METHOD DEVELOPMENT AND VALIDATION OF CARBOPLATIN BY UV SPECTROPHOTOMETRIC METHOD IN BULK AND PHARMACEUTICAL DOSAGE FORM

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Received: February 02, 2019 Accepted: March 25, 2019

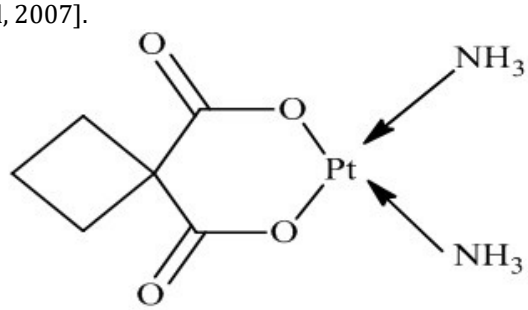
ABSTRACT:*A simple, novel, sensitive and specific spectrophotometric method was developed and validated for the determination of carboplatin in bulk and its pharmaceutical dosage form. A simple UV spectrophotometric method had been developed and validated with different parameters such as linearity, precision, repeatability, limit of detection (LOD), limit of quantification (LOQ), accuracy, robustness and ruggedness. The method A was carried out with 0.1N Hcl and method B was carried out with acetate buffer of pH 4.5. The wavelength maxima 230nm was selected for the estimation of drug using distilled water as a solvent. The absorption minima was found to be 239 nm for first-order derivative. The Beer-lamberts law was obeyed in a concentration range of 20-180µg /ml. The accuracy of the method was assessed by recovery studies and was found between 99.2 to 99.6 for method A and 99.4 to 100.6 for method B, respectively. The LOD and LOQ of carboplatin were found to be 3.3µg/ml and 10µg/ml respectively. The proposed methods are recommended for routine analysis since it was a rapid, simple, accurate and also sensitive.*

Key Words:*Carboplatin, UV Visible spectrophotometer, Zero-order and First-order derivative and ICH guidelines.*

INTRODUCTION

Quantitative analysis is an analysis in which the amount or concentration of an analyte may be determined and stated as a numerical value in appropriate units [Chaitanya and Raja,2018]. UV-visible spectrophotometry is one of the most frequently employed technique in pharmaceutical analysis. It involves measuring the amount of ultraviolet or visible radiation absorbed by a substance in solution [Rahul and Indrajeet ,2015]. The Beer Lambert's 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 advantage of this method takes less time and low labor consumption [Mahfuza et.al,2012]. The usage of UV-Vis spectrophotometry has increased drastically over the last few year especially in the analysis of pharmaceutical dosage form.

Carboplatin is a sold under the trade name paraplatin and it was a second-generation organoplatinum compound with a broad spectrum of antineoplastic properties. It was developed in the 1980s to counteract the side effects of cisplatin mainly the nephrotoxic effects of cisplatin. It is initiated for the initial treatment of advanced ovarian carcinoma in established combination with other approved chemotherapeutic agents. One customary combination treatment consists of paraplatin and cyclophosphamide [Mittal et al, 2007].



Chemical Structure of Carboplatin

Carboplatin is also indicated for the palliative treatment of patients with ovarian carcinoma recurrent after prior chemotherapy, including patients who have been previously treated with cisplatin. The chemical formula and molecular weight of carboplatin are $C_6H_{12}N_2O_4Pt$ and 371.254, respectively. Recently, UV

spectrophotometric methods was reported for estimating carboplatin using solvent like 0.1M NaOH [Hamunyare et.al,2018]. Literature survey revealed that various analytical methods such as HPLC [Hiroshi et.al 2006], LC-MS/MS [Hongliang et.al.2011], and capillary zone electrophoresis [Nawal et al. 2018] were reported for the estimation of carboplatin. In the current study, the efforts were made to develop a simple, easy and economic UV spectrophotometric method using 0.1 N HCl and acetate buffer for the determination of carboplatin in bulk and pharmaceutical dosage forms. The developed method was optimized and validated as per the guidelines of International Conference on Harmonization (ICH) and demonstrated excellent specificity, linearity, precision and accuracy for carboplatin.

