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Separation and Determination of Process Related Impurities in Palbociclib: A Rp-hplc Study

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: A new gradient RP-HPLC method was developed for the separation and determination of process related impurities in Palbociclib.
Methodology: The chromatographic separation was achieved on a Inert sustain swift (C18) column using a mobile phase comprising of perchloric acid and acetonitrile in a gradient mode at a flow rate of 1 mL/min. over a runtime of 50 minutes. All the eluants were monitored at 230 nm. The optimized method was validated as per ICH guidelines for various parameters.
Results: The linearity of the method was proposed in the range of LOQ to 250 % for the drug and its impurities by subjecting the data obtained to statistical analysis using correlation coefficient model ($r > 0.99$). The method also gave acceptable recovery of all the four impurities at each level and was found to be accurate. The % RSD obtained in the method precision and intermediate precision were less than 2% depicting the precision of the method. The LOD and LOQ values were calculated based on the signal to noise ratio and are indicating the sensitivity of the method. The specificity of the method was checked in the presence of process related impurities and also degradants generated by exposing to a variety of forced degradation conditions.
Conclusion: The proposed RP-HPLC method for the determination of process related impurities of Palbociclib could be routinely used in the quality control testing.

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Keywords: Palbociclib; Process related impurities; Gradient; RP-HPLC; ICH guidelines.

1. INTRODUCTION

Palbociclib (Fig. 1) is a piperazine pyridopyrimidine derivative and second generation cyclin-dependent kinase inhibitor [1] approved for the treatment of hormone receptor-positive and human epidermal growth factor receptor type 2 (HER2)-negative breast cancer in combination with aromatase inhibitors like letrazole as initial endocrine-based therapy or following fluvestrant as endocrine therapy in patients with disease progression. Palbociclib was the first Cyclin-Dependant Kinase 4 and Cyclin-Dependant Kinase 6 [2-5] inhibitor. Chemically Palbociclib is 6-Acetyl-8-cyclopentyl-5-methyl-2-((5-(piperazin-1-yl) pyridine-2-yl) amino) pyrido [2,3-d] pyrimidin-7(8H)-one. It is a pale yellow colour solid with a molecular weight of 447.53 g/mol. and is soluble in acetic acid, 0.1 N hydrochloric acid and insoluble in water. Four process related impurities were found to appear in the final drug substance of Palbociclib as given in Table 1.

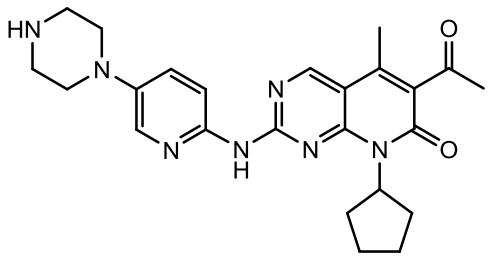


Fig. 1. Chemical structure of Palbociclib

Only few analytical methods were reported for determination of Palbociclib either in dosage forms [6-8] or in biological fluids [9-12] as a single entity or in combination with other drugs. So far no method was reported in the literature for determination of process-related substances of Palbociclib. The current study is aimed at development and Validation for the determination of Palbociclib and its process-related impurities by RP-HPLC method. The method developed was utilized for forced degradation studies of Palbociclib as per ICH guidelines (acid hydrolysis, alkali hydrolysis, oxidation, thermal, photolytic and humidity conditions) for evaluation of its suitability for stability studies of Palbociclib drug substance. A comprehensive spectral study was undertaken for structural confirmation of process impurities by FT-IR, MS and 1H NMR spectroscopy.

2. MATERIALS AND METHODS

2.1 Instrumentation

An integrated Agilent HPLC system with quaternary low-pressure gradient, auto sampler, column thermostat and photodiode array detector or UV detector operating with a computer-based chromatography software (Open Lab CDS) was used in this work. An Inert sustain swift (C18), 4.6 x 250 mm, 5µ column was used.

