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Research Article

Method development and validation for the estimation of carboplatin in pharmaceutical pure and dosage form using UV-Vis method

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ABSTRACT

High incidences and cancer cases in the world for the past decade have resulted in the need to conduct extensive research on the cancer medications. Carboplatin is a platinum compound used mainly in the treatment of lung, head and neck, as well as many other cancers. No method has been reportedly done and developed for the routine analysis of carboplatin using UV-Vis. Many have reported HPLC and Mass spectroscopy. A method was developed and validated for the analysis of carboplatin in the standard and injection form. 10mg of the carboplatin standard was transferred into a 10ml volumetric flask, and diluted up to the mark using 0.1M NaOH. The solution was further diluted to a 1µg/ml solution and scanned using UV-Vis. The ƛ-max was found to be 295nm. A calibration curve was plotted and at 2-10µg/ml Beer's law was obeyed. The Linear equation was y=0.0564x+0.0057. The r^2 value was 0.9996, which was within acceptable limits. Precision using inter-day and intra-day precision were done yielding %RSD values of 0.734 and 0.504 respectively, which were acceptable according to ICH guidelines (≤2). A method was therefore successfully developed and validated for routine use in the analysis of Carboplatin in the standard and parenteral dosage form.

Key words: *Carboplatin; UV-Vis; Method development and validation; ICH guidelines; HPLC*

INTRODUCTION

The World Health Organization has estimated that 32.6 million people are suffering from cancer in the entire world according to 2012 Globocan report[1]. Such a high statistic value suggests a need for availability of medication for cancer treatment, not just medicines but high quality medicines. There are various kinds of medications used for the treatment of cancer, and carboplatin is one such.

Carboplatin is a platinum based antineoplastic drug that was developed in the 1980s to counteract the side effects of cisplatin which was in use at that time, mainly the nephrotoxic effects of cisplatin.

Carboplatin's mechanism of action is by forming intra-strand and inter-strand crosslinks within a cell, which modifies the structure of the DNA and stops synthesis of DNA.

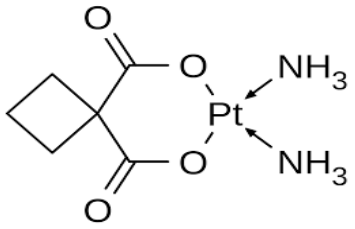

Fig 1. Carboplatin

It is used for the treatment of ovarian cancer, breast,head and neck, neuroblastoma and many other cancertypes. The major side effects of carboplatin are myelosuppession, nausea and vomiting (though more tolerable than cisplatin), diarrhoea, alopecia as well as altered renal function. It is given as an intravenous medication.[2, 3]

Methods have been developed and published on the estimation of Carboplatin using High performance Liquid chromatography and other methods according to the literature review, but no method has been published on method development and validation of UV method[2-4]. UV-Vis spectrophotometry is one of the most employed analytical method currently, which involves the measurement of ultraviolet light, or visible light absorbed by a substance. It is a simple analytical technique that is known to observe the Beer Lambert's law. This law states that the decrease in the intensity of a beam of monochromatic light is directly proportional to the concentration and the path length. The method is validated using ICH guidelines[5].

METHODOLOGY

Carboplatin standard drug was obtained as a gift. The Carboplatin sample (10mg/ml) injection was procured from Shruthi Biotec. Analytical grade NaOH was used. UV-Visible Spectrophotometer was used to carry out the assay.

Standard Working Solution Preparation:

10 mg of Carboplatin was weighed accurately and then transferred into a 10mg volumetric flask. 5 ml 0.1M NaOH was added and shaken to dissolve the Carboplatin, then the 0.1M NaOH was added up to the mark. The resulting 1mg/ml was the working solution.

Determination of the λmax:

1ml solution was taken from the 1mg/ml working solution and put into a 10ml volumetric flask. The solution was further diluted up to the mark using 0.1M NaOH to make 0.1mg/ml concentration. 1ml from this solution was taken and put into a 10ml volumetric flask and diluted to the mark using 0.1M NaOH. The resulting solution was 0.01 mg/ml in concentration. The absorbance was then scanned using UV Spectrophotometer. The λmax was found to be 295nm.