MATERIALS AND METHODS

Carboplatin was obtained as a gift sample from Spectrum lab, Hyderabad. All the chemicals used were of analytical grade and purchased from Thermo Fischer Scientific India private limited. The injection formulations were procured from a local pharmacy.

Instrumentation

UV 1800 double beam UV Visible Spectrophotometer with a pair of 10mm path length matched quartz cells were used for the study. The UV solutions 2.42 software was used.

Preparation of standard drug solution

Carboplatin (10 mg) was accurately weighed and taken in 10 ml clean and dry volumetric flask. Drug was dissolved and diluted up to the mark using distilled water. This was considered as the standard stock solution (1000 µg/ml). From this 1 ml of the stock solution was pipette out and made up to 10 ml (100 µg/ml). It was treated as the working standard [Ishpreet et al,2015]. The working standard solution was further diluted with 0.1N HCL and acetate buffer (pH 4.5) for method A (0.2-1.8 µg/ml) and the method B (0.2-1.8 µg/ml).

Method Development

Determination of λ_{max}

The λ_{max} was determined by appropriate dilutions of standard solution with 0.1N HCl and acetate buffer. Carboplatin (10µg/ml) was scanned in the range of 200-400nm.

Preparation of acetate buffer(pH 4.5)

Sodium acetate trihydrate (2.99 gm) was weighed accurately and transferred into 1000 ml deionised water and. Glacial acetic acid (1.7ml) was added to it and then the pH was adjusted to 4.50 with glacial acetic acid.

Preparation of HCl (0.1N)

Conc. Hydrochloric acid (5mL of 35%) was added to 1000ml volumetric flask which containing 900ml de-ionised water and mixed well. The volume was then made up to 1000ml with de-ionised water.

Validation procedure

Analytical method development and validation play a major role in the discovery, development, and manufacture of pharmaceuticals [Micheli Wrasse et al, 2010]. Validation of the analytical method is the process that establishes by laboratory studies in which the performance characteristics of the method meet the requirements for the intended analytical application. UV spectrophotometric method developed was validated according to International Conference on Harmonization (ICH) guidelines for validation of analytical procedures. The method was validated for the parameters like linearity, accuracy, system precision, intra-day precision, inter-day precision/ intermediate precision/ ruggedness, robustness, limit of detection (LOD) and limit of quantitiation (LOQ) [Hemalatha rathod et al., 2014].

Linearity

Ten points calibration curve were obtained in a concentration range from 20-180 µg/ml for carboplatin. The response of the drug was found to be linear in the investigation concentration range and the calibration curves were constructed by plotting absorbance versus concentration and the linear regression equation were calculated [Siladitya Behera et al, 2012]

Accuracy

The accuracy of the method was determined on three concentration levels by recovery experiments which were carried out at three different levels i.e. 80%, 100% and 120%. A known amount of standard drug solution was added to the pre-analyzed sample solution at three different levels, The absorbance was documented and % recovery was then calculated.

Precision

Precision studies were carried out to ascertain the reproducibility of the proposed method. [Arti Mohanr and Ghosh,2013]. The precision of the assay method was determined by repeatability (intra-day) and intermediate precision (inter-day).

Intra-day precision

Standard stock solution (12,16 &20ml) were taken in a 10 ml volumetric flasks and final volume was made up to the mark with buffer. The absorbance of these solutions were individually measured thrice within a day and recorded.

Inter-day precision

Standard stock solution (12,16 &20 ml) were taken in 10 ml volumetric flasks and volume were made up to the mark with buffer. The absorbance of these solutions were individually measured thrice in three days and recorded.

Limit of detection

Limit of detection decide about the sensitivity of the method. LOD is the lowest detectable concentration of the analyte by the method. LOD was calculated by 3.3 σ/ S. Where σ was the standard deviation of blank and s was slope of calibration [Samina et al, 2011].