2.2 Chemicals and Reagents

Palbociclib and its process related impurities were obtained from Biophore India Pharmaceuticals Private Limited, Hyderabad (India). HPLC grade acetonitrile and perchloric acid (70%) were obtained from Merck. High purity water was prepared by using Milli-Q Elix and then using Milli-Q purification system (Millipore).

2.3 Preparation of Mobile Phase

2 mL of 70 % Perchloric acid was diluted with Milli-Q water in a 1000 mL volumetric flask, mixed, filtered through 0.45µ membrane and degassed (Solvent A). Acetonitrile was used as solvent B.

2.4 Preparation of Diluent

A mixture of solvent A and solvent B in the ratio of 50:50 % v/v was prepared, and the same diluent was used as blank.

2.5 Preparation of Standard Solution

25 mg of Palbociclib standard was weighed and transferred into a 25 mL volumetric flask, about 20 mL of diluent was added, sonicated and made up to the mark with diluent. Further transferred 2.5 mL of the above solution into a 100 mL volumetric flask and made up to the mark with diluent. Further transferred 1 mL of above solution into 25 mL volumetric flask and made up to the mark with diluent.

2.6 Preparation of Test Solution

25 mg of test sample was weighed and transferred into a 25 mL volumetric flask, 20 mL

of diluent was added, dissolve by sonication and made up to the mark with diluent.

2.7 Preparation of Impurity Mixture (stock)

10 mg each of Impurity - A standard, Impurity - B standard, Impurity - C standard and Impurity - D standard was weighed and transferred into a 100 mL volumetric flask, about 70 mL of diluent was added, dissolved by sonication and then made up to the mark with diluent. Further transferred 7.5 mL of above solution in to 100 mL volumetric flask and made up to the mark with diluent.

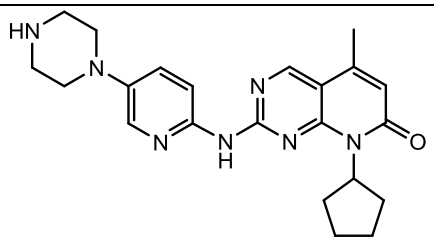
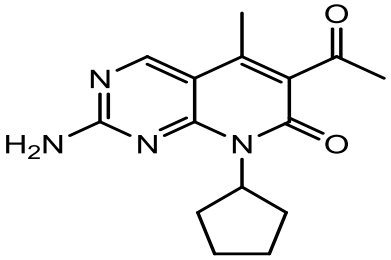
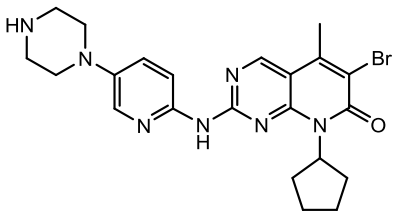
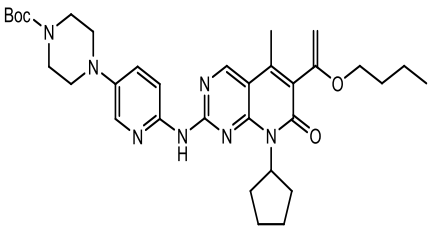
2.8 System Suitability Solution

25 mg of Palbociclib standard was weighed into a 25 mL volumetric flask, 15 mL of diluent was added, sonicated, 5 mL of impurity stock solution was added and made up to the mark with diluent.

2.9 Method Optimization

To develop a suitable and robust RP-HPLC method for the determination of Palbociclib and its process related impurities, different mobile phases and columns were employed to achieve the best separation and resolution. The impurity standards were injected into the chromatographic system separately and later combining with Palbociclib (spiked with sample). After a series of trials, the chromatographic conditions have been optimized. The impurities in the drug substance [13] were identified based on the retention time (RT) and relative retention time (RRT) observed from the spike study.

