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COMPARISON OF VALIDATED UV-SPECTROPHOTOMETRIC AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC (HPLC) METHODS FOR DETERMINATION OF CARBOPLATIN IN INJECTION DOSAGE FORM

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ABSTRACT

Carboplatin is available in injection dosage form; therefore a reliable and economical method is required for its analysis for quality control. The objective of the present study is to develop and validate UV-spectrophotometric method for analysis of Carboplatin and its comparison with already existing method of high performance liquid chromatographic method. The purpose of this comparison is to evaluate the added significance of using HPLC method in the presence of validated UV method.

The validated UV-spectrophotometric method demonstrated an excellent linearity (r^2 = 0.9995) in a range of concentration from 10µg/mL to 50µg/mL, precision (%RSD = 0.388) and a mean percentage recovery of 100.14% indicating an accurate method. The assay results of Carboplatin injection obtained from HPLC and UV-spectroscopic methods are quite comparable.

Since no added benefit of HPLC method is found in the presence of validated economical UV-spectrophotometric method, therefore for the quality control purpose, the UV method may be used for analysis of Carboplatin API as well as in injectable dosage form as an alternative to HPLC.

Key words: Carboplatin, UV-spectrophotometry, HPLC, ICH, Sodium hydroxide

INTRODUCTION

Carboplatin is classified as second generation broad spectrum anti-neoplastic drug containing Platinum(II) in its structure **[1]**. It shows inertness at physiological pH, however the actual mechanism of its activation with respect to its anti-cancer activity is still unknown **[2]**. It is used for the treatment of due to which it is broadly used for treatment of ovarian cancer, lung cancer, carcinoma of squamous cell present in the head & neck region and seminomas. Its efficacy is comparable to Cisplatin in the treatment of ovarian cancer **[3]**.

Carboplatin is colorless crystalline powder, sparingly soluble in water and it is very slightly soluble in 96% ethanol and acetone **[4]**.

The route of administration for Carboplatin in intravenous. Distribution in the tissues corresponding to the concentration of Platinum is seen in liver, kidney, skin cells and tumor cells. However, highest concentration of platinum is found in tumor cells as compared to the surrounding tissues. In cancer patients receiving a dose of 20-1600 mg/m^2, the distribution volume is observed to be 16-20L with a half life of 83-96 minutes. This short half life of Carboplatin (as compared to Cisplatin) is due its low binding with plasma proteins that has been measured from a minimum of 10% to a maximum of 24%. The excretion in terms of platinum is about 50-70 percent in urine in 24 hours of the dose administered **[3]**.

Carboplatin causes cross linking in between different strands and within a strand on nucleophillic sites of DNA as well as it causes the cross linking in DNA-protein. Carboplatin increases the phosphorylation in tumor cells without causing any increase in kidney or liver phosphorylation which indicates that it has greater tumor selectivity **[3]**.

Toxicity profile of Carboplatin includes mild to moderate nausea and vomiting,, neutropenia, leuocpenia and thrombo-cytopenia **[1, 3]**.

For quantitative estimation of Carboplatin in formulation dosage form, HPLC methods have been developed and validated. Though these methods are accurate as well precise but these are costly and are not simple and easy to perform.

MATERIAL AND METHOD

Carboplatin reference was obtained from M/s Helix Pharma, Karachi Pakistan with a purity of 100.1 percent. λmax was found to be 230nm for carboplatin which was determined by dissolving Carboplatin in 0.05M solution of NaOH and then analyzing it is UV spectrophotometer in spectrum mode by taking water for injection as blank. The stock solution was prepared by dissolving 25mg of Carboplatin in 25ml of 0.05M solution of

Sodium Hydroxide (NaOH). Following serial dilutions from 50µg to 5 µg were prepared by 1ml, 2ml, 3ml, 4ml and 5ml from the stock solution and diluting these by adding 0.05M NaOH solution upto 100ml for preparing solution with concentrations 10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml and 50µg/ml respectively.

