Development and Validation of RP-HPLC Method for the Simultaneous Determination of Etoposide and Cisplatin and its Application in Quality Control of Injectable Dosage Forms

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Summary: Etoposide and cisplatin are antineoplastic drugs used in injectable dosage forms in many kinds of cancer separately or in combination therapy. The objective of the work was to develop a simple and rapid RP-HPLC method for direct simultaneous determination of etoposide and cisplatin in injectable dosage forms, aiming its application in quality control analysis. The HPLC method was developed using C_{18} ODS Hypersil column of 250 × 4.6 mm id with 5 µm particle size, mobile phase of water : methanol : acetonitrile (40:35:25, v/v/v, pH 3.5) at a flow rate of 0.75 mL min^{-1} and eluate detected at 283 nm. In the proposed methods, during simultaneous analysis cisplatin showed retention time of 3.12 min and etoposide 5.21 min within a continuous run of 10 min. The CV with-in-batch for low, medium and high concentrations of cisplatin were 0.94%, 0.16% and 0.14%, where as for etoposide these were 2.10%, 0.94% and 0.14% respectively. The CV between batches of cisplatin was 0.48%, 0.19% and 0.13% where as for etoposide 1.25%, 0.48% and 0.13%, respectively. The accuracy of the developed method was 98-99% with RSD of 0.2-0.3% which was within the limits of FDA guidelines.

Keywords: Cisplatin, Etoposide, HPLC, Injectable dosage forms.

Introduction

Etoposide [(4'-demethyl-epipodophylotoxin 9-[4,6-O-(*R*)-ethylidene-β-D-glucopyranoside] is a semi-synthetic derivative of epipodophyllotoxins (Fig. 1) obtained from plant *Mandragora officianarum*. It is used in several human cancers including leukemia, lymphomas and lung cancer [1]. It inhibits DNA repair and replication by affecting uncoiling of DNA which is catalyzed by DNA topoisomerase II [2]. Cisplatin (cis-dichlorodiamine-platinum) belongs to major class platinum complexes of anticancer drugs (Fig. 2) and is used in the treatment of ovarian, testicular, head and neck tumor and small cell lung carcinoma [3-5]. Etoposide is often given in combination with other antineoplastic drugs of different mode of action like cisplatin. *In vitro* studies have suggested possible synergy between etoposide and platinum complexes like cisplatin [6, 7].

Liquid chromatography is a sensitive and accurate method used for the quantitative analysis of drugs. Several methods for the determination of etoposide include HPLC with UV detection [8,9], fluorescence detection [10] and electrochemical detection [11,12]. It has also been determined by LC-MS method [13,14] and ELISA method [15]. LC methods for quantitative determination of cisplatin include bioassay determination [16], LC-MS method [17], HPLC with UV detection [18, 19] and LC-ICP-MS [20]. The previously published methods for individual analysis of cisplatin and etoposide used mixture of expensive organic solvents or demanded costly equipment like LC-MS and for simultaneous analysis, one paper has been published on the derivitized cisplatin [21]. The objective of study was to develop and validate a direct and effective HPLC method for the simultaneous determination of etoposide and cisplatin which could be used in quality control analysis. The present work therefore is a direct method with good linearity and sensitivity for both drugs.

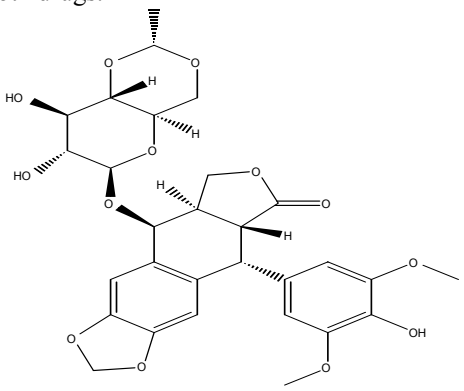

Fig. 1: Structure of etoposide (4'-demethyl-epipodophylotoxin 9-[4, 6-O-(*R*)-ethylidene-β-D-glucopyranoside]). Merck Index.

