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# DEVELOPMENT AND VALIDATION OF A SIMPLE SPECTROSCOPIC METHOD FOR ESTIMATION OF CISPLATIN IN BULK AND IV FORMULATION

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## ABSTRACT

Cisplatin is an important drug of class antitumour agents and is widely used in treatment of many malignancies including Testicular, ovarian, neck, lung, head and bladder. It has a low molar absorptivity in the UV region and has no fluorescence. So a derivatizing reaction is required for its detection in bulk and pharmaceutical dosage form. A simple, precise, rapid spectrophotometric method has been developed for the determination of Cisplatin. This method developed by complexation of the drug with orthophenylene Diamine and the absorbance of green colour solution shown in visible range at 705 nm. This method was validated according to ICH guidelines. The drug obeyed Beers law and showed good correlation. The method linearity observed in the range from 2 to 10 $\mu$g/mL with a correlation coefficient of 0.998. There was no significant difference in the intraday and interday analysis of Cisplatin determined. The results of analysis were validated with respect to recovery, linearity; Limit of detection and limit of quantitation were found to be satisfactory. The proposed method can be applied in routine quality control in the pharmaceutical industries since it is precise, accurate, simple and economic.

**KEYWORDS**: Cisplatin, Complexation, Validated, Precision, Recovery.

## INTRODUCTION

Cisplatin is an important drug of class antitumour agents and is widely used in treatment of many malignancies including testicular, ovarian, bladder, head, neck and lung. It is one of the complexes responsible for cell division inhibition phenomenon.[1] Cisplatin (cis-diamminedichloro-platinum, CDDP) was the first platinum drug to be used clinically. With a wide spectrum of antitumor activities, cisplatin is widely used in the treatment of solid malign cancers, such as thyroid cancers, lung cancers, head and neck cancers and genitourinary cancers.[2] Several methods have been made so far that include detection by phosphorescence of cisplatin in urine and plasma[3], derivative spectrophotometry[4,5], atomic absorption spectrometry[6], electroanalytical methods[7], and highperformance liquid chromatography methods.[8,9,10]

Cisplatin has molar absorptivity is low in the UV region and has no fluorescence. The structure of Cisplatin is unsaturated therefore low absorption so selective derivatizing reaction is required for the detection of drug in biological samples if the optical detection is sought. Derivatization methods have been developed but have some limitations.[11]

Edward et al.[6] also developed the method for the estimation of platinum (IV or II) by using derivatization technique with the help of *o*-phenylenediamine (OPDA). In this study platinum solution was treated with OPDA in presence of dimethylformamide (DMF) at pH 6.5. The solution was heated on boiling water bath for 4min, and the developed light blue color of platinum-OPDA complex was detected at 703 nm against water as blank. This method was basically used to check the effect of pH and temperature on formation of complex and to find out the purity of platinum. Moreover, the method was needed to be validated for the desired purpose.

Anilanmert et al.[4] presented a method for quantification of cisplatin by complexing cisplatin with *o*-phenylenediamine (OPDA). The product was obtained at pH 6.2, in 30 min at $90^0$C, giving a maximum absorbance at 705nm. The method was found specific for formed complex, and the maximum absorbance at 705 nm far beyond the wavelengths of the absorption of cisplatin, *o*-phenylenediamine, and biomolecules in the urine. The detection limit of cisplatin in spiked urine sample was 8.40$\mu$g/mL. The basic limitation of this method includes its higher detection limit, and the method needed to be validated.

Johnson et al.[8] determined cisplatin in urine using derivative spectrophotometry. This method has some disadvantages as the derivatizing reaction was completed

in 24 h and the reagent cannot be found easily in the market.

Most of the methods mentioned above were used for the clinical investigation of cisplatin, and thus the need of developing a simple, rapid, sensitive, cost-effective, and robust method is used to quantify the drug during its regular quality control testing (e.g., assay, dissolution).