Table 1. Process-related impurities of Palbociclib

S. No	Name of the Impurity	Structure	Impurity Code
1.	8-cyclopentyl-5-methyl-2-(5-(piperazin-1-yl) pyridin-2-yl amino) pyrido[2,3-d] pyrimidin-7(8H)-one.		Impurity-A
2.	6-acetyl-2-amino-8-cyclopentyl-5-methylpyrido [2,3-d] pyrimidin-7(8H)-one		Impurity-B
3.	6-bromo-8-cyclopentyl-5-methyl-2-()5-(piperazin-1-yl) pyridin-2-yl) amino) pyrido[2,3-d] pyrimidin-7(8H)-one.		Impurity-C
4.	tert-butyl 4-(6-(6-(1-butoxyvinyl)-8-cyclopentyl-5-methyl-7-oxo-7,8-dihydropyrido[2,3-d]pyrimidin-2-yl amino)pyridin-3-yl)piperazine-1-carboxylate.		Impurity-D

2.10 Method Validation

Analytical method validation is a process that demonstrates the suitability of the proposed procedures for the intended purpose. More specifically, it is a matter of establishing documented evidence providing a high degree of assurance with respect to the consistency of the method and results to evaluate the product against defined specifications. The validation parameters viz., system suitability, linearity, accuracy, precision, limit of detection, limit of quantitation, specificity and robustness, have been evaluated as per the ICH guidelines [14-15].

2.11 System Suitability

This is an integral part of development of a chromatographic method to verify that the resolution and reproducibility of the system are adequate enough for the analysis to be performed. It is based on the concept that the equipment, electronics, analytical operations and samples constituting an integral system could be evaluated as a whole. Parameters such as retention time (RT), relative retention time (RRT), resolution (R_s) and reproducibility (% RSD) were determined. These parameters were studied during the analysis of a "sample" containing the main components and related substances and compared with the recommended limits (% RSD < 10 and R_s>1.5).

2.12 Specificity

Specificity is the ability of the method to measure the analyte response in presence of its process related impurities. The specificity of the developed RP-HPLC method was performed by injecting blank solution, standard solution and process-related impurities separately. The chromatogram of drug with impurities was compared with the blank chromatogram, to verify the blank interference at the retention time of specified impurities and the product peaks.

2.13 Linearity, Accuracy and Precision

Standard solutions at different concentration levels ranging from LOQ to 250 % of specification limit were prepared and analyzed in duplicate at 50 %, 100 % 150% and 200%, while at LOQ, 25 % and 250 % six injections were prepared and analyzed. In order to demonstrate the linearity of detector response for Palbociclib and its impurities, the linearity plot was drawn taking the concentration on X-axis and the mean peak area on Y-axis. The data was subjected to statistical analysis using linear-regression model. Accuracy of the test method was determined by analyzing Palbociclib drug substance spiked with impurities at four different concentration levels of LOQ, 100 %, 150 % and 250 % of each in triplicate at the specified limit. The mean recoveries of all the impurities were calculated. System precision of the method was evaluated by injecting the standard solution six times and percent relative standard deviation (% RSD) for area of Palbociclib peak was calculated. The precision of the method for the determination of impurities related to Palbociclib was studied for repeatability (method precision) and intermediate precision at 100 % level. Repeatability was demonstrated by analyzing six test preparations spiked with impurities. The % RSD for peak area of each impurity was calculated. Intermediate precision was demonstrated by analyzing same sample of Palbociclib by two different analysts on two different days.

2.14 Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ were determined by measuring the magnitude of analytical background by blank samples and calculating the signal-to-noise ratio (S/N) for each compound by injecting a series of solutions until S/N ratio obtained as 3 for LOD and 10 for LOQ.

2.15 Robustness

To determine the robustness of the developed method, some of the chromatographic conditions were deliberately altered. The parameters selected were change in flow rate (± 0.1 ml/min), and change in the column temperature (± 5ºC), the rest of the chromatographic conditions for each alteration study was kept constant.