Limit of quantification

Limit of quantification agree about the sensitivity of the method. LOQ is the minimum quantifiable concentration and it was calculated by 10 σ/ S. Where σ is the standard deviation of blank and s is slope of calibration.

Results

The standard solution of carboplatin in water (10µg/ml) was allowed to a scan individually at series of wavelengths of 200 nm to 400 nm at zero order derivative mode. The first order derivative spectra were taken at a smoothening factor of the instrument using Shimadzu 1800 spectronic UV Visible spectrophotometer. The absorption maximum was found to be at 230nm for zero order derivative and the absorption minima was found to be 239 nm for first order derivative . An overlain spectrum was shown in fig 5-8 and summary of validation parameters was represented in table 1.

Table 1: Summary of validation parameters.

Methods	Order	Correlation coefficient	%Recovery ± SD	Sandell's sensitivity	Molar absorptivity
Method A	Zero-order	0.9992	99.6 (0.12%)	1.189×10^{-1}	8.41×10^{-3}
	First-order	0.9994	99.9 (0.24%)	2.5000	4.00×10^{-3}
Method B	Zero-order	0.9998	99.8 (0.14%)	1.6393×10^{-1}	6.10×10^{-3}
	First-order	0.9996	99.4 (0.32%)	3.4482	2.90×10^{-4}

Linearity

Beer Lambert's law was obeyed in the concentration range of 20-180µg/ml. Calibration curves were shown in fig 1-4.

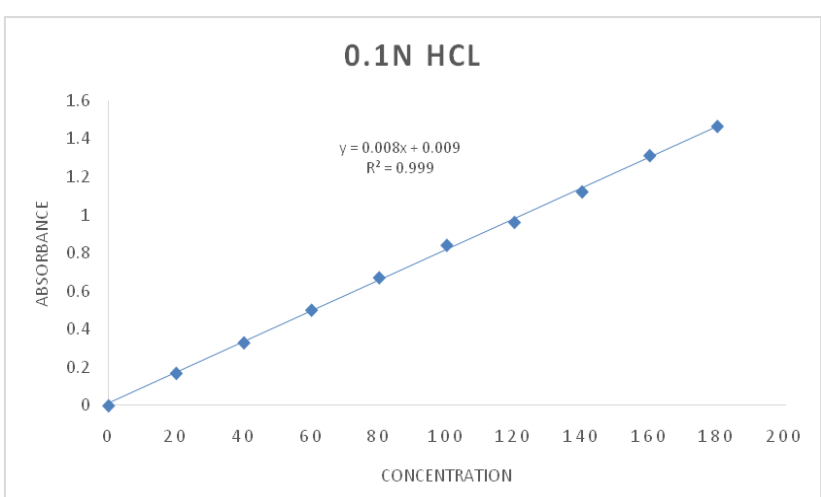


Fig. 1: Calibration curve of method A (zero order).