In the present study we have used the principle of formation of complex of platinum with Ortho Phenylene Diamine (OPDA), to estimate the amount of cisplatin present in IV dosage form. The developed method was validated as per ICH Q2 (R1) guidelines and successfully applied for assay and dissolution studies of Cisplatin.

## EXPERIMENTAL

## MATERIAL AND METHODS

Cisplatin bulk powder was gifted by Khandelwal Laboratories Pvt. Ltd. Thane. The O-phenylene diamine, Potassium dihydrogen ortho phosphate, dimethyl formamide (DMF) was procured from Loba Chemicals Ltd, Mumbai, India. Sodium hydroxide flakes were purchased from Loba Chemie, Mumbai, India. All other chemicals and reagents used were of analytical grade. Double distilled water was used throughout the study.

### Instrument and Apparatus

A Shimadzu model 1800 (Japan) double beam UV/Visible spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell was used to measure absorbance of all the solutions.

### 1. Analytical method Development

### A. Spectral Analysis of Cisplatin

**UV Visible Spectroscopy: Selection of Analytical Wavelength (λmax)**

For UV method, analytical wavelength was selected from the spectra of Cisplatin obtained by using UV spectrophotometer. Stock solution of Cisplatin was prepared by dissolving 10 mg of drug in 100 ml Phosphate buffer pH 7.4. From this solution withdraw 0.6 ml and diluted with 1mL of 1.4 mg/mL of OPDA solution and 2mL of phosphate buffer pH 7.4 were added and heated at 100∘C for 15 min in order to get light green colour solution and adjust the volume with 10 ml Dimethyl Formamide. A concentration of this solution was 6 µg/ml. The working standard solutions were scanned in the UV- Visible range of 400-800 nm, using DMF as blank for obtaining the Absorption Maxima (λmax).

### B. Sample Solutions

Cisplatin IV formulations were opened and volume equivalent to 6 µg/ml of cisplatin was measured 10 mL volumetric flask, and phosphate buffer pH 7.4 was added to adjust the volume. Appropriate dilutions were made using 1mL of 1.4mg/mL of OPDA solution and 2mL of phosphate buffer pH 7.4 and heated at 100∘C for 10 min in order to get light green colour solution. The prepared colored solutions were cooled to room temperature, and finally the volume was made up to 10mL using DMF.

### C. Stability of Cisplatin in Solution.

The stability of Cisplatin standard stock solution in a mixture with 1.4mg/mL of OPDA solution, phosphate buffer pH 7.4, and adjust the volume with Dimethyl formamide at a concentration of 6$\mu$g/mL was investigated at different time intervals using the experimental conditions.

### 2. Validation of the Developed method

### a) Linearity and Range

Calibration curve was performed to measure the linearity of the curve in the concentration range by using solutions of 2, 4, 6, 8, and 10 $\mu$g/mL respectively. Measurements were taken on samples prepared on 3 consecutive days ($n$ = 3). The values are reported as the mean ± confidence interval of the calibration curves. The data was analyzed at a wavelength of 705 nm. Evaluation parameters such as the correlation coefficient were calculated and are presented.

### b) Accuracy

The accuracy of the method was determined at three levels, 50%, 100%, and 150% of the method concentration (8 $\mu$g/mL). A standard IV solution 10 mg/20ml of cisplatin injection BP (Uniplatin 10) was used. From this 20 ml Iv injection, aliquots of 0.01, 0.02, and 0.03mL of this solution (which would yield concentrations of 4,8, and 12 $\mu$g/mL, resp.) were combined with 8ug/ml of sample solution (this would yield a concentration of 12, 16, 20 $\mu$g/mL). Dilutions were made using 1mL of 1.4mg/mL of OPDA solution and 2mL of phosphate buffer pH 7.4 and heated at 100∘C for 15min in order to get light green colour solution. The prepared colored solutions were cooled to room temperature, and finally the volume was made up to 10mL using DMF. Thus the final concentrations were 12, 16 and 20 $\mu$g/mL, which correspond to 50, 100 and 150% of the target concentration, respectively. The mean recoveries of cisplatin expressed in terms of the percentage recovery and relative standard deviation (%R.S.D.), were determined.

