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Advances in Bioresearch

ORIGINAL ARTICLE

Development and validation of stability indicating RP-UPLC method for quantitative estimation of Cisplatin in Cisplatin injection 1mg/mL

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ABSTRACT
A new, simple, rapid, selective, precise and accurate isocratic reverse phase ultra performance liquid chromatography assay method has been developed for estimation of Cisplatin in Cisplatin injection formulations. The separation was achieved by using column waters Acquity HSS T-3 C18 (100 x 2.1 mm, 1.7µm), in mobile phase consisted of pH 5.90 buffer. The flow rate was 0.4 mL/min and the separated Cisplatin was detected using UV detector at the wavelength of 210 nm. The retention time of Cisplatin was noted to be 3.70 min respectively, indicative of rather shorter analysis time. The method was validated as per ICH guidelines. The proposed method was found to be accurate, reproducible, and consistent.
Keywords: *Liquid chromatography, Cisplatin, Forced degradation and Validation.*

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INTRODUCTION

Cisplatin (cis-diamminedichloro-platinum (II), cis-[PtCl2(NH3)2]) (CDDP) is an injectable anticancer agent used frequently in clinical practice [1-3]. The chlorine (Cl) in CDDP is replaced with a water molecule in cells to form a hydrated complex and react with DNA [4]. In addition, CDDP has been reported to change into various forms such as a hydrated complex and hydroxo-bridged complex (OH-dimer) [5].
The literature survey reveals only a few methods were reported to date for the estimation of Cisplatin derivative spectrophotometry [6-9], detection by phosphorescence of Cisplatin in urine and plasma [10], atomic absorption spectrometry [11-13], electroanalytical methods [14], and high performance liquid chromatography methods [15-24].
But there is no simple method for the analysis of Cisplatin. Hence, it is necessary to develop a rapid, accurate and validated RP-UPLC method for the determination of Cisplatin in injection dosage form. The present study illustrates development and validation of a simple, accurate and precise procedure for determination of Cisplatin by RP-UPLC.

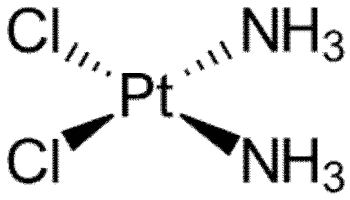

Fig. 1. Chemical structure of Cisplatin

Hence, a new sensitive, economical, stability indicating RP-UPLC method was developed and validated in accordance with ICH guidelines by the author.

MATERIAL AND METHODS

Chemicals and reagents

Analytical-grade 1-octanesulfonic acid sodium salt, Tetrabutylammonium hydrogen sulphate, potassium dihydrogen orthophosphate, sodium chloride and sodium hydroxide, Hydrochloric acid and Hydrogen peroxide reagents and chemicals were procured from Merck Chemicals. Mumbai, India.

Instruments and Equipment

Waters-Acquity H Class equipped with Empower^2 software, Bandelin ultrasonic bath, pH Meter (Thermo Orion Model), Analytical Balance (Metller Toledo Model) were use in the present study.

Method of Analysis

Preparation of 1N sodium hydroxide solution

Accurately weighed and transferred 4.0 g sodium hydroxide pellets into 100 mL volumetric flask, added 50 mL of water sonicated and made up to the mark with water and mixed well.

Preparation of mobile phase

Accurately weighed and transferred 1.08 g of 1-octanesulfonic acid sodium salt and 1.70 g Tetrabutylammonium hydrogen sulphate and 2.72 g of potassium dihydrogen orthophosphate in 950 mL of water. Dissolved and mixed the contents by sonicated for 10 minutes on occasional stirring with a glass rod. Adjusted the pH to 5.90 with 1N sodium hydroxide solution and transferred into a 1000 mL volumetric flask diluted to volume with water. Filtered the solution through 0.45 µm membrane filter and sonicated to degas.

Preparation of diluent

Accurately weighed and transferred 9.0 g sodium chloride into 1000 mL volumetric flask, added 500 mL of water sonicated and made up to the mark with water and mixed well.

Standard solution preparation

Accurately weighed and transferred 50.0 mg of Cisplatin standard into 50 mL volumetric flask, Added 40 mL of diluent, sonicated and made up to the mark with diluent and mixed well to ensure complete dissolution. (Or prepare standard solution of concentration equivalent to 1.0 mg/mL of Cisplatin).

