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Spectrophotometric determination of cisplatin, carboplatin and oxaliplatin in pure and injectable dosage forms.

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Abstract

Objective: A simple and reproducible spectrophotometric method was developed for the estimation of cisplatin, carboplatin and oxaliplatin in pure and injectable dosage forms.
Method: The method is based on the reaction of platinum drug with 1,10-phenanthrolene in the presence of ferric chloride, which formed a red coloured complex that exhibited maximum absorption at 510 nm. The reaction is selective for platinum based drugs and obeys the Beer's law for cisplatin from 0.25-6.0 µg, carboplatin 7.5-180 µg and oxaliplatin 0.5-12.0 µg. The accuracy of the developed method for cisplatin, carboplatin and oxaliplatin was 98.6%, 100.3% and 101.1%, whereas RSD (%) is of 1.1, 1.3 and 1.8, respectively.
Result: The standardization and validation of analytic method was evaluated by the parameters like linearity, accuracy, precision and sensitivity.
Conclusion: The method is useful for the determination of these drugs in pure and dosage forms.

Keywords: Cisplatin, Carboplatin, Oxaliplatin, 1-10, Phenanthrolene, Spectrophotometry

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Introduction

The platinum based drugs are famous family of antiproliferative agents and were identified by Rosenberg and co-workers in 1965. They act like alkylating agents by cross linking DNA. The parent drug cisplatin is widely used platinum based chemotherapeutic agent, whereas the carboplatin and oxaliplatin have improved toxicity profile and provide better results in germ cell, ovarian and colorectal cancers [1].

Cisplatin (Figure 1) belongs to an alkylating class of anticancer drugs and is the first member of platinum containing drugs used as antineoplastic in early 1970's. It is a divalent inorganic, water soluble platinum containing complex and is widely used due to its potent effects in the treatment of ovarian, testicular, head and neck tumor, cervical cancer and small cell lung carcinoma [2,3]. Carboplatin (Figure 1) was approved for clinical use in 1989. The mechanism of action and resistance of carboplatin are similar to cisplatin, but differs significantly in chemical, pharmacokinetic and toxicological properties [4]. Carboplatin is less reactive and well tolerated and is used as intravenous infusion to treat patients who are unable to tolerate cisplatin as front-line treatment for ovarian cancer. It is currently approved by FDA for use in the treatment of advanced ovarian and lung cancer in combination with paclitaxel or cyclophosphamide [5]. Oxaliplatin (Figure 1) is the most recent drug approved by FDA in 2002. It exhibits a wide range of antitumor activities that differ from other platinum drugs. It also suppresses the enzyme thymidylate synthetase which is also target enzyme of 5- fluorouracil, which is the reason of synergy of two drugs. It is used to treat colorectal cancers in combination with 5-fluorouracil and leucovorin [6].

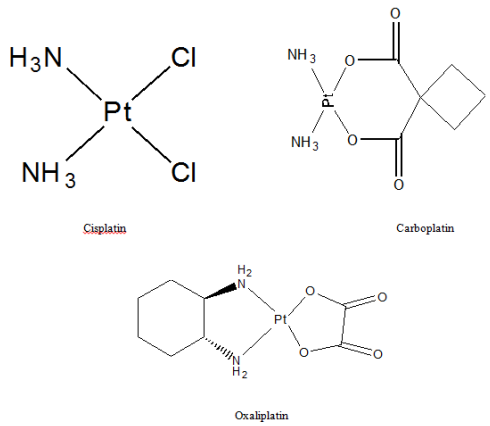

Figure 1. *Structures of platinum based drugs.*

Several methods for the determination of platinum based drugs include HPLC with UV detection [7,8], LC-MS [9] and ICPMS [10,11]. These platinum based drugs have also been determined by spectrophotometric method [12-14]. The previously published methods included derivitization of cisplatin or demanded costly equipment [9-11]. This work demonstrates direct and simultaneous spectroscopic method for

the determination of platinum based drugs by spectrophotometric method.

Materials and Methods

Apparatus

A UV-visible double beam spectrophotometer (Shimadzu 1601, Japan) and 96-well plate reader, Synergy HT, USA were used to measure the absorbance.

Chemicals and drugs

Cisplatin, carboplatin and oxaliplatin were kindly gifted by Pharmedic Laboratories Pvt. Ltd. Lahore, (Pakistan). Ferric chloride, 1,10-phenanthrolene, phosphoric acid and de-ionized double distilled water were of analytic grade. Commercial injectable dosage forms of cisplatin, carboplatin and oxaliplatin were purchased from local market.