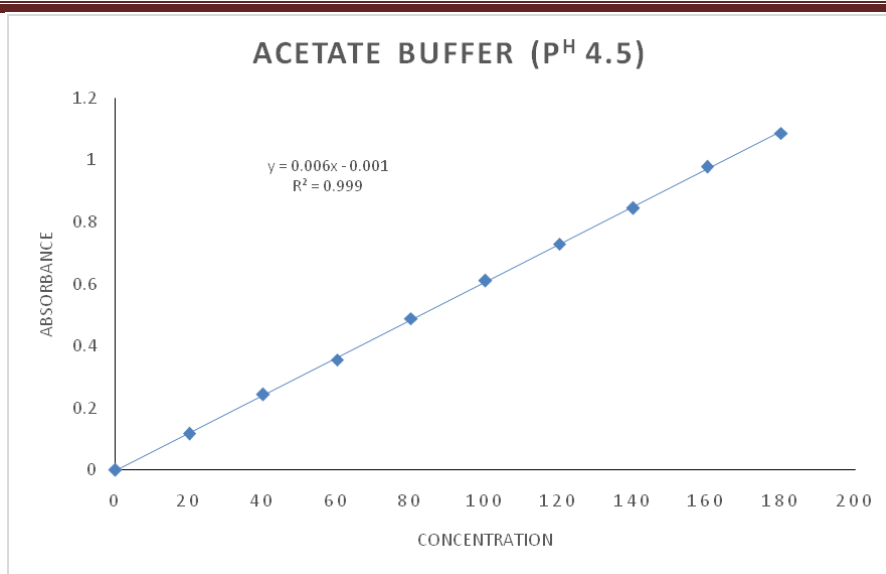


Fig. 2: Calibration curve of method B (zero order).

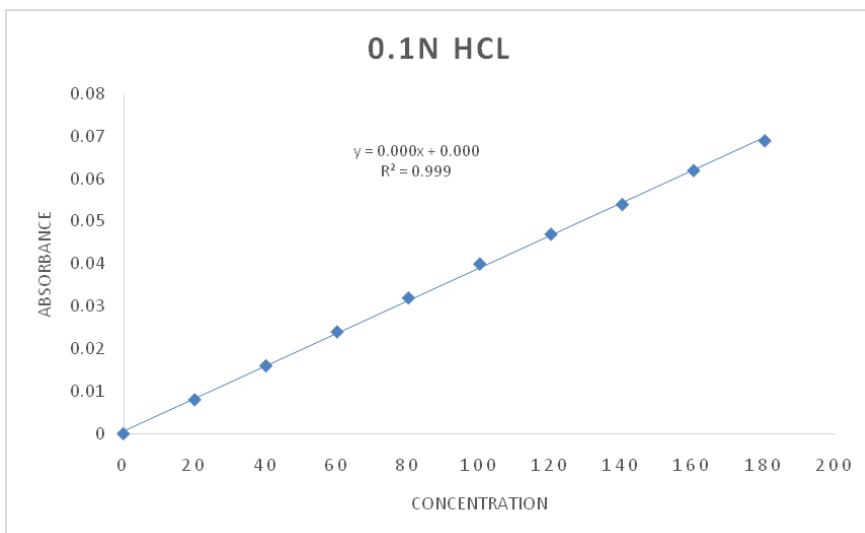


Fig. 3: Calibration curve of method A (first –order derivative).

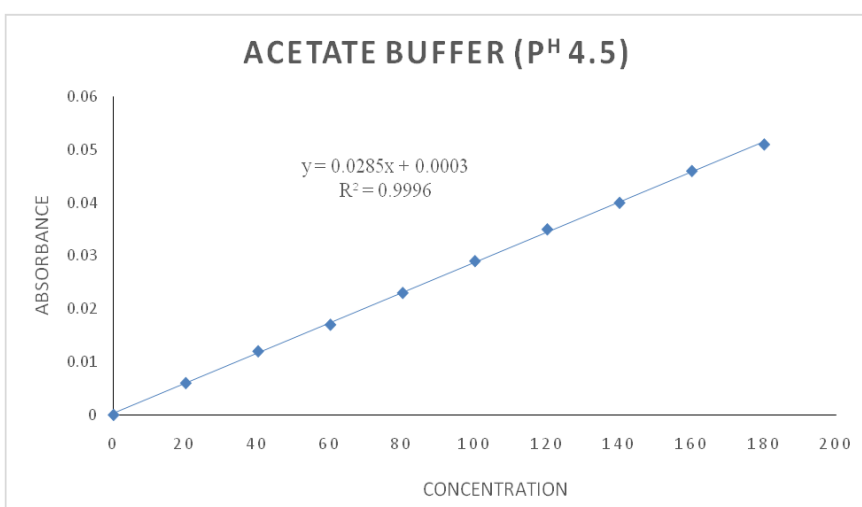


Fig. 4: Calibration curve of method B (first-order derivative).