Preparation of sample solution-1

Inject as such sample solution

Preparation of sample solution-2

Inject as such sample solution

Method development

Spectroscopic analysis of compound Cisplatin showed that maximum UV absorbance (λmax) at 210 nm respectively. To develop a suitable and robust UPLC method for the quantification of Cisplatin, different mobile phases were employed to achieve the best separation and resolution. The method development was started with Acquity HSS T-3 C18 (100 × 2.1 mm, 1.7µm) with the following different mobile phase compositions like that 0.1% OPA pH 1.8 and methanol in the ratio of 95:5 v/v. 0.1% OPA pH adjusted to 3.0 with triethylamine and methanol in the ratio of 90:10 v/v. Phosphate Buffer pH 4.5 and acetonitrile in the ratio of 95:5 v/v. Acetate Buffer pH 3.2 and methanol in the ratio of 98:2 v/v. It was observed that when Cisplatin was injected, higher retention time, Peak Tailing, not satisfactory.

For next trial the mobile phase composition was changed to pH 5.90 buffer containing 1-octanesulfonic acid sodium salt, tetrabutylammonium hydrogen sulphate and potassium dihydrogen orthophosphate respectively as eluent at flow rate 0.4 mL/min. UV detection as performed at 210nm. The retention time of Cisplatin is 3.6 minutes and the peak shape was good. The chromatogram of Cisplatin standard using the proposed method is shown in Figure2. system suitability results of the method are presented in Table 1.

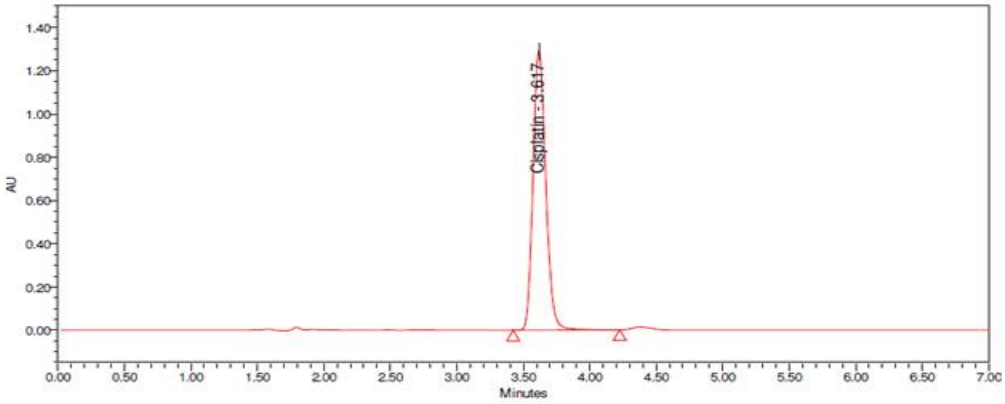

Fig. 2. Typical chromatogram of Cisplatin standard

Optimised chromatographic conditions

Chromatographic analysis was performed on Acquity HSS T-3 C18 (100 × 2.1 mm, 1.7µm), in mobile phase consisted of pH 5.90 buffer. The flow rate was 0.4 mL/min, column oven temperature 30°C, sample cooler temperature 5°C, injection volume was 2 µL, and detection was performed at 210 nm using a photodiode array detector (PDA).

RESULTS AND DISCUSSION

The developed RP-UPLC method extensively validated for assay of Cisplatin using the following parameters.

Specificity and system suitability

Blank and Placebo interference:

A study to establish the interference of blank and placebo were conducted. Diluent and placebo were injected into the chromatograph in the defined above chromatographic conditions and the blank and placebo chromatograms were recorded. Chromatogram of blank solution **Figure 3.** showed no peak at the retention time of Cisplatin peak. This indicates that the diluent used in sample preparation do not interfere in estimation of Cisplatin in Cisplatin injection. Similarly, chromatogram of placebo solution **Figure 4.** showed no peaks at the retention time of Cisplatin peak. This indicates that the placebo does not interfere in estimation of Cisplatin in Cisplatin injection.

