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Original Article

RAPID DETERMINATION OF CARBOPLATIN AND DOCETAXEL USİNG RP-HPLC WİTH PDA DETECTOR

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ABSTRACT

Objective: In the current investigation, to separated and validate the cancer healing drugs (Carboplatin and Docetaxel) through the HPLC (e-2695) instrument containing a PDA detector.

Methods: A simple, selective, validated and well-defined stability that shows isocratic RP-HPLC methodology for the quantitative determination of Carboplatin and Docetaxel. The chromatographic strategy utilized Symmetry C_{18} column of dimensions 150x4.6 mm, 3.5 micron, using isocratic elution with a mobile phase of acetonitrile and 0.1% ortho phosphoric acid (40:60). A flow rate of 1 ml/min and a detector wavelength of 225 nm utilizing the PDA detector were given in the instrumental settings. Recovery, specificity, linearity, accuracy, robustness, ruggedness were determined as a part of method validation and the results were found to be within the acceptable range. Validation of the proposed method was carried out according to an international conference on harmonization (ICH) guidelines.

Results: LOD and LOQ for the two active ingredients were established with respect to test concentration. The calibration charts plotted were linear with a regression coefficient of R^2>0.999.

Conclusion: The proposed method to be fast, simple, feasible and affordable in assay condition. During stability tests, it can be used for routine analysis of production samples and to verify the quality of drug samples during stability studies.

Keywords: Carboplatin, Docetaxel, RP-HPLC, Development, Validation

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INTRODUCTION

In March 1989, the FDA authorised the medication carboplatin. Paraplatin is the trade name for carboplatin, which comes in injection form (150 mg/ml or 10 mg/ml) and is an organo platinum alkylating representative. Carboplatin is a cancer-fighting (chemotherapy) [1, 2] medication [3] that is used to treat ovarian cancer [4, 5]. The treatment of innovative ovarian cancer with a combination of carboplatin and chemotherapeutic agents. Carboplatin resembles cisplatin in terms of vomiting and nephrotoxicity [6, 7]. Carboplatin primarily reacts with alkyl groups on nucleotides [8, 9], which is necessary for DNA fragmentation [10, 11], and simple adducts after restoration enzymes correct the error. Carboplatin and plasma protein [12] have no affinity for each other. Only 40% of free platinum, on the other hand, is irreversibly attached to plasma proteins. Carboplatin is mostly eliminated in the same way as the unmodified mother compound is removed. Patients who are overdue for carboplatin may experience severe hepatotoxicity [13, 14] and neutropenia [15, 16].

Docetaxel, also known as Taxotere, is a chemotherapy medication that comes in injection (10 and 20 mg/ml) form and is used to treat a variety of cancers. Breast cancer [17, 18], head and neck cancer [19, 20], stomach cancer [21], prostate cancer [22, 23], and non-small cell lung cancer [24, 25] are some of the most common cancers. It can be used alone or in combination with other chemotherapy medications. It's given as a gradual injection into a vein. Hair loss, cytopenia (low blood cell count), numbness, shortness of breath [26], vomiting, and muscle soreness [27] are all common adverse effects. Allergies and potential malignancies are two more significant adverse effects. People with liver disorders [28, 29] are more likely to experience side effects. Using it when pregnant can harm the foetus. Docetaxel belongs to the taxane family of drugs. It operates by interfering with the normal function of microtubules [30, 31], preventing cell division. Both drug structures are shown in fig. 1. The aim of the study is to separate the pharma ingredients carboplatin and docetaxel by using RP-HPLC.