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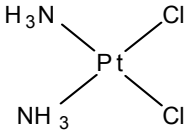

Fig. 2: Structure of cisplatin (cis-dichlorodiamine-platinum). Merck Index

Results and Discussion

Peak Identification

The peaks were identified by comparison of retention times of sample and standard solution with an increase or decrease in the size of the peak with change of concentration of standard solution. During simultaneous analysis, cisplatin showed retention tine of 3.12 min and etoposide 5.21 min (Fig. 3).

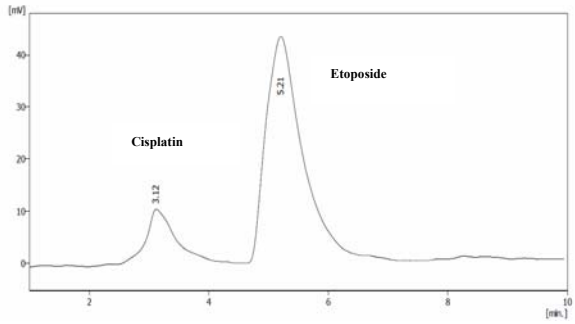

Fig. 3: A representative chromatograph of cisplatin and etoposide.

Method Validation

Following parameters were evaluated step by step to ensure the validity of the methods according to good analytical practice guidelines.

Linearity and Range

Linearity was determined by constructing calibration curve to find out the relationship between instrumental response and known concentrations of pure drug samples. The standard stock solution of cisplatin was diluted with 0.9% NaCl to prepare a set of solutions of different concentrations ranging from 3.9 to 1000 µg mL^{-1} (Fig. 4). The calibration curve of cisplatin was prepared in the 0.9% NaCl solution. Each drug concentration was run in triplicates and data was plotted to calculate the parameters of standard curve (Fig. 4). The values of slope, intercept and r^2 for cisplatin were 0.6029, 5.9477 and 0.999, respectively (Fig. 5). Likewise, the standard stock solution of etoposide was diluted with mobile phase to prepare a set of solutions of different concentrations ranging from 0.25 to 62.5 µg mL^{-1} (Fig. 5). The calibration curve of etoposide was prepared in the selected mobile phase. Each drug concentration was run in triplicates and data was plotted to calculate the parameters of standard curve (Fig. 5). The values of slope, intercept and r^2 for etoposide were 63.565, 4.608 and 0.9982, respectively. The values of these parameters were found to be consistent with FDA guidelines of method validation.

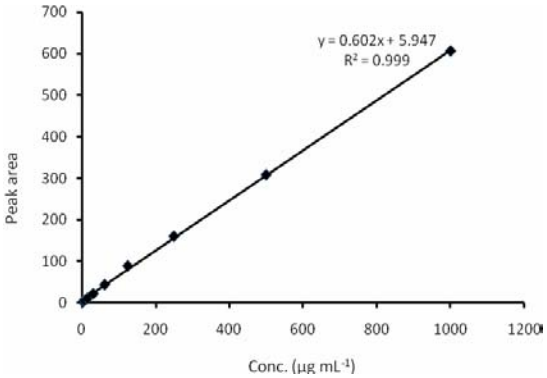

Fig. 4: Calibration curve of cisplatin.

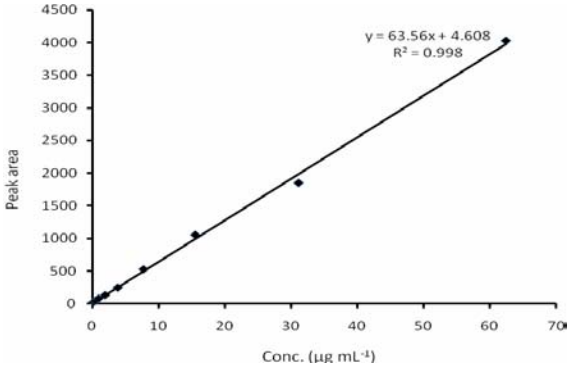

Fig. 5: Calibration curve of etoposide.