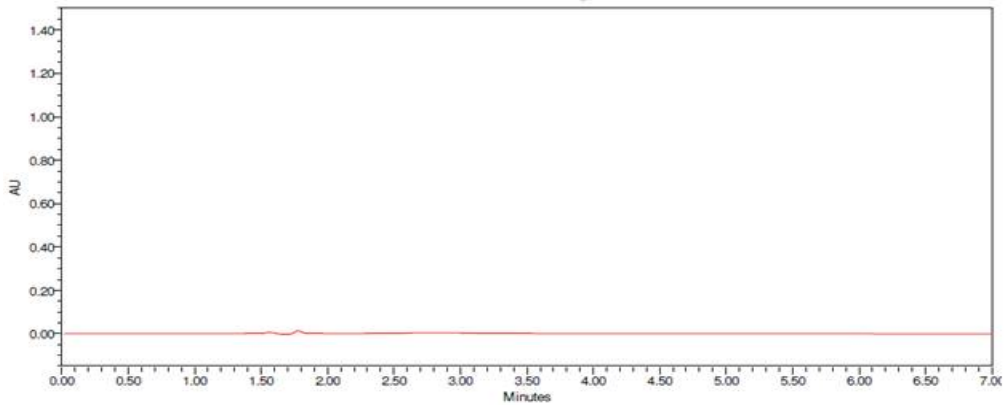

Fig. 3. Typical chromatogram of blank

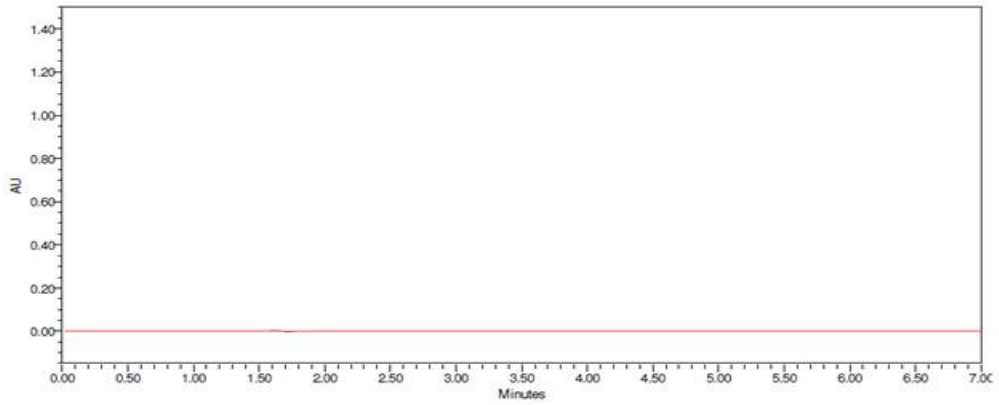

Fig. 4. Typical chromatogram of placebo

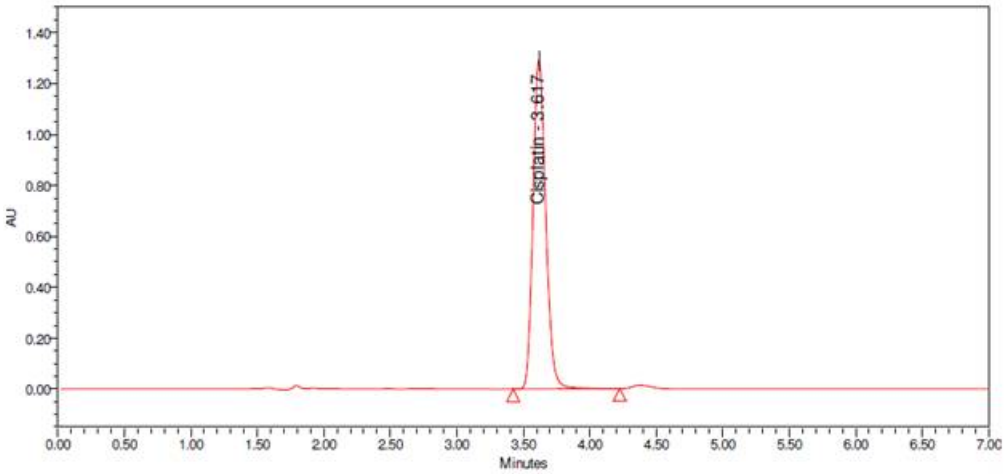

Fig. 5. Typical chromatogram of standard

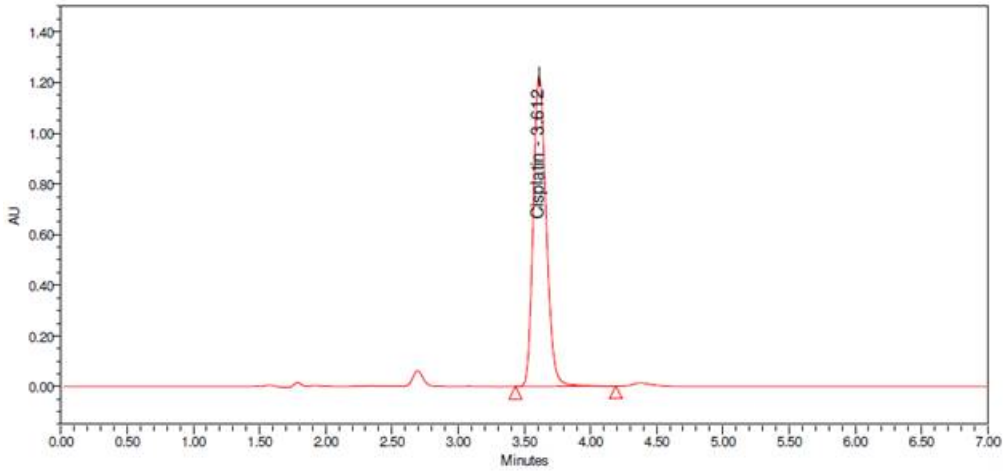

Fig. 6. Typical chromatogram of sample

Table 1. Specificity results

S.No	Name	Retention Time (min)	Blank	Placebo
1	Blank	ND	NA	NA
2	Placebo solution	ND	NA	NA
3	Standard solution	3.617	No	No
4	Sample solution	3.612	No	No

Table 2. System suitability results

S. No	Name	Retention time	USP Tailing	USP Plate Count
1	Cisplatin	3.617	1.2	6882

Force degradation studies

A study was conducted to demonstrate the effective separation of degradants/impurities from Cisplatin. Separate portions of sample and placebo solutions were exposed to the following stress conditions to induce degradation. Stressed and unstressed samples were injected into the UPLC system with a PDA detector. The degradation study results were presented in Table 3.

Table 3. Forced degradation results

Stress condition	Degradation condition	% Assay	% Degradation
As such	Control sample	100.0	0.24
Acid	1.0N HCl/60°C/1 Hour	99.8	0.27
Alkali	1.0N NaOH/BT/30 minutes	98.1	2.1
Oxidative	3% H_2O_2/BT/ 30 minutes	98.5	1.9
Photolytic	1.2 million Lux hours, 200-watt hours/m^2 for 7 days	94.7	5.73
Humidity	90%RH/ Exposed for 2 days	99.7	0.31
Thermal	60°C/ Exposed for 2 days	91.18	10.51

