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Research Article

RP-HPLC METHOD FOR THE QUANTIFICATION OF OXALIPLATIN IN FORMULATIONS

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ABSTRACT

A simple, precise and accurate RP-HPLC method was developed and validated for rapid assay of Oxaliplatin tablet dosage form. Isocratic elution at a flow rate of 1ml/min was employed on a symmetry Chromosil C18 (250x4.6mm, 5µm in particle size) at ambient temperature. The mobile phase consisted of Methanol : Acetonitrile 75: 25 v/v(0.1% OP 0.5ml). The UV detection wavelength was 240nm and 20µl sample was injected. The retention time for Oxaliplatin was 8.33min. The percentage RSD for precision and accuracy of the method was found to be less than 2%. The method was validated as per the ICH guidelines. The method was successfully applied for routine analysis of Oxaliplatin tablet dosage form and bulk drug.

Key Words: Oxaliplatin, RP-HPLC, UV detection, recovery, precise, 240nm

INTRODUCTION

Oxaliplatin is a platinum-based antineoplastic agent that is used in cancer chemotherapy. oxaliplatin is the platinum-containing complex similar to cisplatin. This agent was recently approved for use as a second line therapy in metastatic colorectal cancer. Neurotoxicity is its dose limiting side effect and characterized by peripheral sensory neuropathy.

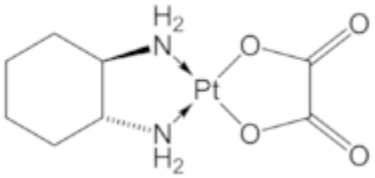


Figure 1: Structure of Oxaliplatin

Oxaliplatinum is a platinum compound with antineoplasmatic properties and is registered in the Netherlands since 2005. Oxaliplatin is used in combination with 5-fluorouracil and folinicacid in the treatment of metastatic colorectal cancer. It is also indicated as adjuvant therapy in the treatment of colon carcinoma (stage III) after a complete resection of the primary tumor [1]. The platinum compound in oxaliplatin forms a complex with 1,2-diaminocyclohexane and an oxalate group [1] which interferes with DNA synthesis [2]. The complex is more hydrophobic than the complex formed by cisplatin and carboplatin, other platinum compounds and is therefore less nephro-toxic than cisplatin and less myelotoxic than carboplatin [3].side effectsof the Oxaliplatin is diarrhea, dizziness, fatigue, gas, hair loss, headache, heart burn, hiccups, mild stomach pain, muscle or joint achaes.

EXPERIMENTAL

Materials:

Working standard of Oxaliplatin was obtained from well reputed research laboratories. HPLC grade water, Methanol was purchased from E. Merck (Mumbai, India).

Apparatus:

A Series HPLC [6-11] system PEAK LC 7000 isocratic HPLC with PEAK 7000 delivery system. Rheodyne manual sample injector with switch (77251), Analytical column Chromosil C18. 250×4.6mm, Electronic balance-DENVER (SI234), manual Rheodyne injector with a 20 µl loop was used for the injection of sample. PEAK LC software was used. UV 2301 Spectrophotometer was used to determine the wavelength of maximum absorbance.

Determination of wavelength of maximum absorbance:

The standard solutions of Oxaliplatin were scanned in the range of 200 -400 nm against mobile phase as a blank. Oxaliplatin showed maximum absorbance at 240nm. So the wavelength selected for the determination of Oxaliplatin was 240nm.

Chromatographic equipment and conditions:

To develop a High Pressure Liquid Chromatographic method for quantitative estimation of OXALIPLATINan isocratic PEAKHPLC instrument with Zodiac C18 column (250 mm x 4.6 mm, 5µ) was used. Theinstrument is equipped with a LC 20AT pump for solvent delivery and variable wavelength programmable LC – 7000 UV-detector. A 20µL Rheodyne inject port was used for injecting the samples. Data was analyzed by using PEAK software.

The mobile phase consisted of Methanol : ACN 75: 25 v/v(0.1% OP 0.5ml). Injections were carried out using a 20 µl loop at room temperature (20 + 2 °C) and the flow rate was 1 ml/min. Detection was performed at 240nm with 10min runtime.