Accuracy

Accuracy was determined by selecting low, medium and high concentrations of etoposide in triplicate. The accuracy [low (LC), medium (MC) and high conc. (HC)] for cisplatin were 99.74 %, 99.94 % and 99.78 % and for etoposide these values were 99.16%, 99.74% and 99.78%, respectively (Table-1).

Precision

The precision was determined by elution of analytes at three different concentrations (LC, MC and HC) in range in triplicates. This assay was repeated for three different days. The CV with-in-batch for low, medium and high concentrations of cisplatin were 0.94 %, 0.16 % and 0.14 %, where as for etoposide 2.10%, 0.94% and 0.14% respectively. The CV between batches of cisplatin were 0.48%, 0.19% and 0.13% , where as for etoposide 1.25%, 0.48% and 0.13%, respectively, which were <3% and within the range of FDA guidelines (Table-1, 2).

Table-1: Data of between-batches precision and accuracy for cisplatin and etoposide solutions of three Batches.

Cisplatin				Etoposide			
Batch code	*LC µmL^{-1}	**MC µg mL^{-1}	***HC µmL^{-1}	Batch code	*LC µg m^{1}	**MC µg mL^{-1}	***HC µg m^{1}
Cis-01	3.91	15.61	62.42	Eto-01	0.251	3.91	62.42
	3.89	15.58	62.39		0.249	3.89	62.39
	3.90	15.60	62.23		0.248	3.90	62.23
Cis-02	3.88	15.59	62.45	Eto-02	0.251	3.88	62.45
	3.91	15.62	62.37		0.246	3.91	62.37
	3.87	15.58	62.46		0.241	3.87	62.46
Cis-03	3.88	15.57	62.31	Eto-03	0.247	3.88	62.31
	3.86	15.55	62.41		0.248	3.86	62.41
	3.91	15.61	62.25		0.250	3.91	62.25
Mean	3.890	15.59	62.366	Mean	0.248	3.890	62.366
SD	0.0187	0.03	0.0840	SD	0.0031	0.0187	0.0840
N	9	9	9	N	9	9	9
nominal	3.90	15.60	62.50	nominal	0.25	3.90	62.50
% CV	0.4809	0.1924	0.1347	% CV	1.2506	0.4809	0.1347
%Accuracy	99.74	99.94	99.78	%Accuracy	99.16	99.74	99.78

*Low concentration, ** Medium concentration, ***High concentration.

Table-2: Data of within-batch precision and accuracy for etoposide solution of batch (E01).

Cisplatin				Etoposide			
Batch (01)	*LC µg mL^{-1}	**MC µg mL^{-1}	***HC µg mL^{-1}	Batch (01)	*LC µg mL^{-1}	**MC µg mL^{-1}	***HC µg mL^{-1}
01	3.86	15.55	62.25	01	0.244	3.86	62.25
02	3.81	15.56	62.38	02	0.246	3.81	62.38
03	3.88	15.57	62.21	03	0.241	3.88	62.21
04	3.81	15.61	62.41	04	0.242	3.81	62.41
05	3.89	15.55	62.33	05	0.249	3.89	62.33
06	3.82	15.54	62.42	06	0.255	3.82	62.42
Mean	3.8450	15.56	62.3333	Mean	0.2462	3.8450	62.3333
SD	0.0362	0.0256	0.0869	SD	0.0052	0.0362	0.0869
N	6	6	6	N	6	6	6
Nominal	3.90	15.60	62.50	Nominal	0.25	3.90	62.50
%CV	0.9413	0.1645	0.1394	%CV	2.1095	0.9413	0.1394
%Accuracy	98.59	99.76	99.73	%Accuracy	98.47	98.59	99.73

*Low concentration, ** Medium concentration, ***High concentration.

Sensitivity

Limit of detection (LOD) and limit of quantification (LOQ) values were determined with suitable accuracy and precision between batches and within batches of both drugs. The LOD and LOQ of cisplatin and etoposide were 2.0 µg mL^{-1} and 0.03 µg mL^{-1} respectively (Table-3).

Table-3: Different parameters regarding the simultaneous HPLC analyses of cisplatin and etoposide.