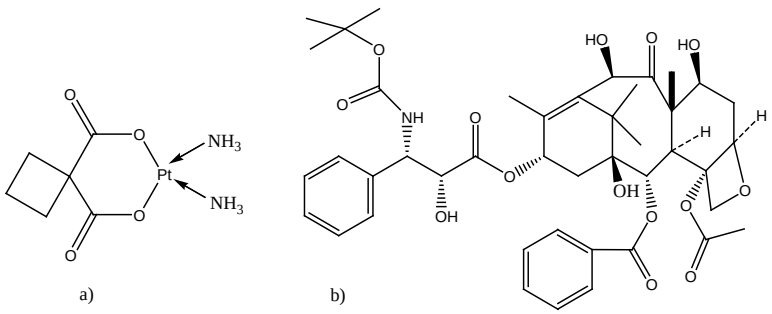


Fig. 1: Structure of (a) Carboplatin and (b) Docetaxel

Till today there are no HPLC method was reported in the literature. Hence we developed method for the simultaneous quantification of carboplatin and docetaxel. The developed HPLC method was utilized for the estimation of the combined drugs by *in vitro* method.

MATERIALS AND METHODS

Chemicals

Acetonitrile, HPLC-grade orthophosphoric acid, water were purchased from Merck India Ltd, Mumbai, India. APIs of Carboplatin and Docetaxel standards were procured from Glenmark, Mumbai.

The Instrumentation

Waters alliance liquid chromatography (model 2695) [32] monitored with empower 2.0 data handling system and a detector of photo diode array (model 2998) [33] was used for this study.

Method optimization

To optimize the chromatographic conditions, different ratios of phosphate buffer and the acetonitrile in the mobile phase with isocratic and gradient mode was tested. However the mobile phase composition was modified at each trial to enhance the resolution and also to achieve acceptable retention times. Finally 0.1% OPA buffer and acetonitrile with isocractic elution was selected because it results in a greater response of active pharmacy ingredients. During the optimization of the method various stationary phases such as C_8, C_{18} phenyl and amino, Symmetry C_{18} columns were tested. From these trials the peak shapes were relatively good with a Symmetry C_{18} column of 150 x 4.6 mm, 3.5 µ with a PDA detector. The mobile phase flow rate has been done at 225 nm in order to obtain enough sensitivity. By using above conditions we get retention times of Carboplatin and Docetaxel were about 2.772 and 7.386 min with a tailing factor of 1.12 and 1.04. The number of theoretical plates for Carboplatin and Docetaxel were 3072, 10999 which indicate the column's successful output. The proposed approach suggests that it is extremely precise. According to ICH guidelines, the method established was validated.

Validation procedure

According to ICH Q2 (R1) guidelines [34, 35], analytical parameters were validated [36-40] such as system appropriateness, precision, specificity, accuracy, linearity, robustness, LOD, LOQ, forced deterioration, and stability.

Preparation of buffer

1 ml of ortho phosphoric acid was added to 1 L of HPLC grade water and filtered through 0.45 µ filter paper.

Chromatographic conditions

The HPLC analysis was performed on reverse phase HPLC system with isocratic elution mode using a mobile phase of acetonitrile and 0.1% OPA and Symmetry C_{18} column (150x4.6 mm, 3.5 µ) column with a flow rate of 1 ml/min.

Diluent

Mobile phase was used as diluent.

Preparation of the standard stock solution

For standard stock solution preparation, 70 ml of diluents was added to 50 mg of Carboplatin and 20 mg of Docetaxel taken in a 100 ml volumetric flask and sonicated for 10 min to dissolve the contents completely and then volume was made upto the mark with diluent.

Preparation of Standard solution

5 ml of solution was drawn from the above stock solution into a 50 ml volumetric flask and diluted up to the mark.

RESULTS AND DISCUSSION

The main analytical challenge during development of a new method was to separate active Pharma ingredients. In order to provide a good performance the chromatographic conditions were optimized.

System suitability

In System suitability [41] injecting standard solution and reported USP tailing and plate count values are tabulated in table 1.

Specificity

In this test method placebo, sample and standard solutions were analyzed individually to examine the interference [42]. The below fig. shows that the active ingredients were well separated from blank and their excipients and there was no interference of placebo with the principal peak. Hence the method is specific.

Linearity

The area of the linearity peak versus different concentrations has been evaluated for Carboplatin, Docetaxel as 10, 25, 50, 100, 125, 150 percent respectively [43, 44]. Linearity was performed in the range of 5-75µg/ml of Carboplatin and 2-30µg/ml of Docetaxel. The correlation coefficients achieved greater than 0.999 for all.