Standard and sample solutions:

A 10 mg amount of Oxaliplatin reference substance was accurately weighed and dissolved in 10 ml mobile phase in a 10 ml volumetric flask to obtain 1000 ppm concentrated solution. Required concentrations were prepared by serial dilution of this solution.

A (ELOXATIN-50mg) injection powder was prepared by grinding them to a fine, uniform size powder. 10 mg of Oxaliplatin was accurately weighed and quantitatively transferred into a 100 ml volumetric flask. Approximately 25 ml mobile phase were added and the solution was sonicated for 15 min. The flask was filled to volume with mobile phase, and mixed. After filtration, an amount of the solution was diluted with mobile phase to a concentration of 120ppm.

Method validation:

Method validation was performed following ICH specifications for specificity, range of linearity, accuracy, precision and robustness.

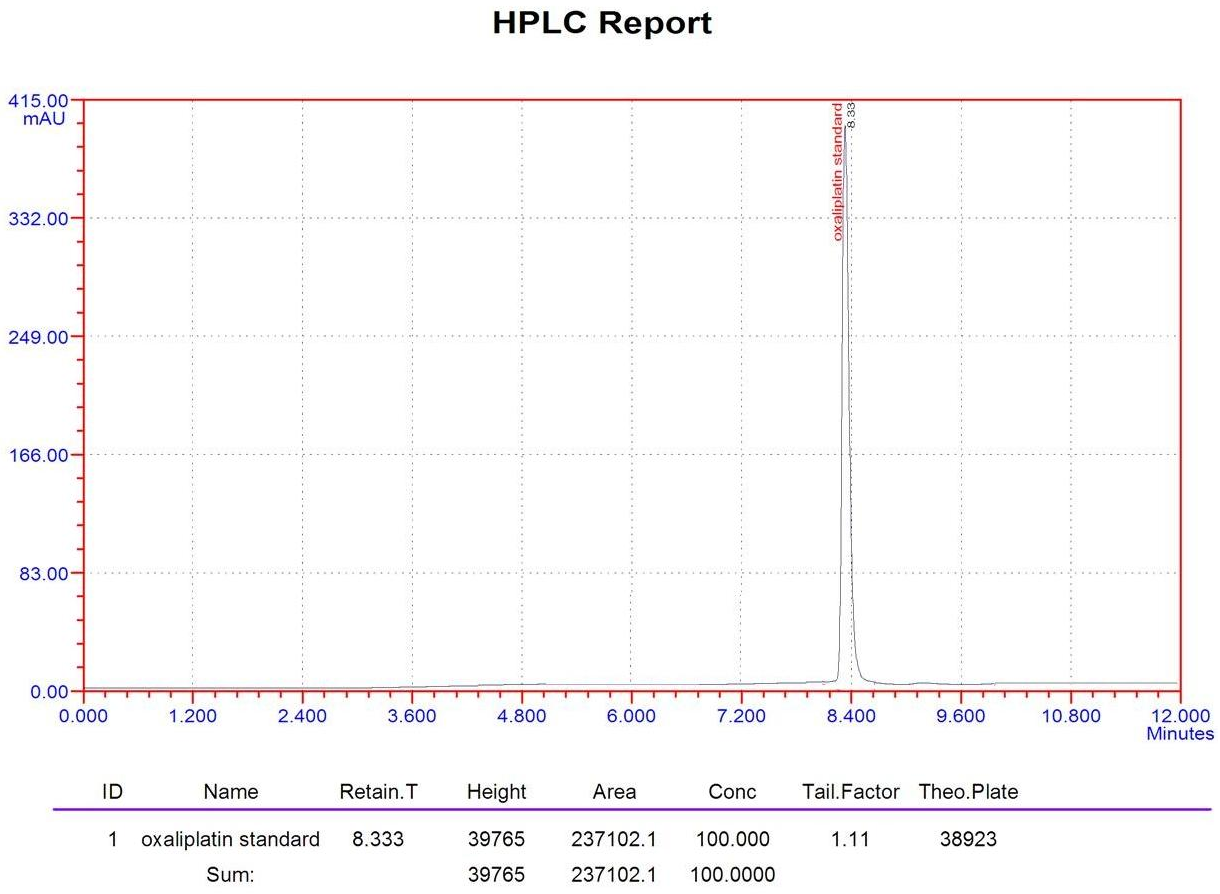
RESULTS AND DISCUSSION

System Suitability:

Having optimized the efficiency of a chromatographic separation, the quality of the chromatograph was monitored by applying the following system suitability tests: capacity factor, tailing factor and theoretical plates. The system suitability method acceptance criteria set in each validation run were: capacity factor >2.0, tailing factor ≤2.0 and theoretical plates >2500. In allcases, the relative standard deviation (R.S.D) for the analytic peak area for two consecutive injections was < 2.0%. A chromatogram obtained from reference substance solution is presented. System suitability parameters were shown in Table.1. Standard chromatogram was given in Figure.2

Api Concentration	120 ppm
Mobile Phase	Methanol: ACN 75: 25 v/v(0.1% OP 0.5ml).
Wavelength	24nm
Column	C_{18} Column
P^H	4.8
Concentration	120ppm
Retention Time	8.33
Run Time	12min
Area	237102
Th. Plates	38923
Tailing Factor	1.12
Pump Pressure	12.5MPa

Table 1:System suitability parameters of OXALIPLATIN



ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate
1	oxaliplatin standard	8.333	39765	237102.1	100.000	1.11	38923
	Sum:		39765	237102.1	100.0000		

Figure 2: Standard chromatogram of Oxaliplatin

Range of linearity:

Standard curves were constructed daily, for three consecutive days, using seven standard concentrations in a range of 30, 60, 90, 120, and 150ppm for Oxaliplatin. The linearity of peak area responses versus concentrations was demonstrated by linear least square regression analysis. The linear regression equation was y = -2163.64+ 1977.907x (r. 0.999747 Linearity values can show in Table: 2.

S.No	Concentration (µg/ml)	Area
1	30	62740
2	60	115511
3	90	172663
4	120	237102
5	150	291256
6	180	357227
	Slope	1977.901
	Intercept	-2163.64
	CC	0.999747

Table 2: Linearity results of Oxaliplatin

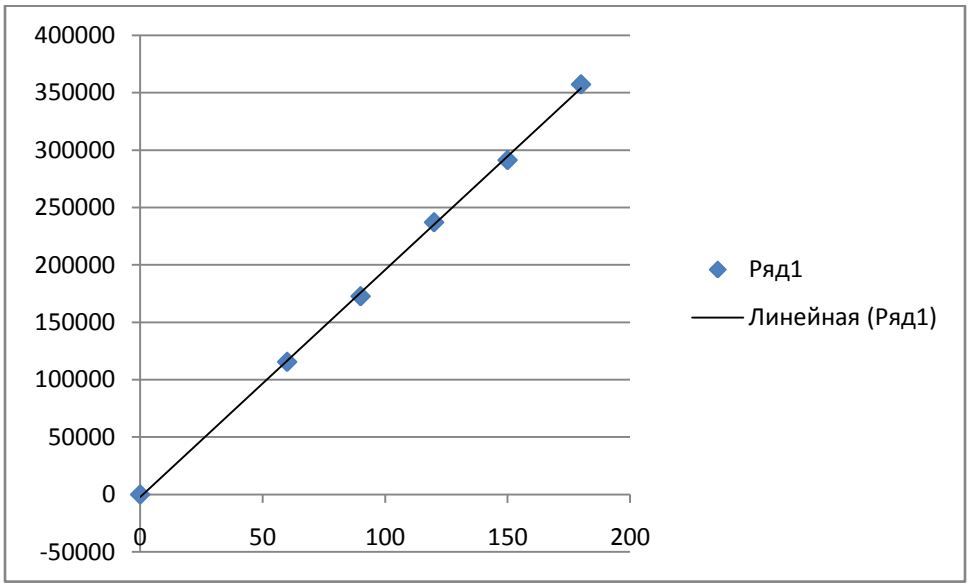


Figure 3: Calibration curve of Oxaliplatin

Precision:

To study precision, six replicate standard solutions of Oxaliplatin (120ppm) were prepared and analyzed using the proposed method. The percent relative standard deviation (% RSD) for peak responses was calculated and it was found to be which is well within the acceptance criteria of not more than 2.0%. Results of system precision studies are shown in Table.3 and Table.4.