2.16 Forced Degradation Study

Stability testing of an active substance or finished product provide evidence on how the quality of a drug substance or drug product varies with time influenced by a variety of environmental conditions like temperature, humidity and light etc. Knowledge from stability studies enables understanding of the long-term effects of the environment on the drugs. Stability testing provides information about degradation

mechanisms, potential degradation products, possible degradation path ways of drug as well as interaction between the drug and the excipients in drug product. Forced degradation study was carried out by treating the sample under the following conditions and eluents were detected using a photodiode array detector.

2.17 Acid degradation

25 mg of sample was weighed and transferred into a 25 mL volumetric flask and 5 mL of 1.0 N hydrochloric acid was added to it. The solution was warmed on a water bath at 60°C for 10 hrs. and then neutralized with 5 mL of 1.0 N sodium hydroxide. The neutralized solution was made up to the volume with diluent and further analyzed.

2.18 Alkali degradation

25 mg of sample was weighed and transferred into a 25 mL volumetric flask and 5 mL of 1.0 N sodium hydroxide was added to it. The solution was warmed on a water bath at 60°C for 10 hrs. and then neutralized with 5 mL of 1.0 N hydrochloric acid. The neutralized solution was made up to the volume with diluent and analyzed.

2.19 Thermal Degradation

200 mg of the sample was taken in a watch glass and kept in an oven at 105°C for 168 hr. From that sample 25 mg was accurately weighed and transferred into a 25 mL volumetric flask, dissolved and the volume was made up with diluent.

2.20 Humidity Degradation

200 mg of the sample was exposed to 90 % RH for 168 hrs. From that sample 25 mg was accurately weighed and transferred into a 25 mL volumetric flask, dissolved and the volume was made up with diluent for further analysis.

2.21 Photolytic Degradation

200 mg of the sample was exposed to 1200 Klux hrs. in a photo stability chamber. From that sample 25 mg was accurately weighed and transferred into a 25 ml volumetric flask, dissolved and the volume was made up with diluent.

2.22 UV Degradation

200 mg of the sample was exposed to 200 Watt Hours/Square meter in a UV stability chamber. From that sample 25 mg was accurately weighed and transferred into a 25 mL volumetric flask, dissolved, made up to the volume with diluent and injected into the chromatographic system.

2.23 Peroxide degradation

25 mg of the sample was weighed and transferred into a 25 mL volumetric flask and 5 mL of 10.0 % hydrogen peroxide solution was added to it. The mixture was kept at room temperature for 3 hrs. The above mixture was kept aside for few minutes, volume was made up with diluent and injected in replicates.

2.24 Solution stability

The stability of the standard and test solutions at room temperature was evaluated at three different time intervals (initial, 24 hours and 48 hours) and % RSD for the peak area response was calculated for the impurities and Palbociclib peaks in all the solutions.

3. RESULTS AND DISCUSSION

A simple and sensitive RP-HPLC was developed and validated for the quantification of Palbociclib, its four known process related impurities and Unknown impurities in the drug substance. The specification limits for known impurities (Impurity-A, Impurity-B, Impurity-C and Impurity-D) and unknown impurities were 0.15% w/w and 0.10% respectively.

3.1 Method Optimization

To develop a suitable and robust RP-HPLC method for the determination of Palbociclib and its process related impurities, different mobile phases and columns were employed to achieve the best separation and resolution. The method development was initiated with C18 column using a mobile phase containing water and acetonitrile as organic modifier where broad peaks were observed. The aqueous phase was made acidic using various acids to modify the peak shapes. Since the palbociclib and its process related impurities possess varying polarities, a gradient elution mode was proposed for better elution. Optimisation of the method was performed using different gradient programs, various C18

columns, optimizing the pH of mobile phase and organic modifier strength and wavelength. The optimized chromatographic conditions resulted in sharp peaks with minimum tailing and fronting, good resolution between impurities and product peak without any blank interference. The optimized chromatographic conditions are shown in Table 2a and 2b.

3.2 Method Validation

The system suitability solution prepared above was injected in six replicates at the beginning of each study and the data is given in Table 3.