ANALYTICAL METHOD DEVELOPMENT AND ITS VALIDATION:

Calibration curve was plotted by taking 5 different readings for each dilution and value of correlation coefficient (R2) was calculated by using equation for regression in microsoft excel 2012. Then, the accuracy and precision including inter-day (repeatability) and intra-day (reproducibility) were determined.

Following formulas were used to calculate the values for limit of Detection (LOD) and Limit of quantitation (LOQ) by putting the required values using LINEST function of Microsoft Excel 2012.

LOD=3.3 σ/ S

LOQ=10 σ/ S

For determination of Robustness, dilution with concentration 20µg/ml was used. The analysis was performed by setting UV-spectrophotometer on 220nm, 230nm and 240nm and results were compared which were found satisfactory.

RESULTS AND DISCUSSION

System suitability

System suitability tests were performed. Six identical dilutions of concentration 20µg/ml were prepared separately and readings for absorbance were noted down. Then % RSD of the readings was calculated which was found to be according to the acceptance criteria that is ±5% **[5]**. The results are given in **Table 1**.

Linearity

Absorbance for all the prepared dilutions of Carboplatin in 0.05M NaOH solution was measured for constructing the calibration curve as shown in **(Figure 1)**.

The absorbance pattern by the prepared dilutions shows that Beer-Lambert law was obeyed. Calibration curved was constructed by taking value of absorbance on y-axis against the concentration of each dilution on x-axis (**Figure 1**).

The equation for regression was calculated as (Y= 0.079X + 0.0101) where as 0.0785 is value of slope and 0.0101 is the value for y-intercept. The value calculated for correlation coefficient (R^2) 0.9995. A calibration curve having a value of correlation coefficient equal to 0.99 is believed to be linear in nature and even greater values of curves than 0.9 are considered to be more reliable **[6]**.

Accuracy

3 dilutions were prepared in order to determine mean percentage recovery and

results are mentioned in **Table 3**. For an accurate method, the mean percentage recovery should fall in the range of ±2% **[7]**. The results show that the mean percentage recovery satisfies the stated criteria. However, **Table 1** also shows that the mean percentage recovery is within the acceptance criteria.

Precision:

A dilution with a concentration of 20µg/ml was prepared and 5 different reading were taken for determination of precision including Inter-day that is Repeatability as well as Intra-day that is Reproducibility. The results for precision are satisfactory and are given in **Table 4**.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Limit of Detection (LOD) and Limit of Quantification (LOQ) were determined by using Microsoft excel and the results are summarized in **Table 1**.

Robustness

The robustness of the method was determined by changing the wavelength of the UV light. No significant change in absorbance was observed upon slight change in wavelengths, however, changing the wavelength to larger extent can change the absorbance significantly. The results are given in **Table 4**. Moreover, by changing the molarity of NaOH solution no significant change in the absorbance was noted. The analysis may be performed by preparing 0.01M to 0.05M solution of NaOH.

Analysis of Carboplatin Injection by UV spectrophotometry and HPLC method

HPLC Method

Chromatographic conditions

Stainless steel column (30cm×3.9 mm) packed with *aminopropylsilyl silica gel for chromatography* (10 µm) (µ-Bondapak-NH_2 is suitable) was used. The flow was kept at 2ml/min. Detection wavelength was set at 230nm and injection volume of 20µL along with the ambient column temperature was maintained. The mobile phase was prepared by adding 130 volumes of water and 870 volumes of acetonitrile **[8]**.

System suitability:

Calibration curve was prepared by performing system suitability tests considering the required values of certain parameter. Such as number of theoretical plates should not be less than 5000 and symmetry/tailing factor should be less than 2 **[8]**.

Assay

Carbotional Injection (carboplatin 150mg/15ml) was purchased from the local market. !ml from the vial was taken and transferred to a volumetric flask and then the injection was diluted by adding water to make concentration of the solution to 1mg/ml. The standard solution was prepared by taking 10mg of Carboplatin standard in 100ml flask and dissolving in

small amount of solvent. The final volume was so made to get a concentration of 1mg/ml **[9]**. The typical chromatogram is shown in the **Figure 3**. The calculated assay values for Carboplatin Injection (Carbotinol 150mg/15ml) was 97.279% which qualifies the acceptance criteria of 90-105% **[9]**.