Table 1: Results of system suitability

System suitability parameter	Acceptance criteria	Carboplatin		Docetaxel	
		Mean	Std dev	Mean	Std dev
USP Plate Count	NLT 2000	3067	11.089	10971	26.912
USP Tailing	NMT 2.0	1.09	0.044	1.08	0.221
USP Resolution	NLT 2.0	-	-	19.23	0.089
Retention time	NLT 2.0	2.774	0.003	7.385	0.002

n=6

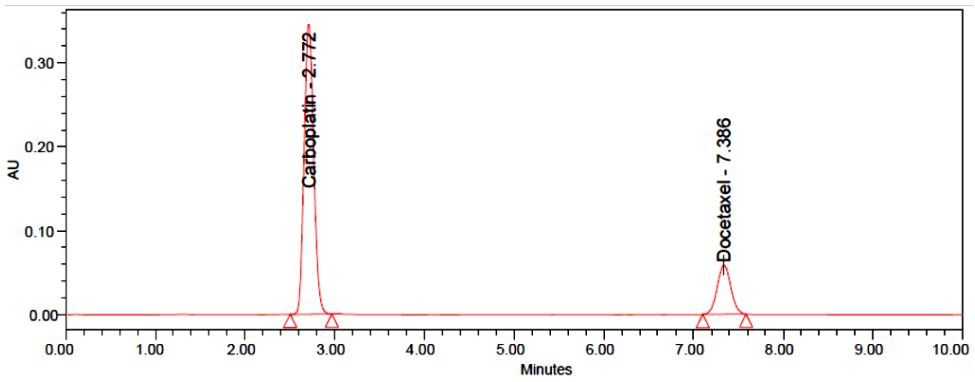


Fig. 2: Chromatogram of system suitability

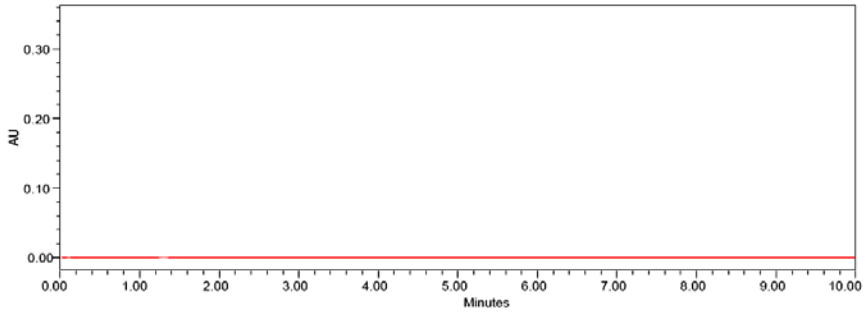
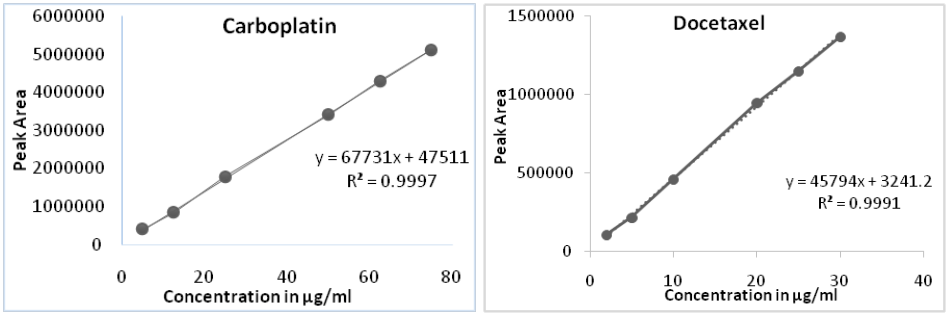


Fig. 3: Chromatogram of blank

Table 2: Linearity of carboplatin and docetaxel

S. No.	Parameter	Obtained values	
		Carboplatin	Docetaxel
1	Residual sum of squares	0.9997	0.9991
2	Slope	67731	45794
3	Y-Intercept	47511	3241.2



A **B**

Fig. 4: Calibration plots of (A) Carboplatin (B) Docetaxel

Accuracy

In this method, Accuracy was conducted in triplicate by analyzing active pharma ingredient sample solution at three kinds of concentration levels of 50, 100 and 150% of each at a specified limit. Percentage recoveries were measured and found to be within the limit. The accuracy and reliability of the developed method were established. The percentage recovery values were found to be in the range of 98.56%-99.75% for Carboplatin and 101.05-101.56% for Docetaxel. The results are given in table 3.