### c) Precision

Precision was evaluated by the repeatability and intermediate precision. Repeatability was tested by three determinations at three levels, 50%, 100%, and 150% of the method concentration (6 $\mu$g/mL) on the same day and under the same experimental conditions. Intermediate precision was evaluated by performing the analysis on three different days (interday) on the same levels as mentioned above and by the three analysts performing the analysis in the same laboratory and under the same experimental conditions (between analyst). Three replicates at concentrations of 2, 6, and 10 $\mu$g/mL were prepared and assayed at 705 nm. The percentage of relative standard deviation (R.S.D.) of the analytical responses was calculated.

***d)*** **Robustness**

The robustness of this method is measure of its ability to remain unaffected by small changes. In the present study robustness has been calculated by varying the pH and taking the absorbance at pH 7 and pH 7.4 and pH 7.8 of the sample solution 8 $\mu$g/mL. Moreover, the robustness of the method was determined by analyzing a change of 2 nm in the wavelength of analysis. Three replicates of the working standard solution and sample solution were prepared at the same concentration (6 $\mu$g/mL), and the assays were carried out at 703, 705, and 707nm.The percentage relative standard deviation (R.S.D.) of the quantitation of cisplatin in the IV was calculated.

**e) Limit of Detection (LOD) and Limit of Quantification (LOQ)[11]**

The standard deviation of response ($\sigma$) and slope of calibration curve ($S$) was used for the estimation of LOD and LOQ. Standard deviation of $Y$ intercepts of regression line was used as standard deviation. Equations (1) and (2) for LOD and LOQ, respectively, are as follows.

**LOD = 3.3$\sigma$ / S**
**LOQ = 10$\sigma$ / S**

## RESULT AND DISCUSSION

**1. Method Development:** The reported methods which were used for the determination of cisplatin were complex, time consuming and costly. Precolumn derivatization of cisplatin (as it lacks chromophore) followed by HPLC analysis makes the process tedious and expensive. In this present work, phosphate buffer pH 7.4, OPDA, and DMF were chosen to obtain an inexpensive, simple, and environment friendly colorimetric method for the quantification of cisplatin in IV injection.

**1. U.V. Analysis**

**A. Selection of Wavelength**

A solution of 6 µg/ml solution of drug was prepared using complex derivatization method and scanned in the range of 400-800 nm, using the above solvent as blank. The maximum absorbance is at 705 nm shown in figure 1.

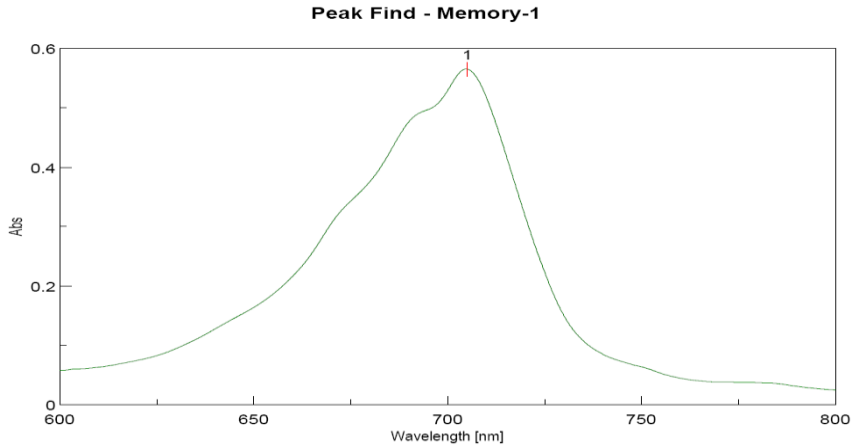


**Figure 1: Spectra of Cisplatin.**

**B. Stability of Cisplatin in Solution**

The results from the stability study indicated that the cisplatin stock standard solution was stable at room temperature for at least one week (Table 1).