UV-spectrophotometric Method

The same injection of Carboplatin (Carbotinol 150mg/15ml) was analyzed by using the validate UV method by preparing a solution of 20µg/ml of standard solution and test solution with the same concentration. The calculated assay value for Carboplatin was 98.72%. The typical chromatogram is given in **Figure 4**.

Table 1: System Suitability Data Sheet for Carboplatin

No. of Samples	Absorbance
Sample 1	0.162
Sample 2	0.163
Sample 3	0.163
Sample 4	0.164
Sample 5	0.163
Mean $\bar{X} = \frac{\sum x}{n}$	0.163
Standard Deviation: $s = \sqrt{\frac{\sum (x-\bar{x})^2}{n-1}}$	0.001
RSD % = 100S/$\bar{x}$	0.613

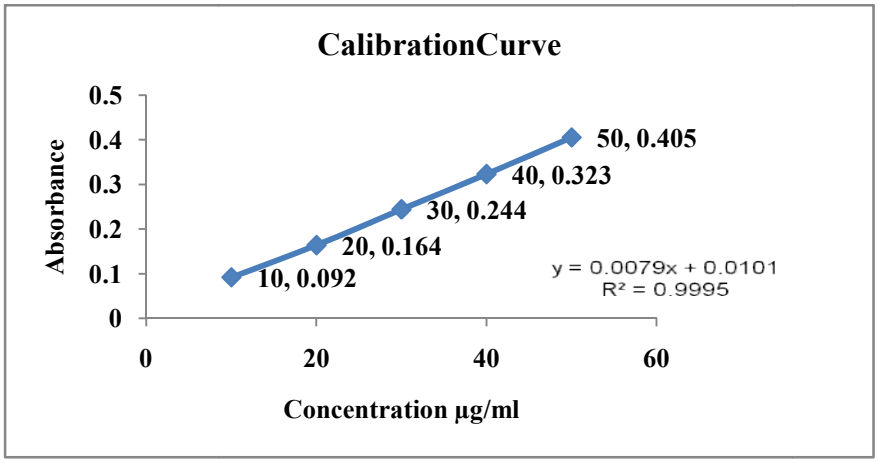

Figure 1: Calibration Curve for Serial Dilutions of Carboplatin

Table 2: Linearity Data Sheet for Serial Dilutions of Carboplatin

S. No.	Concentration (X)	Mean Absorbance	Percentage Recovery
1.	10 µg /ml	0.092	104%
2.	20µg/ml	0.164	98%
3.	30µg/ml	0.244	99.3%%
4.	40µg/ml	0.323	99.65%
5.	50µg/ml	0.405	100.6%
Mean	30 µg /ml	0.2456	100.31%
Linearity Range		10-50µg/ml	
Coefficient of correlation (R^2)		0.9995	
Intercept (b)		0.0101	
Slope (m)		0.00785	
LOD (µg/ml)		1.344785	
LOQ (µg/ml)		4.075106	

Table 3: Accuracy Data Sheet for Serial Dilutions of Carboplatin

Conc. Prepared	Conc. µg/ml	Found Amount µg/ml	Abs. of 3 replicates	Standard Deviation	Relative Standard Deviation	Mean Abs.of 3 replicates	(%) Recovery
50%	10	9.796	0.087 0.088 0.087	0.00058	0.661	0.087	97.96%
100%	20	19.876	0.160 0.163 0.164	0.00208	1.282	0.162	99.38%
250%	50	49.69	0.405 0.405 0.405	0.00	0.00	0.405	248.46%

Table 4: Precision Data Sheet for Serial Dilutions of Carboplatin

	Absorbance (Intra-day)	Absorbance (Inter-day)
Sample Concentration (Carboplatin= 20µg/ml)	0.163	0.164
	0.164	0.165
	0.163	0.165
	0.162	0.165
	0.163	0.165
Mean $\bar{X} = \frac{\sum x}{n}$	0.163	0.165
Standard Deviation: $s = \sqrt{\frac{\sum(x-\bar{x})^2}{n-1}}$	0.001	0.0004
RSD % $= 100S/\bar{x}$	0.613	0.242