Preparation of 1,10-phenanthroline solution: 1,10-Phenanthroline monohydrate (0.4 gram) was dissolved in 100 ml double distilled water to form 0.4% solution.

Preparation and standardization of phosphoric acid solution: A fixed volume (25 µl) of phosphoric acid of different molarity was mixed with cisplatin (150 µg/ml), carboplatin (450 µg/ml) and oxaliplatin (150 µg/ml) followed by the addition of 40 µl of ferric chloride (0.5%) and 100 µl of 0.2% or saturated solution (0.4%) of 1, 10-phenanthrolene solution and absorbance was measured at 510 nm.

Ferric chloride solution: Ferric chloride (0.5 gram) was dissolved in 100 ml double distilled water to constitute a 0.5% solution.

Standard stock solution preparation: Stock solutions of cisplatin and oxaliplatin (1000 µg/ml) were prepared by dissolving accurately weighed 10 mg in double distilled water and making the volume to 10 ml in standard volumetric flask. Carboplatin was prepared by dissolving accurately weighed 30 mg in 10 ml of double distilled water to get a concentration of 3.0 mg/ml. Working solutions of lower concentration was prepared by further dilution of standard stock solutions with de-ionized double distilled water.

Sample preparation: A commercial brand of cisplatin injection (Unistin-Korea), carboplatin injection (Cytocarb-Cipla) and oxaliplatin injection (Oxilitin- Pharm Evo) were chosen.

Results

Absorption spectrum of coloured complex

The absorption spectra of cisplatin, carboplatin and oxaliplatin are shown in Figures 2-5.

Chemistry of coloured complex

The present method was based on the formation of red coloured complex with ferric chloride after its reduction to ferrous form. This complex resulting from platinum based drugs may be due to the fact that each of the two nitrogen atoms has an unshared pair of electrons that can be shared with Fe (II) ion formed by the reaction of platinum with Fe (III). Three molecules of 1,10-phenanthroline attach themselves by means of dative bonds to form ferroin complex.

Method Development

A spectroscopic method was developed in pure and applied on injectable brands of platinum based drugs as follows.

Procedure

Aliquots of working standard solutions (cisplatin, carboplatin and oxaliplatin) of 5, 10, 20, 40 and 60 µl were added in triplicate in 96 well-plates with 40 µl of 0.5% ferric chloride and 100 µl of 0.2% or saturated solution (0.4%) of 1,10-phenanthrolene. Contents were incubated at 60ºC for 45 minutes, cooled at room temperature followed by addition of 25 µl of 15 mM phosphoric acid. Finally, the solution was mixed thoroughly and absorbance was measured at 510 nm against the blank prepared under same identical conditions and calibration curves were constructed (Figure 2). The system was attached with computer software (Gen 5) to analyze and calculate data (Tables 1 and 2).

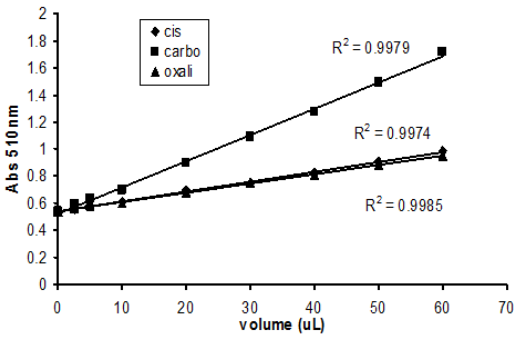

Figure 2. *Calibration curve of cisplatin, carboplatin and oxaliplatin.*

Table 1. *Spectrophotometric determination of platinum based drugs.*

Cisplatin (µg)	Abs. of cisplatin at 510 nm	Carboplatin (µg)	Abs. of carboplatin at 510 nm	Oxaliplatin (µg)	Abs. of oxaliplatin at 510 nm
0	0.543	0	0.543	0	0.543
0.25	0.563	7.5	0.591	0.5	0.564
0.5	0.589	15	0.638	1	0.583
1	0.609	30	0.703	2	0.599
2	0.701	60	0.898	4	0.679
3	0.749	90	1.091	6	0.751

4	0.828	120	1.276	8	0.808
5	0.905	150	1.493	10	0.878
6	0.987	180	1.717	12	0.952

Table 2. *Absorbance (510 nm) of cisplatin, carboplatin and oxaliplatin vs concentration (µg /ml).*