**Table 1: Stability of the cisplatin 6 $\mu$g/Ml concentration in mixture of 1.4mg/mL of OPDA solution, phosphate buffer pH 7.4, and DMF.**

| | 0.5hr | 1hr | 2hr | 6hr | 12 hr | 24 hr | Mean | SD | %RSD |
|---|---|---|---|---|---|---|---|---|---|
| **Responses at 705nm** | 0.341 | 0.345 | 0.340 | 0.338 | 0.346 | 0.338 | 0.341 | 0.003 | 0.87 |
| | 0.344 | 0.342 | 0.338 | 0.342 | 0.340 | 0.346 | 0.342 | 0.002 | 0.58 |
| | 0.340 | 0.340 | 0.344 | 0.347 | 0.348 | 0.348 | 0.344 | 0.003 | 0.87 |

**2. Method Validation**

After the method development, the analytical method was validated according to ICH recommendations.

**1. Linearity and Range**

For the analysis of the linearity the standard stock solution of Cisplatin was assessed by calibration curve using 2 µg/ml to 10 µg/ml respectively. Measurements were taken on samples ($n$ = 3). The values are reported as the mean ±confidence interval of the calibration

curves. The data was analyzed at a wavelength of 705 nm. The absorbance was plotted against the corresponding concentrations to obtain the Calibration graph.

**Table 2: Observation of Calibration of cisplatin.**

| Conc. | Abs. I | II | III | AVG | SD | % RSD |
|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 0.135 | 0.135 | 0.137 | 0.135 | 0.002 | 1.04 |
| 4 | 0.209 | 0.207 | 0.205 | 0.207 | 0.002 | 0.96 |
| 6 | 0.341 | 0.344 | 0.340 | 0.341 | 0.0045 | 0.6 |
| 8 | 0.457 | 0.455 | 0.457 | 0.456 | 0.0037 | 0.83 |
| 10 | 0.565 | 0.567 | 0.561 | 0.564 | 0.0030 | 0.54 |

**Table 3: Linear Regression Data for calibration curve.**

| Parameters | Results |
|---|---|
| λ max (nm) | 705 nm |
| Linearity Range, µg/mL | 2-10 µg/mL |
| Slope (m) | 0.055957 |
| Intercept (c) | 0.004048 |
| Correlation coefficient | 0.998101 |

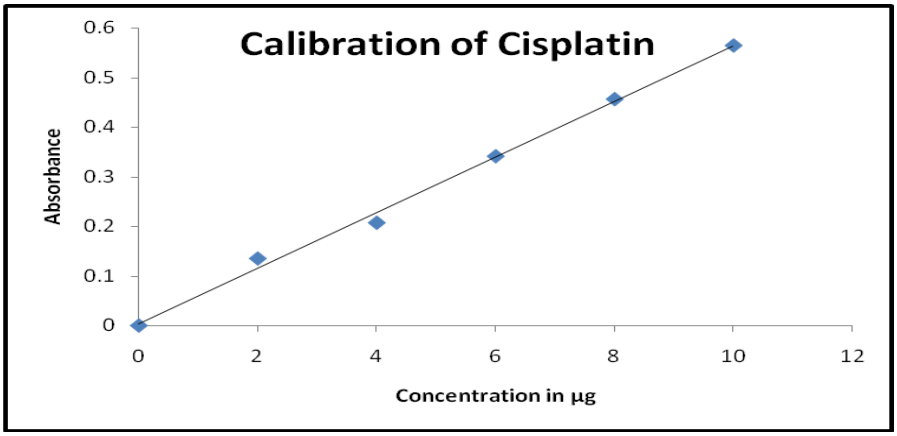


**Figure 2: Calibration curve of Cisplatin.**

**2. Precision**

The precision studies for the present method was carried out as per ICH guideline at the given wavelength (705nm). The intraday and interday precisions of the proposed methods were determined by estimating the corresponding responses 3 times on the same day and on 3 different days. The data obtained was integrated and found within the specified limits (shown in Table 5 and 6).