Calibration Curve Plotting:

After finding the λmax to be 295nm, a calibration curve was plotted, using concentrations2-10µg/ml and the respective absorbances of the respective concentrations and it was a linear graph.

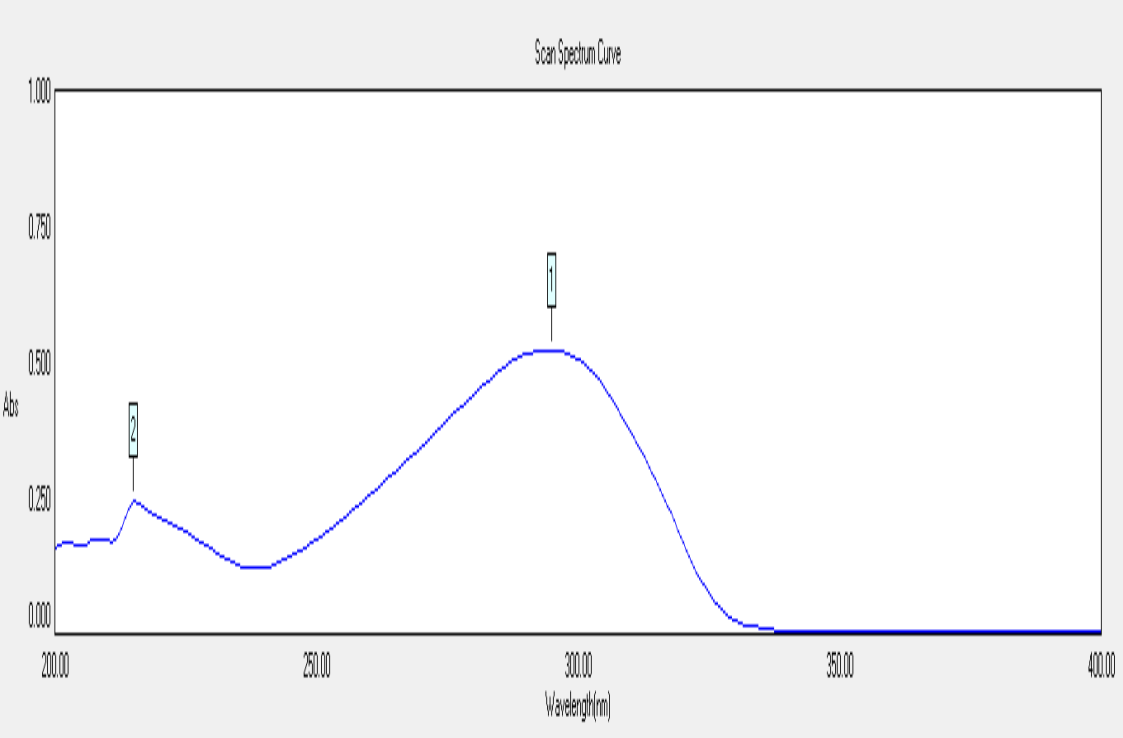

Fig 2. Λ-max for Carboplatin

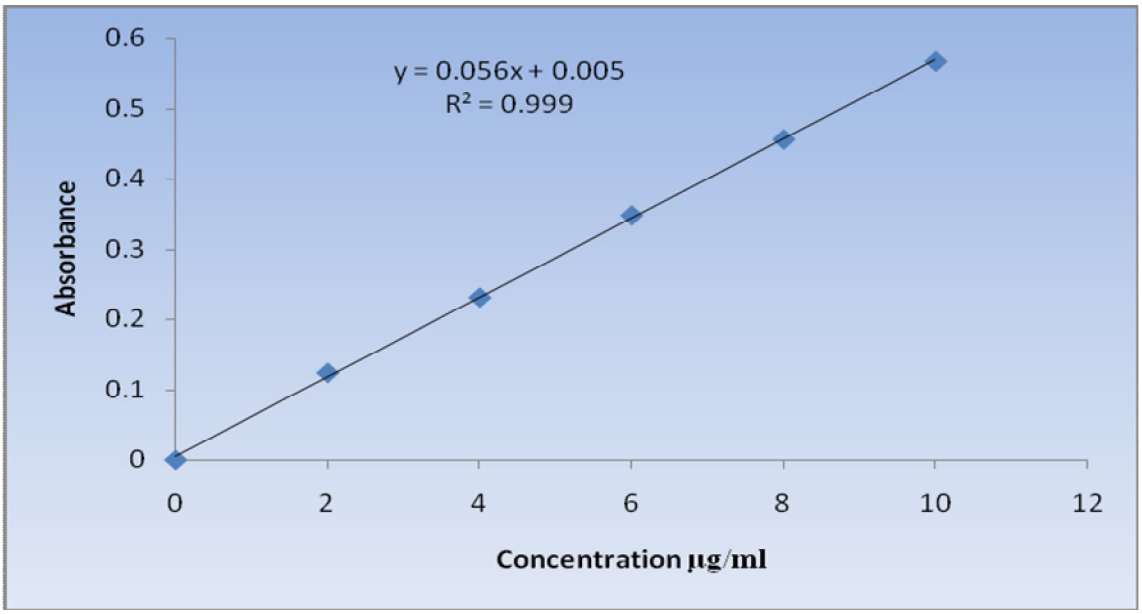

Fig 3. Linearity and calibration curve

Determination of Active Ingredients in an Injection Sampl:

The proposed method was used to analyze the quantity and for the identification of the drug from an injection consisting of 10mg/ml Carboplatin. 1ml was taken from the 10mg/ml solution and put into a 10ml volumetric flask and further diluted up to the mark with 0.1M NaOH. The resulting 1mg/ml solution was further diluted into 0.01 mg/ml concentration by dilution of 1ml of the solution into a 10 ml volumetric flask, and repeating the same process again. The resulting working sample solution was 0.01mg/mlsolution of Carboplatin. 5 concentrations of 2-10 mg/ml were assayed. The results are shown in Table I.

Accuracy of Standard:

Preparation of Solutions was one in triplicate at levels 120%, 100% and 80%. The absorbance was recorded for each solution. The recovery results were recorded, and the Standard deviation as well as the %RSD were calculated.

Accuracy of the Injection Sample:

1mg of the standard working solution was transferred and added to 1ml of the sample solution of concentration 10mg/ml in a 100ml volumetric flask. The volume was made up to 100ml using 0.1M NaOH and shaken. 50 ml of the solution was taken into another 100ml volumetric flask and 0.1M NaOH was added. The solution was shaken, and the resulting concentration of 10µg/ml, for which the absorbance was taken in triplicate to check for accuracy. The results are shown in table III.

Precision:

The precision was demonstrated through repeatability, inter-day and intraday studies.

Repeatability:

six samples were taken from the same concentration of 10µg/ml and the absorbance was observed. The mean and the %RSD were calculated for which the acceptance limit is supposed to be 2%.

Inter-day Precision:

six solutions of the same concentration of 10µg/ml were subjected to analysis by UV-VIS. The absorbance and mean of the %RSD were calculated.

Intraday Precision:

6 different solutions of the same concentration of 10µg/ml were analyzed three times a day at different times and %RSD and the average of the %RSD were calculated.

Effect of Temperature:

The effect of temperature was observed on 10µg/ml solution of the carboplatin at three different temperatures and the results were taken in triplicate.

Effect of Different Wavelengths of Light:

10µg/ml of the carboplatin solution was subjected to different wavelengths from the UV-Vis and their respective absorbances were noted.

Table I. Concentration vs. absorbance for Carboplatin injection.