Parameter	Etoposide	Cisplatin
Retention time (simultaneously)	5.21 ± 0.2 min	3.12 ± 0.1 min
	Sensitivity	
LOD	0.03 µg mL^{-1}	2.0 µg mL^{-1}
LOQ	0.25 µg mL^{-1}	3.9 µg mL^{-1}
Linearity	0.25-62.5 µg mL^{-1}	3.9-1000 µg mL^{-1}
Linear Equation	Y=63.5x + 4.6	Y=0.6x + 5.9
Regression Equation (r^2)	0.9982 ± 0.0005	0.9995 ± 0.0005
Slope	63.565 ± 0.005	0.6029 ± 0.0005
Precision (n=6) mean±SD	*L.C. 0.246 ± 0.005 **M.C.3.845 ± 0.036 ***H.C.62.333 ± 0.0869	*L.C. 3.845 ± 0.036 **M.C.15.560 ± 0.025 ***H.C.62.333 ± 0.086
RSD (%) (n=6)	0.3	0.2

*Low concentration, ** Medium concentration, ***High concentration.

Robustness

The method was found robust under varied conditions of flow rate (± 0.02 mL min^{-1}), mobile phase compositions (± 2.0 mL of both organic components) and wavelength (± 6 nm). Since no marked changes were observed in the elution profiles, the data is not shown here.

Applications

The developed method has an importance in the quality control and quality assurance of cisplatin and etoposide formulations during manufacturing in industry as well as monitoring the quality of different brands of cisplatin and etoposide formulations especially the injectables at drug testing laboratories or other regulatory agencies. This method is recommended for industrial and quality control analysis of cisplatin and etoposide. The developed method was applied successfully on two different injectable brands of cisplatin and etoposide. The application of this method is clearly demonstrated in Table-4 and 5.

Table-4: Percent recovery of cisplatin by forecast formula (y = a + b x). Cisplatin quantity quoted in dosage form was 1000 µg mL^{-1}.

Serial No.	Sample	Experiments	Etoposide quantity found (µg mL^{-1})	Accuracy (%)
1		1	998.19	99.8
2		2	995.21	99.5
3		3	987.43	98.7
4		4	996.12	99.6
5	Brand 1	5	995.26	99.5
6	(Med)	6	992.71	99.2
Mean			994.15	99.4
SD			3.73	-
RSD			0.37	-
N			6	6
1		1	1003.24	100.3
2		2	997.98	99.7
3		3	995.32	99.5
4		4	993.01	99.3
5	Brand 2	5	997.95	99.7
6	(CCL)	6	998.85	99.8
Mean			997.72	99.7
SD			3.32	-
RSD			0.33	-
N			6	6

Table-5: Percent recovery of etoposide by forecast formula (y = a + b x). Etoposide quantity quoted in dosage form was 1000 µg mL^{-1}.

Serial No.	Sample	Experiments	Cisplatin quantity found (µg mL^{-1})	Accuracy (%)
1		1	991.75	99.1
2		2	995.28	99.5
3		3	984.43	98.4
4		4	992.19	99.2
5	Brand 1	5	993.16	99.3
6	(Med)	6	994.31	99.4
Mean			991.85	99.1
SD			3.86	-
RSD			0.38	-
N			6	6
1		1	993.24	99.3
2		2	995.18	99.5
3		3	992.11	99.2
4		4	993.51	99.3
5	Brand 2	5	997.09	99.7
6	(CCL)	6	998.01	99.8
Mean			994.85	99.4
SD			2.52	-
RSD			0.25	
N			6	6