Sample (µl)	Water (µl)	0.5 % $FeCl_3$ (µl)	0.4 % 1,10-Phenanthrolene (µl)		15 mM H_3PO_4 (µl)
0	60	40	100		25
2.5	57.2	40	100		25
5	55	40	100		25
10	50	40	100		25
20	40	40	100		25
30	30	40	100		25
40	20	40	100		25
50	10	40	100		25
60	0	40	100	Heated at 60ºC for 45 minutes	25

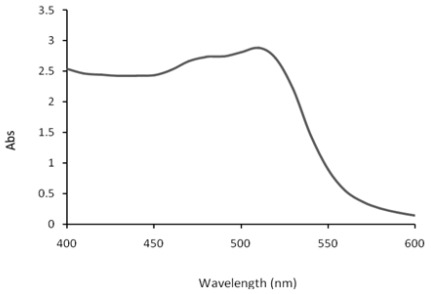

Figure 3. *Spectrum of cisplatin (λ_{max} 510 nm)*

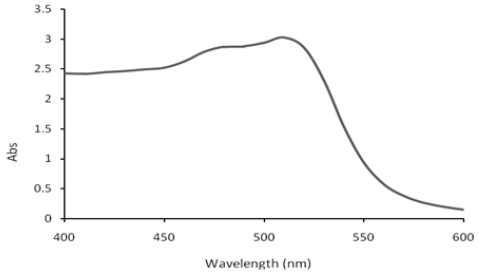

Figure 4. *Spectrum of carboplatin (λ_{max} 510 nm).*

Method validation

The developed spectrophotometric method was standardized validated with following parameters.

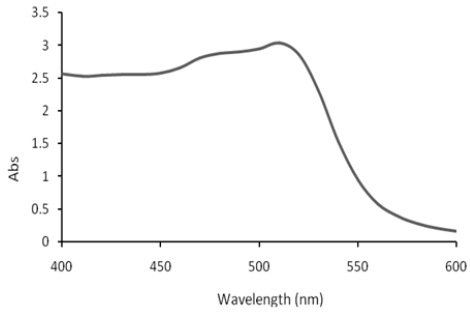

Figure 5. *Spectrum of oxaliplatin (λ_{max} 510 nm).*

Linearity and range: Linearity of the method was determined by constructing calibration curves for cisplatin, carboplatin and oxaliplatin (Figure 2). The standard stock solution of cisplatin, carboplatin and oxaliplatin (1.0 µg/ml) were prepared in distilled water (Table 3).

Table 3. *Standard curve parameters for platinum based drugs (mean values, n=3).*

Drug	Slope	Intercept	r^2
Cisplatin	0.072	0.544	0.997
Carboplatin	0.006	0.52	0.996
Oxaliplatin	0.33	0.543	0.998

Sensitivity: The LOQ values of cisplatin, carboplatin and oxaliplatin were 0.25, 7.5 and 0.5 µg/ml and LOD values of cisplatin, carboplatin and oxaliplatin were 0.12, 3.75 and 0.25 µg/ml, respectively (Table 4).

Table 4. *Parameters and sensitivity (mean values, n=3).*

Parameters	Cisplatin	Carboplatin	Oxaliplatin
Colour	Red complex	Red complex	Red complex
λ_{max}	510 nm	510 nm	510 nm
Range	0.25-6.0 µg	7.5-180 µg	0.5-12 µg
LOD	0.12 µg/ml	3.75 µg/ml	0.25 µg/ml
LOQ	0.25 µg/ml	7.5 µg/ml	0.5 µg/ml
r-square	0.9974	0.9979	0.9985
slope	0.0724	0.0065	0.0337
intercept	0.5443	0.5209	0.5433
Accuracy	98.6	100.3	101.1
RSD (%)	1.1	1.3	1.8

Accuracy: Accuracy was determined by selecting low, medium and high concentrations of cisplatin, carboplatin and oxaliplatin in triplicate. The mean values (low, medium and high) of accuracy for pure drugs were given in Tables 5 and 6

for injectable dosage forms of cisplatin, carboplatin & oxaliplatin were given in Table 7.

Table 5. Determination of between-batches accuracy.

Drug	Parameters	LC	MC	HC
	Mean	0.2427	2.925	5.84
	S.D.	0.007	0.079	0.121
	N	9	9	9
	Nominal	0.25	3	6
	% CV	2.986	2.69	2.069
Cisplatin	% Accuracy	97.07	97.51	97.33
	Mean	7.2812	97.267	176.061
	S.D.	0.1413	0.7886	1.085
	N	9	9	9
	Nominal	7.5	100	180
	% CV	1.9407	0.8108	0.6163
Carboplatin	% Accuracy	97.08	97.27	97.81
	Mean	0.4892	5.891	11.809
	S.D.	0.0125	0.1331	0.322
	N	9	9	9
	Nominal	0.5	6	12
	% CV	2.5546	2.2598	2.7264
Oxaliplatin	% Accuracy	97.84	98.18	98.41

Table 6. Determination of with-in batch precision.