The specificity of the developed RP-HPLC method was performed by injecting blank solution, standard solution and process-related impurities separately. No blank interferenec was observed at the retention time of Palbociclib and its impurities. Hence, the method was found specific for the determination of process related impurities in Palbocilib. The corresponding chromatograms of the drug and individual impurities are given in Fig. 1a – 1f.

Standard solutions at different concentration levels ranging from LOQ to 250% of specification limit were injected into the chromatographic system and the response was found linear as observed from the calibration curves obtained for the drug and impurities analyzed separately. The data obtained for linearity study, LOD and LOQ is summarized below in table 4.

The accuracy of the developed method was established in terms of the amount of impurities recovered in the spiked drug substance at four different levels (LOQ, 100 %, 150 % and 250 %). The percentage recovery data is given in Table 5. The method was proved to be precise as observed from the % RSD (0.3 – 1.1) values (for peak areas) obtained in method precision and intermediate precision study given in Table 6. A deliberate variation in the optimized chromatographic conditions such as flow rate (± 0.1 mL/min) and column temperature (± 5ºC) did not show any significant changes in the system suitability parameters and proved the robustness of the method. The standard and test solutions were observed for the solution stability and found to stable over a period of 48 hours as calculated from the impurities content (Table 7).

Table 2a. Optimized chromatographic conditions

Column	**:**	**Inertsustain swift (C18), 4.6 x 250 mm, 5µ**
Mobile phase A	:	2 mL of 70 % Perchloric acid in1000 mL Milli Q water
Mobile phase B	:	Acetonitrile
Flow rate	:	1.0 mL/min
Wave length	:	230 nm
Injection volume	:	10.0 µl
Flow mode	:	Gradient
Run time		50 min.
Column temperature	:	40ºC

Table 2b. Gradient program

Time (min.)	**Mobile phase A (%)**	**Mobile phase B (%)**
0	75.0	25.0
5	75.0	25.0
40	30.0	70.0
42	75.0	25.0
50	75.0	25.0

Table 3. System suitability data

Name	**RT**	**RRT**	**Resolution**(R_s)	**Reproducibility (% RSD)**
Impurity-A	12.75	0.89	-	0.6
Impurity-B	13.51	0.94	2.99	0.2
Palbociclib	14.32	1.00	2.29	1.1
Impurity-C	17.91	1.25	10.54	0.4
Impurity-D	30.10	2.11	50.15	1.3

Table 4. Linearity data, LOD and LOQ for Palbociclib and impurities

Level	Palbociclib		Impurity A		Impurity B		Impurity C		Impurity D	
	Conc. (µg/mL)	Mean peak area	Conc. (µg/mL)	Mean peak area	Conc. (µg/mL)	Mean peak area	Conc. (µg/mL)	Mean peak area	Conc. (µg/mL)	Mean peak area
LOQ	0.1813	93148	0.2635	82125	0.0882	92548	0.2125	80296	0.2841	72494
25 %	0.2501	110357	0.3771	124299	0.3776	390186	0.3775	135980	0.3790	118633
50 %	0.5001	219791	0.7542	245271	0.7551	770371	0.7550	269619	0.7580	232801
100 %	1.0002	444289	1.5084	491584	1.5102	1541622	1.5099	537464	1.5159	470439
150 %	1.5003	670062	2.2626	740071	2.2653	2318753	2.2649	806143	2.2739	704021
200 %	2.0004	887805	3.0168	977602	3.0204	3066273	3.0198	1065665	3.0318	929124
250 %	2.5005	1124640	3.7710	1230576	3.7755	3858228	3.7748	13339169	3.7898	1160396
Slope	446220.9		326040.8		1019083.9		353163.9		308053.3	
Intercept	1466.53		-833.43		2948.18		3799.94		-2677.69	
r	0.9999		1.0000		1.0000		1.0000		0.9999	
LOD (µg/mL)	0.0544		0.0790		0.0265		0.0638		0.0852	