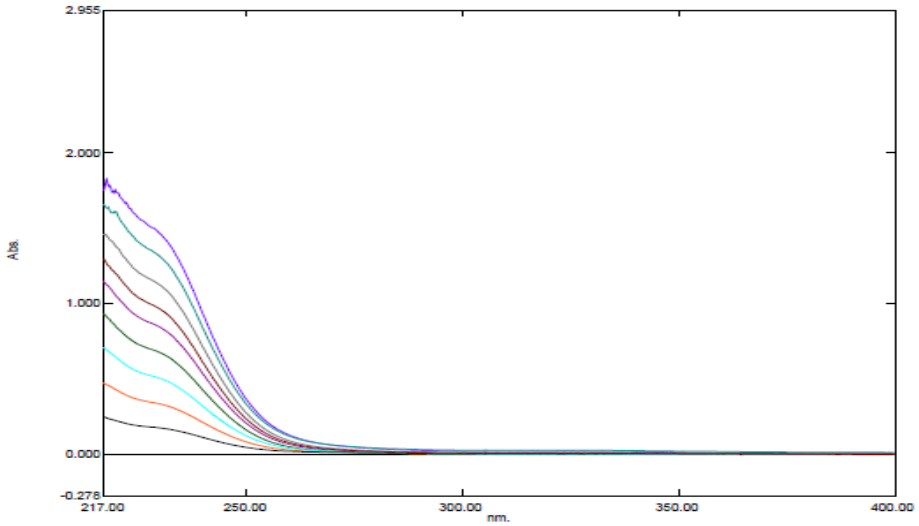


Fig.5: Overlain spectrum of method A (zero order).

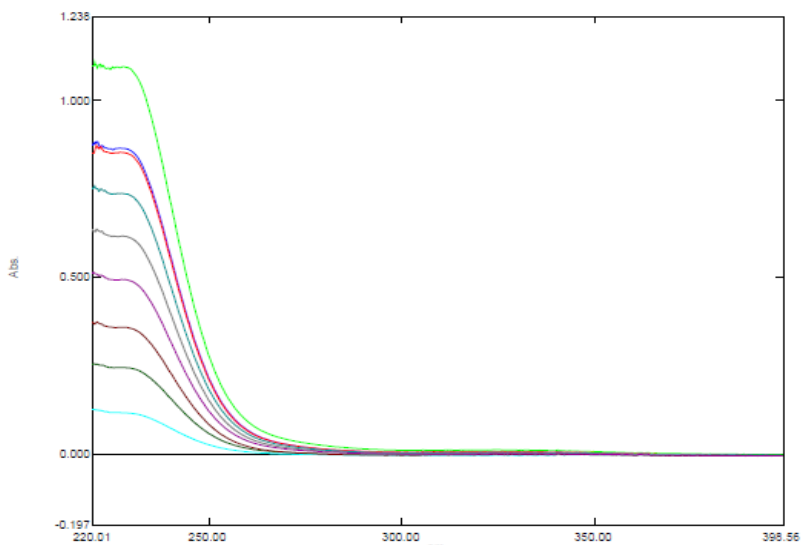


Fig.6 : Overlain spectrum of method B (Zero order).

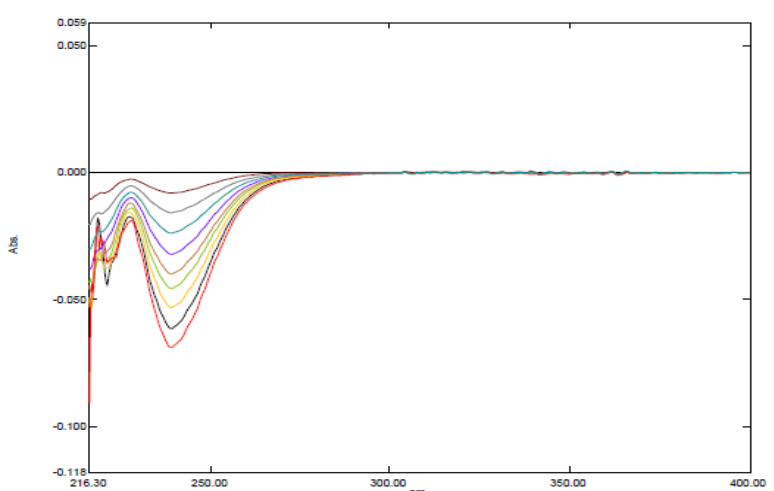


Fig. 7: Overlain spectrum of method A (First order).

