injection. Based on peak purity results, obtained from the analysis of force degraded samples using described method, it can be concluded that the absence of coeluting peak along with the peak of carboplatin indicated that the developed method is specific for the estimation of carboplatin in presence of degradation products. Further the proposed RP-HPLC method has good sensitivity, precision and reproducibility. Even though no attempt was made to identify the degraded products, proposed method can be used as a stability indicating method for assay of carboplatin injection.

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