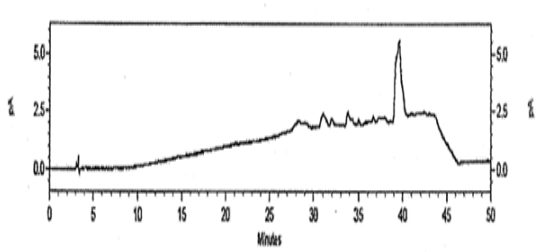


Fig. 1a. Chromatogram of blank

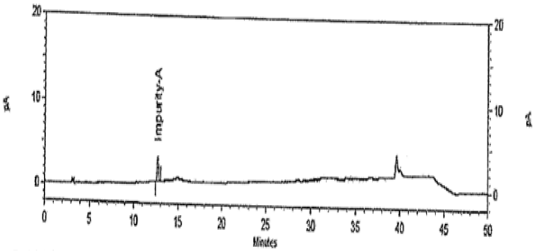


Fig. 1b. Chromatogram of Impurity A Standard, RT at 12.71 min

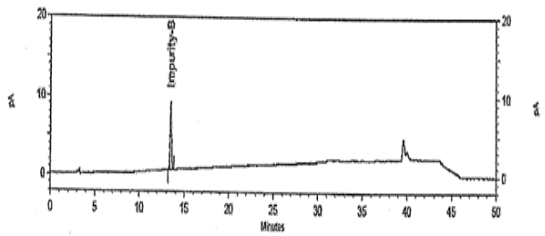


Fig. 1c. Chromatogram of Impurity B Standard, RT at 13.51 min.

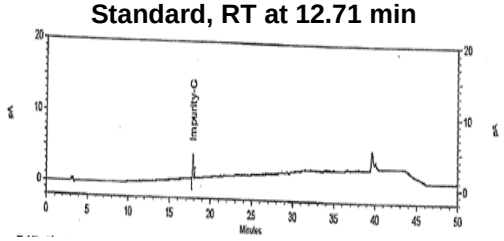


Fig. 1d. Chromatogram of Impurity C Standard, RT at 17.87 min

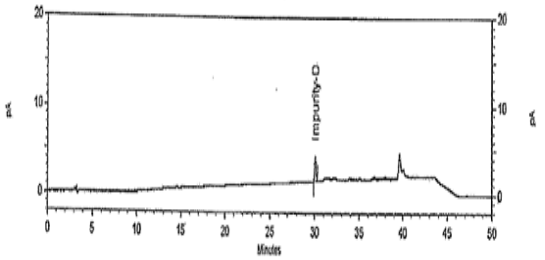


Fig. 1e. Chromatogram of Impurity D Standard, RT at 30.07 min

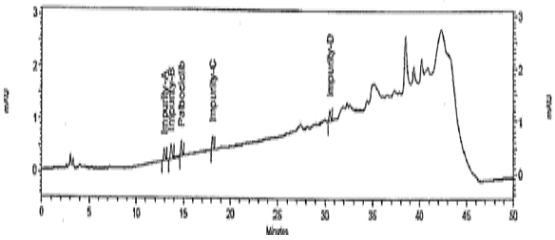


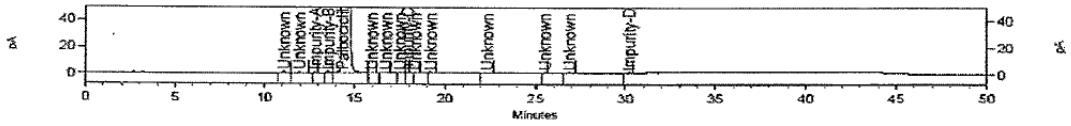
Fig. 1f. Typical chromatogram for Palbociclib (RT at 14.78 min.) spiked with impurities

Fig. 1a – 1f. Typical chromatograms of blank, individual impurity A, B, C, D and Palbociclib spiked with impurities

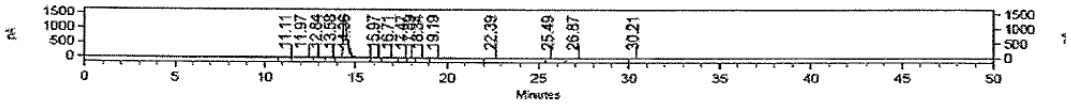
Table 5. Accuracy studies for impurities of Palbociclib