SI. No	Concentration µg/ml	Absorbance
1	2	0.124
2	4	0.231
3	6	0.348
4	8	0.456
5	10	0.569

Table II. Accuracy of standard

SI. No	**Preparation**	**Absorbance**	**Concentration**	**Recovery percentage**	**Mean± SD**	**%RSD**
1	120%	0.682 0.684 0.679	12.078 12.113 12.023	100.639881 100.9375 100.1934524	**100.590± 0.374**	0.372
2	100%	0.587 0.587 0.579	10.380 10.380 10.237	103.8035714 103.8035714 102.375	**103.327± 0.825**	0.798
3	80%	0.452 0.458 0.455	7.970 8.077 8.023	99.62053571 100.9598214 100.2901786	**100.290± 0.670**	0.668

Table III. Accuracy of sample

Amount of Drug from the formulation (µg/ml)	**Amount of drug added (µg/ml)**	**% Drug added**	**Total Amount of Drug**	**Absorbance (nm)**	**Amount recovered**	**% Recovery**
5	5	100	10	0.568	10.041	100.410
5	5	100	10	0.563	9.952	99.519
5	5	100	10	0.565	9.986	99.875
					Mean± SD =99.935±0.45 **%RSD** =0.450	

Table IV. Intraday precision

	ABSORBANCE		
Concentration µg/ml	**Time- 9am**	**Time-12:30pm**	**Time-2:30pm**
10	0.561	0.563	0.559
10	0.562	0.561	0.561
10	0.567	0.566	0.564
10	0.567	0.569	0.563
10	0.564	0.563	0.562
10	0.561	0.559	0.559
% RSD	0.502674807	0.638885969	0.371754356
Average % RSD	0.504		

Table V. Inter-day precision results

Drug	Concentration (µg/ml)	Amount found (mg)	Mean± SD	%RSD
Carboplatin 150mg injection	10	148.42		
	10	150.19		
	10	149.74	150.01±1.101	0.734
	10	149.67		
	10	151.82		
	10	150.21		

Table VI. Effect of change of temperature on absorbance

SI. No	**Temp. ^{0}C**	**Absorbance**	**Concentration (µg/ml)**	**Mean±SD**	**%RSD**
1	25	0.563	9.951	**99.280±** 0.206	0.208
	25	0.561	9.916		
	25	0.561	9.916		
2	30	0.582	10.291	**102.85±** 0.449	0.437
	30	0.584	10.327		
	30	0.579	10.238		
3	35	0.581	10.273	**102.851±** 0.103	0.100
	35	0.582	10.291		
	35	0.582	10.291		

Table VII. Effect of change in ʎ-max

SI. No	Conc (µg/ml)	ʎmax (nm)	Absorbance
1	10	295	0.682
2	10	350	0.003
3	10	275	0.391
4	10	250	0.171
5	10	225	0.186
6	10	200	0.155

Table VIII. System suitability

Drug	Linearity range	Regression equation	R^2	Slope	Intercept
Carboplatin (10µg/ml)	2-10	y =0.0564+0.0057	0.9998	0.0564	0.0057

DISCUSSION

According to Subhashini[2] the ʎ-max for carboplatin used was 225nm, but in this study Carboplatin was subjected to UV-Vis analysis and the ʎ-max was found to be 295nm. The other wavelengths could not give the maximum absorbance as shown in the results when the comparison of the wavelengths and the respective absorbances was done. Linearity was achieved in the range 2-10µg/ml and therefore Beer's law was obeyed. The equation of the line was y=0.0564x+0.0057, and the r^2 value was found to be 0.9996 which is acceptable according to the guidelines. System suitability was therefore proven. Inter-day, intra-day were performed, and yielded %RSD values of 0.734 and 0.504 respectively, which is acceptable according to the ICH guidelines. An increase in the temperature resulted in an increase in the absorbance. Therefore in the proposed method it is recommended to warm the 1µg/ml solution for 2

minutes before scanning with the UV-Vis. With the system suitability results and all the results being in range, the method was therefore considered well validated.

CONCLUSION

A method for the analysis of Carboplatin in standard and injection form was therefore developed and validated according to ICH guidelines. The proposed method can therefore be used for the routine analysis of Carboplatin in standard form as well as the parenteral form.

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