Table 5: Robustness Data Sheet for Serial Dilutions of Carboplatin

Parameter	Absorbance
Reference Wavelength (230nm)	0.166
Decreased Wavelength (220nm)	0.016
Increased Wavelength (240nm)	0.145

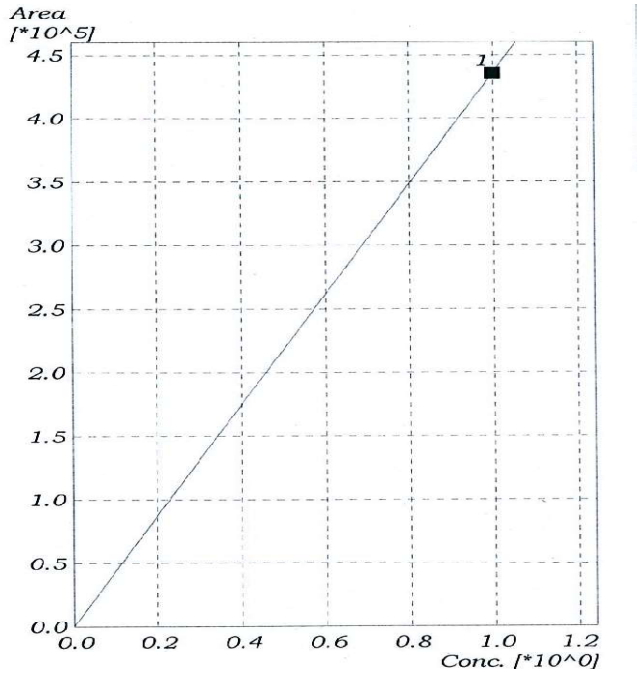

Figure 2: Calibration Curve of Pure Carboplatin

Table 6: Data Sheet for Calibration Curve of Pure Carboplatin

Area		Theoretical plates	Tailing factor
435005		5829	0.957
435581		5816	0.960
434704		5814	0.962
435729		5807	0.964
436229		5803	0.960
Mean Area		435450	
RF%RSD	0.138613		