Significant degradation was observed in the thermal and photolytic stress conditions. Hence it can be concluded that Cisplatin is sensitive to thermal and photolytic.

Method precision

The precision of test method was evaluated by doing assay for six samples of Cisplatin injection as per method. The content in % assay for Cisplatin for each of the test preparation was calculated. The average content of the six preparations and % RSD for the six observations were calculated. The method precision study results were presented in Table 4.

Table 4. Method precision results

S.No.	No. of preparations	% Assay
1	Preparation-1	99.25
2	Preparation-2	100.08
3	Preparation-3	100.11
4	Preparation-4	99.47
5	Preparation-5	100.52
6	Preparation-6	100.68
Average		100.0
STDV		0.5644
%RSD		0.56

Linearity

The standard curve was obtained in the concentration range of 100.12-1500.05 µg/mL for Cisplatin. The linearity of this method was evaluated by linear regression analysis. Slope, intercept and correlation coefficient [r^2] of standard curve were calculated and given in Figure 7. to demonstrate the linearity of the proposed method. From the data obtained which given in Table 5 the method was found to be linear within the proposed range.

Table 5. Linearity studies for Cisplatin

S. No	Linearity Level (%)	Concentration (ppm)	Area response
1	10	100.12	859412
2	20	200.42	1719654
3	50	500.19	4311512
4	80	800.21	6897451
5	100	1000.13	8604821
6	120	1200.27	10427987
Correlation coefficient (r^2)			0.9999
Intercept			-20830.4122
Slope			8666.8963
% Y-intercept			-0.24