Drug	Parameters	LC	MC	HC
	Mean	0.2427	2.915	5.861
	S.D.	0.004	0.085	0.166
	N	6	6	6
	Nominal	0.25	3	6
	% CV	1.535	2.922	2.831
Cisplatin	% Accuracy	97.07	97.17	97.68
	Mean	7.2788	97.093	175.336
	S.D.	0.148	0.553	0.396
	N	6	6	6
	Nominal	7.5	100	180
	% CV	2.027	0.569	0.226
Carboplatin	% Accuracy	97.05	97.09	97.41
	Mean	0.497	5.8512	11.823
	S.D.	0.01	0.129	0.159
	N	6	6	6
	Nominal	0.5	6	12
	% CV	2.101	2.211	1.342
Oxaliplatin	% Accuracy	99.37	97.52	98.52

Precision: Precision of the method was performed at three different concentrations (low, medium and high) in the range in triplicates. The CV with-in-batch and between batches for low, medium and high concentrations of cisplatin, carboplatin and oxaliplatin were given in Tables 5 and 6. The values were less than 3% and within the range of FDA guidelines.

Discussion

The present study was designed to develop a spectrophotometric method for determination of platinum based drugs. Already there was a single method only for determination of oxaliplatin 14 but no single method for simultaneous determination of cisplatin, carboplatin and oxaliplatin. Hence, a direct, simple and reproducible spectrophotometric method was developed for the estimation of cisplatin, carboplatin and oxaliplatin in pure and dosage forms.

The method is based on the reaction of platinum with saturated solution (0.4%) of 1,10-phenanthrolene in the presence of ferric chloride, which forms a red coloured complex on heating at 60ºC for 45 minutes and added 15 mM phosphoric acid.

The formed complex exhibited maximum absorption at 510 nm. This method was successfully applied on commercial brand of cisplatin injection (Unistin-Korea), carboplatin injection (Cytocarb-Cipla) and oxaliplatin injection (Oxilitin-Pharm Evo). The method had wide linear range with good accuracy and precision (Tables 2-4).

The applicability of the developed method for assay of injectable pharmaceutical dosage form was examined and results were summarized in Table 7. The results are highly reproducible.

Table 7. Determination of concentration of the injectable brands of cisplatin, carboplatin and oxaliplatin by forecast formula (y = a + b x).

Serial No.	Sample	File name	Absorbance at 510 nm	Quantity quoted in dosage forms (µg/ml)	Quantity determined (µg/ ml)	Accuracy (%)
	Cisplatin					
1	**Brand (Unistin)**	Unistin 1	0.901	5	4.94	98.8

2		Unistin 2	0.898	5	4.9	98
3		Unistin 3	0.904	5	4.99	99.8
4		Unistin 4	0.894	5	4.85	97
5		Unistin 5	0.902	5	4.96	99.2
Mean					4.928	98.6
SD					0.054	-
RSD					1.1	-
N					5	5
1		Cytocarb 1	1.187	100	101.02	101
2		Cytocarb 2	1.171	100	98.66	98.6
3		Cytocarb 3	1.194	100	102.05	102
4		Cytocarb 4	1.183	100	100.43	100.4
5		Cytocarb 5	1.176	100	99.39	99.4
Mean					100.31	100.3
SD					1.334	-
RSD	**Carboplatin**				1.3	-
N	**brand (Cytocarb)**				5	5
1		Oxalitin 1	0.881	10	9.94	99.4
2		Oxalitin 2	0.879	10	9.89	98.9
3		Oxalitin 3	0.889	10	10.17	101.7
4		Oxalitin 4	0.894	10	10.32	103.2
5		Oxalitin 5	0.891	10	10.23	102.3
Mean					10.11	101.1
SD					0.187	-
RSD					1.8	-
N	**Oxaliplatin brand (Oxalitin)**				5	5

Conclusion

The present spectrophotometric method is found to be economical, direct, more sensitive and unique method for the simultaneous analysis of platinum based drugs (cisplatin, carboplatin, oxaliplatin) in pure and injectable dosage forms. The statistical parameters show that method has importance in quality analysis of injectable dosage forms of platinum based drugs.

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