Table 3: Results of accuracy

S. No.	% Level	Carboplatin		Docetaxel	
		Mean % recovery	Std dev	Mean % recovery	Std dev
1	50	99.11	0.596	101.38	0.272
2	100	99.75	0.628	101.56	0.277
3	150	98.56	0.139	101.05	1.155

n=3

Precision

In method precision study prepare six different standards in the concentration of Carboplatin (50 ppm) and Docetaxel (20 ppm) are injected into HPLC system. These results are given below table 4.

Intraday precision

Six replicates of a standard solution containing carboplatin (50µg/ml) and docetaxel (20µg/ml) were analysed on the same day. Peak areas were calculated, which were used to calculate mean, SD and % RSD values.

Inter-day precision

Also called Intermediate precision. In this six replicates of a standard solution containing Carboplatin (50µg/ml) and Docetaxel (20µg/ml) were analysed on a different day. Peak areas were calculated which were used to calculate mean, SD and %RSD values. The present method was found to be precise as the RSD values were less than 2% and also the percentage assay values were close to be 100%.

LOD and LOQ

The LOD concentrations for Carboplatin are 1.515 µg/ml and s/n values is 7 and Docetaxel 0.606 µg/ml and s/n value 3. The LOQ concentration for Carboplatin 5.0 µg/ml and its s/n value is 28 and Docetaxel 2.0 µg/ml and s/n value is 23. The method is validated as per the ICH guidelines [45].

Robustness

The conditions of the experiment were designed to test the robustness of established system intentionally altered, such as flow rate, mobile phase in organic percentage in all these varied conditions [46]. Robustness results for Carboplatin and Docetaxel found to be within the limit and results are tabulated in table 7.

Table 4: Intraday precision results of carboplatin and docetaxel

S. No.	% Assay			
	Carboplatin		Docetaxel	
	M. P.	I. P.	M. P.	I. P.
1	101.30	101.97	100.10	100.34
2	100.01	102.18	100.21	100.14
3	99.99	101.52	100.31	99.82
4	100.26	101.20	99.93	100.23
5	101.01	102.23	100.16	100.31
6	100.69	101.89	99.82	100.45
Mean (n=6)±SD	100.54±0.496	101.83±0.364	100.09±0.165	100.22±0.196
%RSD (n=6)	0.494	0.356	0.164	0.195

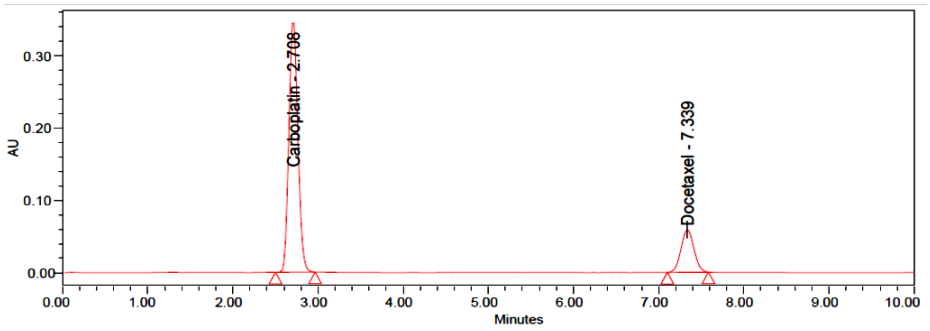


Fig. 5: Chromatogram of method precision

Table 5: LOD and LOQ for carboplatin and docetaxel

Carboplatin				Docetaxel			
LOD		LOQ		LOD		LOQ	
Concentration	s/n	Concentration	s/n	concentration	s/n	Concentration	s/n
1.515 µg/ml	7	5.0 µg/ml	28	0.606 µg/ml	3	2.0 µg/ml	23