**Table 5: Precision study.**

| | Concentration µg/mL | Analytical Response | | | R.S.D % |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | |
| **Repeatability** | 2 | 0.135 | 0.135 | 0.137 | 1.04 |
| | 6 | 0.341 | 0.344 | 0.35 | 0.60 |
| | 10 | 0.565 | 0.567 | 0.561 | 0.54 |

**Table 6: Intraday Precision study.**

| Intermediate Precision | Concentration µg/mL | Analytical Response | | | R.S.D % |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | |
| **Day 1** | 2 | 0.137 | 0.135 | 0.137 | 1.25 |
| | 6 | 0.345 | 0.340 | 0.352 | 1.74 |
| | 10 | 0.565 | 0.56 | 0.555 | 0.89 |
| **Day 2** | 2 | 0.137 | 0.135 | 0.135 | 0.85 |
| | 6 | 0.345 | 0.352 | 0.342 | 1.48 |

| | | | | | |
|---|---|---|---|---|---|
| | 10 | 0.565 | 0.562 | 0.560 | 0.44 |
| **Day 3** | 2 | 0.136 | 0.135 | 0.133 | 1.13 |
| | 6 | 0.345 | 0.351 | 0.348 | 0.86 |
| | 10 | 0.565 | 0.559 | 0.56 | 0.57 |

**3. Accuracy**

The accuracy of the proposed method was assessed by determining the average recoveries of samples using the standard addition method. As shown in Table 7, the mean percentage recovery of cisplatin hydrochloride IV (10 mg/20 ml) was 99.93%, and the % relative standard deviation (%R.S.D.) was 1.08%. The results were in accordance with fixed limits from 98.0% to 102.0%, indicating the suitability of the developed method in quantifying the concentration of cisplatin in IV injection. The accuracy value of the current method was found to be excellent.

**Table 7: Method accuracy results Cisplatin IV injection.**

| Sample µg/ml Cisplatin | Reference standard concentration added ($\mu$g/mL) | Total concentration of solution ($\mu$g/mL) | Concentration of drug found ($\mu$g/mL) | Recovery (%) | R.S.D. (%) ($n$ = 3) | Mean recovery% |
|---|---|---|---|---|---|---|
| 6 µg/ml | 2 µg/ml | 8 µg/ml | 7.99 | 99.10 | 0.14 | 99.93 |
| | 6 µg/ml | 12 µg/ml | 12.10 | 100.6 | 1.0 | |
| | 10 µg/ml | 16 µg/ml | 16.00 | 100.1% | 0.82 | |

**4. Limit of Detection (LOD) & Limit of Quantitation (LOQ).**

LOD and LOQ for the method was calculated separately based on the standard deviation response of the calibration curve. Results shown in Table 8.

**Table 8: LOD & LOQ of Cisplatin.**

| Parameter | µg/mL |
|---|---|
| LOD(limit of detection) | 0.1459 |
| LOQ (limit of quantitation) | 0.4423 |

**5) Robustness*:*** The robustness was found reliable, as determined by %R.S.D. (<2%). It was observed that the constancy of the absorbance with changes in the experimental parameter of wavelength resulted in a %R.S.D. of 1.29 and wavelength changes resulted in a %R.S.D. of 0.56 (Table 9). The minor changes which are occurred during the analysis did not affect the absorbance intensity of the samples.

**Table 9: Robustness of test results.**

| Sample | pH | Wavelength nm | Content % | RSD% |
|---|---|---|---|---|
| Cisplatin IV formulation | 7.0 | --- | 101.64 | 1.29 |
| | 7.4 | --- | 99.24 | |
| | 7.8 | --- | 101.32 | |
| | --- | 703 | 100.16 | 0.56 |
| | --- | 705 | 99.82 | |
| | --- | 707 | 100.76 | |

## CONCLUSION

From the study it can be concluded that the method described in this paper for the determination of Cisplatin from bulk and formulation has the simple, accurate, sensitive, reproducible and low cost conditions. All validation parameters were found to be highly satisfactory, including linearity, accuracy, precision, robustness, and adequate detection and quantitation limits The proposed validated method could be applied for routine analysis in quality control laboratories.

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