Name	Spike level (%)	Concentration spiked (µg/mL)	Concentration recovered (µg/mL)	*Recovery (%)
Impurity-A	LOQ	0.261924	0.233905	89.30
	100	1.542600	1.475055	95.62
	150	2.313900	2.234974	96.59
	250	3.856500	3.727305	96.65
Impurity-B	LOQ	0.088255	0.091966	104.20
	100	1.531800	1.593240	104.01
	150	2.297700	2.411934	104.97
	250	3.829500	3.863670	100.89
Impurity-C	LOQ	0.210189	0.241610	114.95
	100	1.533750	1.443800	94.14
	150	2.300625	2.231657	97.00
	250	3.834375	3.772968	98.40
Impurity-D	LOQ	0.281428	0.271554	96.49
	100	1.535100	1.583946	103.18
	150	2.302650	2.391119	103.84
	250	3.837750	3.906464	101.79

** Mean of three replicates*



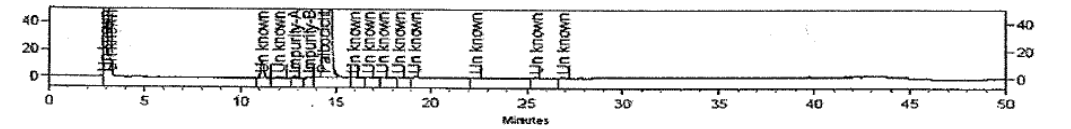
NORMALIZED CHROMATOGRAM



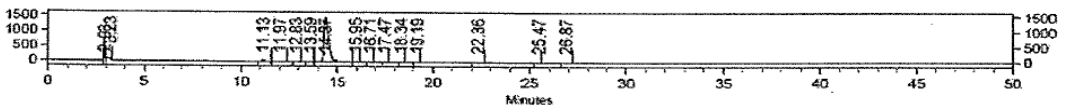
DAD: Signal A, 230.0 nm/Bw:4.0 nm Results

S.No	Name	RT	Area	Area Percent	Relative RT	Peak purity
1	Unknown Impurity	11.11	1809390	0.06	0.77	1.00
2	Unknown Impurity	11.97	840869	0.03	0.83	1.00
3	Impurity-A	12.84	203924	0.01	0.89	1.00
4	Impurity-B	13.58	1460313	0.05	0.95	1.00
5	Palbociclib	14.36	2966771081	99.68	1.00	1.00
6	Unknown Impurity	15.97	343863	0.01	1.11	1.00
7	Unknown Impurity	16.71	598038	0.02	1.16	1.00
8	Unknown Impurity	17.47	697950	0.02	1.22	1.00
9	Impurity-C	17.89	155982	0.01	1.25	1.00
10	Unknown Impurity	18.34	407769	0.01	1.28	1.00
11	Unknown Impurity	19.19	1304364	0.04	1.34	1.00
12	Unknown Impurity	22.39	289782	0.01	1.56	1.00
13	Unknown Impurity	25.49	272355	0.01	1.77	1.00
14	Unknown Impurity	26.87	728889	0.02	1.87	1.00
15	Impurity-D	30.21	285296	0.01	2.10	1.00
Totals			2976169865	100.00		