Various methods for the determination of cisplatin and etoposide were previously developed individually [8, 9], whereas there was only one indirect method on the simultaneous determination of these drugs [21]. In this study, we have developed the method for the simultaneous determination of both these drugs. The studies were conducted on cisplatin and etoposide in pure form and injectable brands commercially available in the market using RP-HPLC analytical technique. The method is successfully applied on two injectable brands of etoposide and cisplatin. The validation results and statistical data shows that the developed method has short analysis time of < 8 min for etoposide with LOD and LOQ of 0.03 µg mL^{-1} and 0.250 µg mL^{-1}, respectively. Similarly, the LOD and LOQ values of cisplatin were 2.0 µg mL^{-1} and 3.9 µg mL^{-1}, respectively. During the application of this newly developed simultaneous method, cisplatin was eluted at 3.12 min and etoposide at 5.21 min. The mean values of accuracy for bulk cisplatin 99.74%, 99.94% and 99.78% and for etoposide were 99.16%, 99.74% and 99.78%, respectively. This method is better than the published one [21] where derivatives of cisplatin with sodium diethyldithio-carbamatetrihydrate were detected at variable wavelengths and fluorescence detector. In this study, nickel chloride was used as internal standard. The linear range for etoposide in plasma and tissue was 0.5-5 µg mL^{-1} and for cisplatin was 0.05-5 µg mL^{-1}. The RSD for both intraday and interday was < 10%. In contrast, the present work has % RSD of cisplatin 0.25 and 0.33 of etoposide.

This simultaneous method is important in the quality control and quality assurance of cisplatin and etoposide formulations during manufacturing in industry and monitoring the quality of different brands of cisplatin and etoposide formulations at drug testing laboratories or other regulatory agencies. The main advantages of the present developed method is a direct, simplicity of the mobile phase at a flow rate of 0.75 $mL.min^{-1}$ and short column run time to perform large number of assays. The developed method is found highly selective, sensitive and reproducible. The accuracy of the developed method was 98- 99% with RSD of 0.2-0.3%, within the limits of FDA guidelines.

Experimental

Cisplatin and etoposide were kindly supplied by Pharmedic Laboratories Pvt. Ltd. Lahore, Pakistan, with 99.64% and 98.60% purity, respectively. Methanol and acetonitrile were of HPLC grade from Merck, Germany. The column C_{18} (ODS Hypersil) was purchased from Thermo Electron Corporation, UK.

Instrumentation and HPLC Conditions

A Sykam series chromatographic system consisting of solvent delivery system (S-2100), Injector value Bracket (S 5111) and UV/Vis detector (S-3210) with C_{18} ODS Hypersil column having dimensions 250 × 4.6 mm with 5µm particle size was used. The detection was set at 283 nm. The mobile phase consisted of methanol, acetonitrile and water (40:35:25, v/v/v) at pH 3.5. The flow rate was maintained at 0.75 mL min^{-1}. Aliquots of drug were injected in loop of 20 µL. Total run time was 10 min. The system was attached with a computer soft ware (Clarity ver. 2.5) to analyze and calculate data.

Preparation of Mobile Phase

Mobile phase was prepared by mixing HPLC grade methanol and acetonitrile with double distilled filtered water (40:35:25) and contents filtered through 0.45 µm membrane filter (Sartorius Stedim, Germany). The pH was adjusted to 3.5 with 0.2N HCl. Mobile phase was used after degassing.

Standard sample preparation

Stock solutions (1mg mL^{-1}) each of cisplatin and etoposide were prepared in 0.9 % NaCl and mobile phase, respectively. The different solutions were prepared by the dilution of stock solutions. Further dilutions were made with mobile phase. Fresh solutions were prepared daily, filtered, and degassed by sonication.

Analytical Method Development

HPLC methods were developed and standardized for the analysis of two injectable brands of etoposide (Etoside-Medinet, Lymphoside-CCL) and cisplatin (Unistin-Korea, Ceplatin-Pharmedic). In the developed processes, various systems of mobile phase with several combinations were tested. Finally, methanol, acetonitrile and water (40:35:25 v/v/v) at pH 3.5 was found to be the most suitable at flow rate of 0.75 $mL.min^{-1}$. Run time of every elution was 10 min [22, 23].

Conclusion

The developed HPLC method is simple, direct, good linear, sensitive, accurate and robust for the quality analysis of cisplatin and etoposide individually or simultaneously. This method can be used in routine analysis of active ingredients as well as pharmaceutical injectable dosage forms. Moreover, it is recommended to perform analysis of these two drugs in the plasma of small cell lung cancer patients which are either individually or collective using these drugs for bioequivalence or pharmacokinetic studies.

Acknowledgements

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