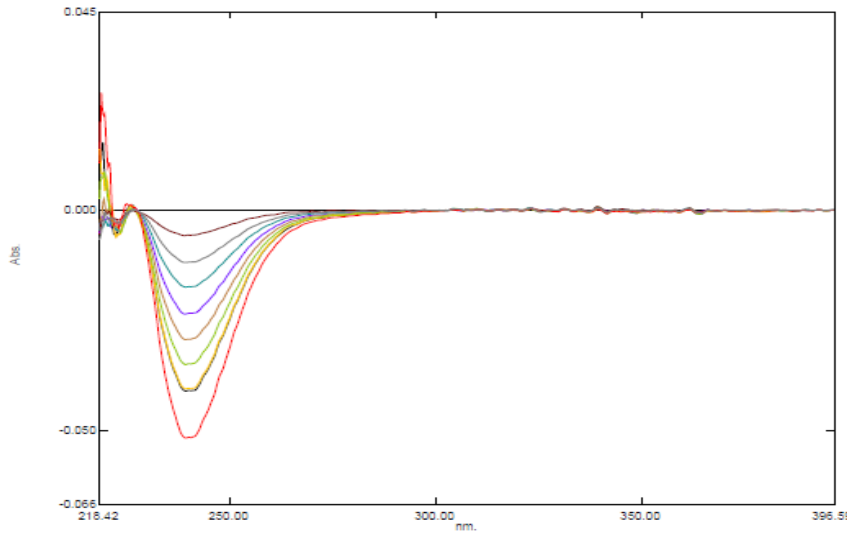


Fig. 8: Overlain spectrum of method B (First order).

Accuracy

The mean % recovery of concentrations ranging from 80%, 100%, 120% was found to be 99.2 to 99.6 for method A and 99.4 to 100.6 for method B, respectively. The results were shown in table 2&3.

Table 2: Accuracy data of method A

Method A	Initial amount (µg/ml)	Amount added (µg/ml)	Amount recovered (µg/ml, n=3)	*% Recovery ± SD (%RSD)
Zero order	25	12.5	37.36	99.6 ± 0.1193 (0.32%)
	25	50	74.41	99.2 ± 0.3643 (0.49%)
	25	68.75	93.41	99.6 ± 0.4086 (0.44%)
First order	25	12.5	37.34	99.6 ± 0.1358 (0.36%)
	25	50	74.55	99.4 ± 0.4651 (0.62%)
	25	68.75	93.32	99.5 ± 0.5187 (0.56%)

Table 3: Accuracy data of method B

Method B	Initial amount (µg/ml)	Amount added (µg/ml)	Amount recovered (µg/ml, n=3)	*% Recovery ± SD (%RSD)
Zero order	25	12.5	37.35	99.6 ± 0.1514 (0.41%)
	25	50	75.09	100.1 ± 0.1206 (0.16%)
	25	68.75	93.6	99.8 ± 0.2425 (0.26%)
First order	25	12.5	37.74	100.6 ± 0.2616 (0.69%)
	25	50	74.99	100.0 ± 0.3869 (0.52%)
	25	68.75	93.22	99.4 ± 0.2030 (0.22%)

Precision

The %RSD for the inter-day and intra-day precision were reported to be 0.24 & 0.12 for method A and 0.29 & 0.10 for method B, respectively. The results of precision were shown in table 4-7.

Table 4: Intra-day precision data of method A.