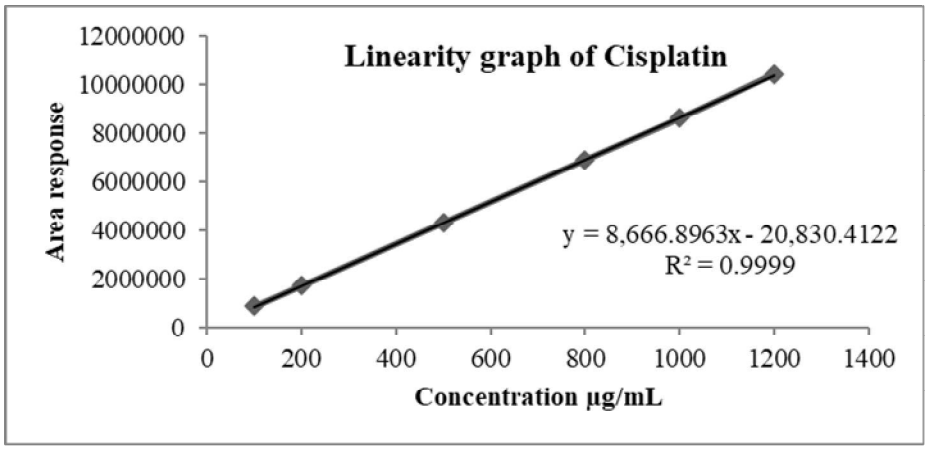

Fig. 7. Calibration curve for Cisplatin

Accuracy

The accuracy of the method was demonstrated by preparing recovery samples of Cisplatin at 50% to 120% of the target concentration level. The recovery samples were prepared in triplicate preparations on Cisplatin API spiked to placebo, and analysed as per the proposed method for each concentration level except 50% and 120%. The above samples were chromatographed and the percentage recovery of each sample was calculated for the amount added. The data obtained which given in Table 6. and the method was found to be accurate.

Table 6. Recovery studies for Cisplatin

Recovery Level	Amount Added (mg)	Amount recovered (mg)	Percentage recovery	Average Recovery (%)	% RSD
50%	0.5052	0.5101	101.0	101.6	0.5
	0.5012	0.5111	102.0		
	0.5024	0.511	101.7		
100%	1.016	1.0043	98.9	99.1	0.2
	1.012	1.0027	99.1		
	1.010	1.0023	99.3		
120%	1.234	1.2120	98.2	98.9	0.7
	1.228	1.2136	98.9		
	1.220	1.2149	99.5		

Solution stability of analytical solutions

Solution stability standard and sample solutions was established at various conditions such as bench top at room temperature and in refrigerator 2-8°C. The stability of standard and sample solutions was determined by comparison of initial prepared standard and sample solutions with freshly prepared standard solutions. The solution stability study results were presented in Table 7-9.

Table 7. Solution stability of standard

Time interval	Similarity factor	
	Room temperature	Refrigerator
Initial	NA	NA
18 hrs	1.01	1.00

Table 8. Solution stability of sample at room temperature

Time interval	%Assay	%Assay difference
Initial	99.3	NA
18 hrs	98.5	0.8

Table 9. Solution stability of sample in refrigerator

Time Interval	%Assay	%Assay difference
Initial	99.3	NA
18 hrs	99.1	0.2

Standard and sample solutions are stable for 18 hours when stored at room temperature and 2-8°C.

Robustness studies

To validate the method robustness the chromatographic performance at changed conditions was evaluated compared to nominal conditions of the method. Standard solution was injected at each of the following changed conditions. The robustness study results were presented in Table 7-9.

Table 10. Robustness studies Results

Parameter		Theoretical plates	Tailing factor	%RSD of peak area
Flow variation ± 10%	0.40 mL	6882	1.2	0.15
	0.44 mL	7215	1.1	0.11
	0.36 mL	6231	1.3	0.10
Temperature variation ± 5°C	25°C	6882	1.2	0.15
	30°C	7998	1.0	0.09
	20°C	5471	1.4	0.39
pH variation ± 0.2	5.9	6882	1.2	0.15
	6.1	6532	1.0	0.07
	5.7	6784	1.2	0.16

CONCLUSION

A validated RP-UPLC method for stability indicating assay of Cisplatin was developed and validated as per ICH guidelines. The results obtained indicate that the proposed method is rapid, accurate, selective, and reproducible. It can be used for the routine analysis of formulations containing any one of the drugs or their combinations without any alteration in the assay. The main advantage of the method is the common chromatographic conditions adopted for all formulations. Therefore, the proposed method reduces the time required for switch over of chromatographic conditions, equilibration of column and post column flushing that are typically associated when different formulations and their individual drug substances are analyzed.

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CONFLICT OF INTERESTS

The authors claim that there is no conflict of interest.

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