Table 6: Robustness data of carboplatin and docetaxel

Parameter name	Carboplatin % assay		Docetaxel % assay	
	Mean	Std dev	Mean	Std dev
Flow minus (0.8 ml/min	99.7	0.252	99.7	0.201
Flow plus (1.2 ml/min)	99.5	0.265	99.9	0.379
Organic minus (36:64)	100.2	0.101	100.1	0.208
Organic plus (44:56)	100.1	0.265	99.9	0.265

n=3

Table 7: Stability results of carboplatin and docetaxel

Stability	Carboplatin		Docetaxel	
	Purity	% deviation	Purity	% deviation
Initial	99.9	0.1	99.9	0.1
6 h	99.6	0.4	99.7	0.3
12 h	99.2	0.8	99.3	0.7
18 h	98.9	1.1	99.0	1.0
24 h	98.5	1.5	98.6	1.4
Mean	99.2	0.8	99.3	0.7
Std dev	0.554	0.554	0.524	0.524

Stability

The standard solution was kept at room temperature and at 2-8 °C up to 24 h. Then these solutions were pumped into the device and calculate the % of deviation from initial to 24 h [47]. There was no significant deviation observed and confirmed that the solutions were stable up to 24 h percentage of the assay was not quite 2%. There is no effect in storage conditions for Carboplatin and Docetaxel drugs. The results are given below table 7.

Degradation studies

The Docetaxel and Carboplatin standard was subjected into various forced degradation conditions to effect partial degradation of the drug. Studies of forced degradation [48] have carried out to find out that the method is suitable for products of degradation [49, 50]. In addition, the studies provide details about the conditions during which the drug is unstable, in order that the measures are often taken during formulation to avoid potential instabilities [51].

Acid degradation

Acid degradation was done at 1N HCl and degradation was formed 16.89% for Carboplatin and 15.94% for Docetaxel.

Alkali degradation

Alkali degradation was done at 1N NaOH and degradation was formed 15.53% for Carboplatin and 15.14% for Docetaxel.

Peroxide degradation

Peroxide degradation was done at 20% hydrogen peroxide and degradation was formed 12.66% Carboplatin and 13.88% for Docetaxel.

Reduction degradation

In reduction degradation, 11.81% Carboplatin and 12.08% Docetaxel degradation was observed.

Thermal degradation

In thermal degradation the standard was degraded to 10.02% of Carboplatin and 7.95% of Docetaxel.

Hydrolysis degradation

In hydrolysis degradation the standard was degraded to 8.88% of Carboplatin and 8.66% of Docetaxel.

All degradation results are tabulated in table 9.

Table 9: Forced degradation results of carboplatin and docetaxel

Degradation condition	Carboplatin % deg		Docetaxel % deg	
	Mean	Std dev	Mean	Std dev
Control degradation	0.18	0.038	0.22	0.026
Acid degradation	16.89	0.231	15.94	0.049
Alkali degradation	15.53	0.035	15.14	0.029
Peroxide degradation	12.66	0.021	13.88	0.033
Reduction degradation	11.81	0.03	12.08	0.018
Thermal degradation	10.02	0.02	7.95	0.011
Hydrolysis degradation	8.88	0.021	8.66	0.027

n=3

CONCLUSION

We present in this article simple, selective, validated and well-defined stability that shows isocratic RP-HPLC methodology for the quantitative determination of Carboplatin and Docetaxel. All the products of degradation formed during the stress conditions and active pharma ingredients are well separated and peaks were well resolved from each other and separate with an appropriate retention time indicating that the proposed method to be fast, simple, feasible and affordable in assay condition. Therefore the developed method during stability tests, it can be used for routine analysis of production samples and to verify the quality of drug samples during stability studies.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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