Method A	Concentration (µg/ml)	Amount found (µg/ml)	Mean ± SD (µg/ml, n=3)	%RSD
Zero order	12	11.97	100.26 ±0.41	0.42
	16	15.77	99.75±1.39	1.14
	20	20.17	100.41 ±0.53	0.54
First order	12	11.97	99.63 ±0.36	0.36
	16	15.49	99.69 ±0.24	0.24
	20	19.81	99.07 ±0.28	0.28

Table 5: Inter-day precision data of method A.

Method A	Concentration (µg/ml)	Amount found (µg/ml)	Mean ± SD (µg/ml, n=3)	% RSD
Zero order	12	11.98	99.89 ±0.41	0.41
	16	15.97	99.81±0.55	0.56
	20	19.91	99.58 ±0.19	0.20
First order	12	11.94	99.37 ±0.12	0.13
	16	15.78	99.36 ±0.65	0.66
	20	19.81	99.12 ±0.39	0.14

Table 6: Intra-day precision data of method B

Method B	Concentration (µg/ml)	Amount found (µg/ml)	Mean ± SD (µg/ml, n=3)	% RSD
Zero order	12	11.47	100.20 ±0.74	0.48
	16	14.40	99.81±1.02	1.02
	20	19.85	100.90 ±0.53	0.49
First order	12	11.92	99.69 ±0.41	0.41
	16	14.90	99.33 ±1.02	1.62
	20	19.84	99.07 ±0.29	0.29

Table 7: Inter-day precision data of method B

Method B	Concentration (µg/ml)	Amount found (µg/ml)	Mean ± SD (µg/ml, n=3)	% RSD
Zero order	12	11.48	100.70 ±0.81	0.39
	16	14.97	99.71±0.55	0.46
	20	19.90	99.46 ±0.19	0.10
First order	12	11.93	99.23 ±0.12	0.12
	16	14.67	99.26 ±0.55	0.55
	20	19.82	99.06 ±0.35	0.12

LOD& LOQ

The limit of detection and the limit of quantification were determined to be 3.3 µg/ml and 10µg/ml for method A & method B.

DISCUSSION

In recent years, the development of analytical method development and statistical validation of UV spectrophotometric method for carboplatin in bulk and pharmaceutical dosage form has acquired considerable attention due to their importance in quality control testing of drugs and their products. Due to their wide availability and suitability [Ghulam and Shujaat Ali, 2011]. Since no method was reported in literature for the method development and statistical validation of UV spectrophotometric method for carboplatin in bulk and pharmaceutical dosage form tablets for the routine quality control assay in ordinary laboratories. The development of such method could be appreciable.Carboplatin is an UV-absorbing molecule with precise chromophores in the structure that absorb at a certain wavelength and this circumstance was successfully employed for their quantitative determinations using the UV spectroscopic method [Talekar et al,2000]. The spectral analysis showed the λ max of carboplatin to be 230nm. Carboplatin obeyed Beer's law in the concentration range of 20-180µg/ml(Method A & B).The mean % recovery was validated as per the ICH guidelines.The precision (measurements of intraday and interday) results showed good reproducibility with percentage relative standard deviation (% RSD) is below 2.0.The evaluation of accuracy of the method was performed by standard addition method. This indicated that method is highly precise.The regression analysis of the calibration curves and the optical characteristics such as Beer's law limits, molar absorptivities and Sandell's sensitivities were also determined [Sowjanya et al,2012].The LOD and LOQ of carboplatin were determined by using the standard deviation of response and slope approach as defined by ICH guidelines.

CONCLUSION

It could be concluded that the developed method for estimation of carboplatin in pharmaceutical dosage form and in bulk is simple sensitive, accurate, precise, reproducible, and economical. The proposed method can be used for routine quality control analysis of carboplatin in bulk and pharmaceutical formulation.

ACKNOWLEDGEMENTS

The authors are thankful for the management of GITAM (Deemed to be University) Visakhapatnam, Andhra Pradesh, India, for providing necessary facilities to carry out the research work.

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