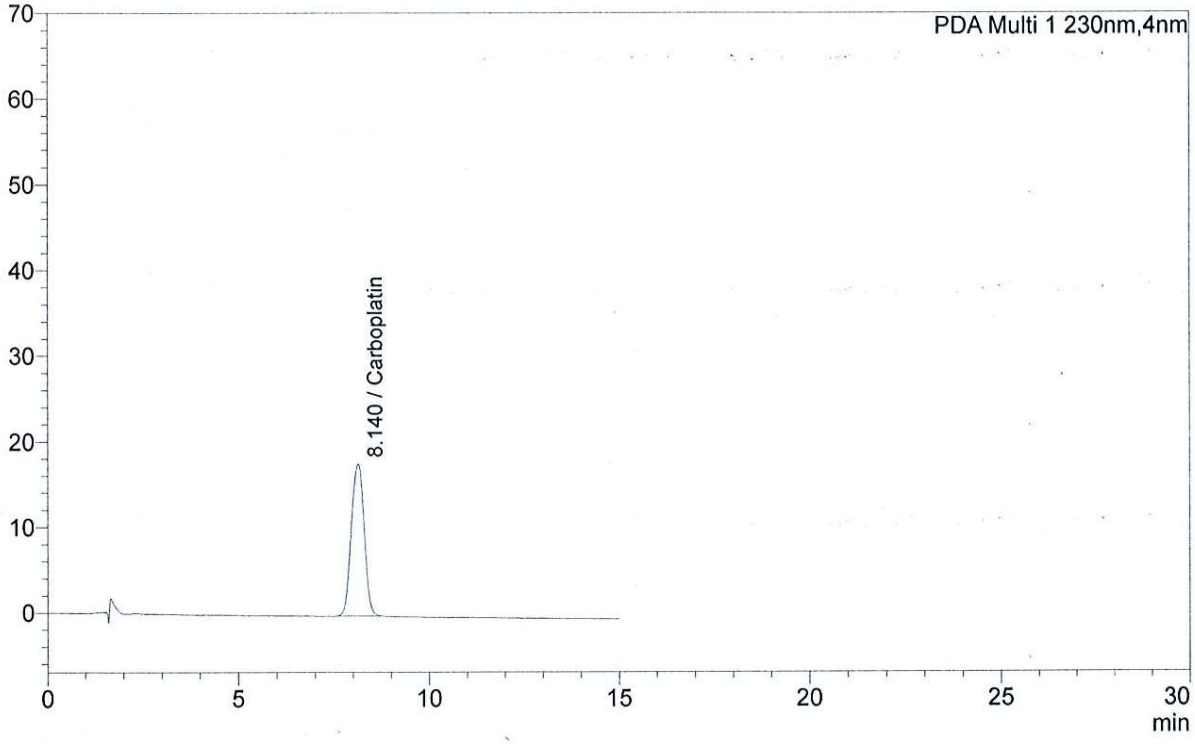

Figure 3: Assay of Carbotinol Injection through HPLC

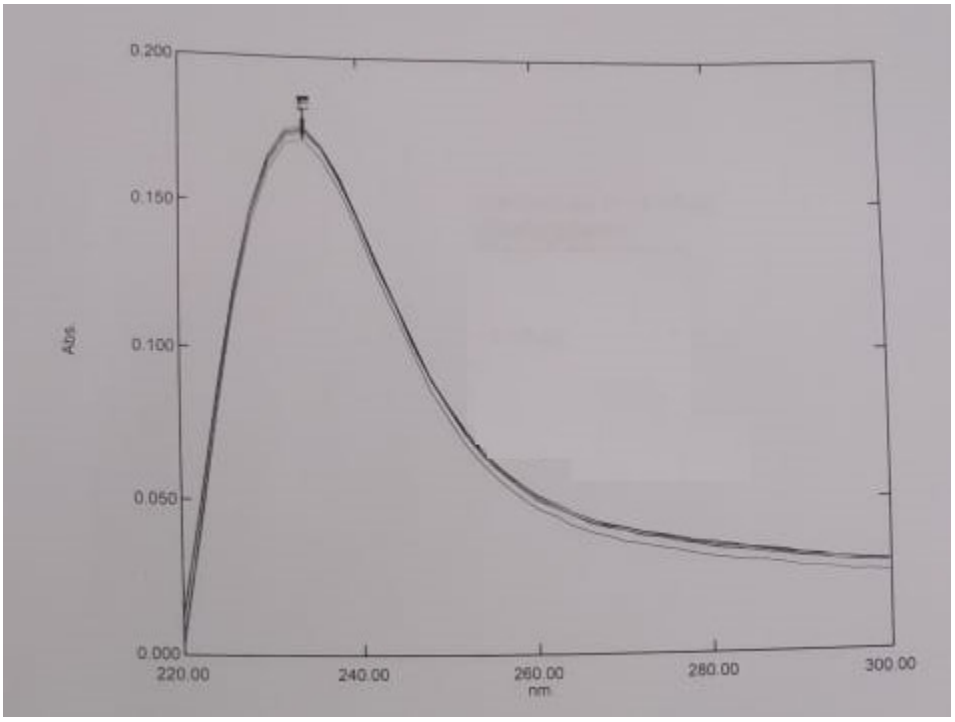

Figure 4: Assay of Carbotinol Injection through UV Spectrophotometer

CONCLUSION

The assay value as per certificate of analysis (COA) for Carboplatin injection (Corbotinol 150mg/15ml) is 99.5%. The assay values calculated by using validated UV-spectrophotometric method is even more close to the actual value given in the COA. Therefore it can be concluded that in the presence of validated UV-spectrophotometric method there is no significant added benefit of HPLC method for estimation of carboplatin in injectable dosage form.

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