Sample (µg/ml)	Area	Sample (µg/ml)
1	228437	1
2	231457	2
3	227306	3
4	231232	4
5	230253	5
6	228369	6

Table 3: Intraday Precision Results for Oxaliplatin

Sample (µg/ml)	Area
1	227694
2	229424
3	229742
4	227473
5	227276
6	227694
RSD	0.94

Table 4: Inter day Precision results of Oxaliplatin

Limit of Detection and Limit of Quantification:

To determine the Limit of Detection (LOD) sample was dissolved by using Mobile phase and injected until peak was disappeared. After 0.2ppm dilution Peak was not clearly observed, based on which 0.2ppm is considered as Limit of Detection and Limit of Quantification is 0.065ppm.

Parameter	Measured Value
Limit of Quantification	0.03ppm
Limit of Detection	0.01ppm

Table5: LOD and LOQ results of Oxaliplatin:

Robustness:

Typical variations in liquid chromatography conditions were used to evaluate the robustness of the assay method. The robustness study was performed by slight modification in flow rate of the mobile phase, composition of the mobile phase and wavelength of the detector. Oxaliplatin at standard concentration was analyzed under these changed experimental conditions. It was observed that there were no marked changes in chromatograms, which demonstrated that the developed method was robust in nature. The robustness acceptance criteria set in the validation were the same established on system suitability test describe above. Results were shown in table 6.

S.NO	Parameter	change	assay	area	%of change
1	standard				
2	Mp		100.31	236672	0.1
3			99.81	239834	0.19
4	pH		101.15	237059	1.15
5			100.23	239138	0.23
6	WL		100.85	235638	0.85
7			99.12	236672	0.88

Table6: Robustness results of Oxaliplatin

Ruggedness:

Ruggedness was performed by using six replicate injections of standard and sample solutions of concentrations which were prepared and analyzed by different analyst on three different. Ruggedness also expressed in terms of percentage relative standard deviation.

SAMPLE	CONC (PPM)	INJECTION NO	PEAKS AREA	R.S.D (Acceptance criteria ≤ 2.0%)
Oxaliplatin	120	1	226592	**1.77**
		2	237909	
		3	232797	
		4	233856	
		5	237439	
		6	235690	

Table 7: Ruggedness results of Oxaliplatin

Recovery:

The accuracy of the method was determined by standard addition method. A known amount of standard drug was added to the fixed amount of pre-analyzed tablet solution. Percent recovery was calculated by comparing the area before and after the addition of the standard drug. Recovery test was performed at 3 different concentrations i.e. 90ppm, 120ppm, 150ppm. The percent recovery was calculated and results are presented in Table. Satisfactory recoveries ranging from 98.07 to 101.14 were obtained by the proposed method. This indicates that the proposed method was accurate. Results are given in table.8

% Recovery	Oxaliplatin				
	Target Conc., (ppm)	Spiked conc, (ppm)	Final Conc, (ppm)	Conc., Obtained	% of Recovery
50%	60	30	90	88.73	98.58
50%	60	30	90	89.94	99.93
50%	60	30	90	88.51	98.34
100%	60	60	120	119.52	99.6
100%	60	60	120	120.43	100.35
100%	60	60	120	117.78	98.15
150%	60	90	150	148.36	98.9
150%	60	90	150	149.42	99.61
150%	60	90	150	148.51	99

Table 8: Recovery results of Oxaliplatin

Formulation	Dosage	Concentration	Amount found	% Assay
ELOXATIN injection powder	50mg	120ppm	118.20	98.50

Table 9: Formulation Analysis

CONCLUSION

The proposed method for the assay of Oxaliplatin in tablets or capsules is very simple and rapid. It should be emphasized it is isocratic and the mobile phase do not contain any buffer. The method was validated for specificity, linearity, precision, accuracy and robustness. Although the method could effectively separate the drug from its products, further studies should be performed in order to use it to evaluate the stability of pharmaceutical formulations.

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