Fig. 2a. Chromatogram for acid degradation



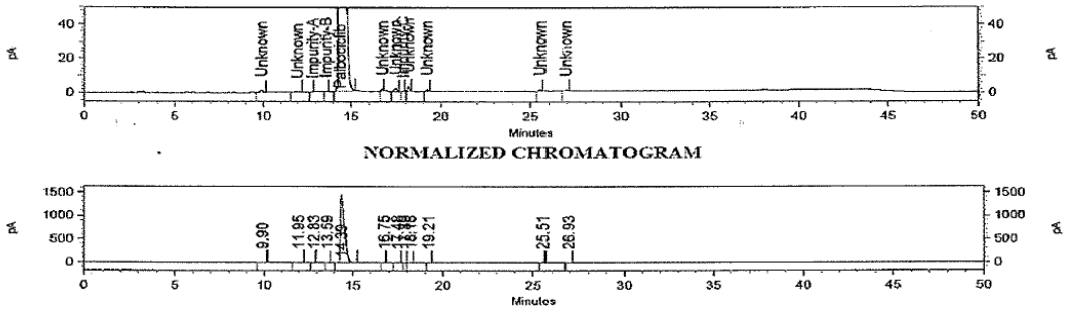
NORMALIZED CHROMATOGRAM



DAD: Signal A, 230.0 nm/Bw:4.0 nm Results

S.No	Name	RT	Area	Area Percent	Relative RT	Peak purity
1	Un known Impurity	2.93	320768873	9.45	0.20	1.00
2	Un known Impurity	3.23	70228521	2.07	0.23	1.00
3	Un known Impurity	11.13	16770895	0.49	0.77	1.00
4	Un known Impurity	11.97	532461	0.02	0.83	1.00
5	Impurity-A	12.83	1193264	0.04	0.89	1.00
6	Impurity-B	13.59	931248	0.03	0.95	1.00
7	Palbociclib	14.37	2979613112	87.78	1.00	1.00
8	Un known Impurity	15.95	319748	0.01	1.11	1.00
9	Un known Impurity	16.71	292851	0.01	1.16	1.00
10	Un known Impurity	17.47	570578	0.02	1.22	1.00
11	Un known Impurity	18.34	318266	0.01	1.28	1.00
12	Un known Impurity	19.19	1402395	0.04	1.34	1.00
13	Un known Impurity	22.36	298904	0.01	1.56	1.00
14	Un known Impurity	25.47	270699	0.01	1.77	1.00
15	Un known Impurity	26.87	717254	0.02	1.87	1.00
Totals			3394229069	100.00		

Fig. 2b. Chromatogram for alkali degradation

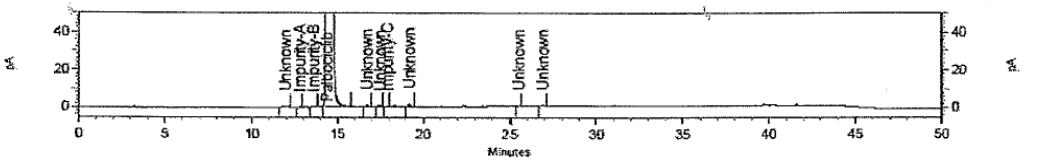


NORMALIZED CHROMATOGRAM

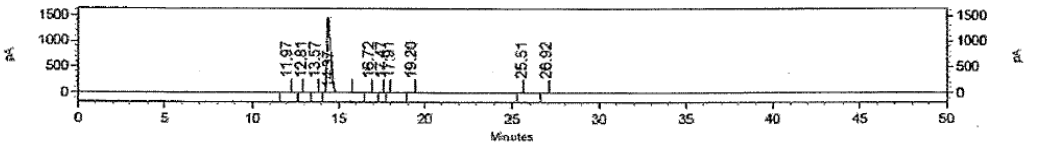
DAD: Signal A, 230.0 nm/Bw:4.0 nm Results

S.No	Name	RT	Area	Area Percent	Relative RT	Peak purity
1	Unknown	9.90	2092082	0.07	0.69	1.00
2	Unknown	11.95	688068	0.02	0.83	1.00
3	Impurity-A	12.83	143091	0.00	0.89	1.00
4	Impurity-B	13.59	385494	0.01	0.94	1.00
5	Palbociclib	14.39	2956260478	99.56	1.00	1.00
6	Unknown	16.75	1102156	0.04	1.16	1.00
7	Unknown	17.48	2328319	0.08	1.22	1.00
8	Impurity-C	17.89	89676	0.00	1.24	1.00
9	Unknown	18.18	3294880	0.11	1.26	1.00
10	Unknown	19.21	1299319	0.04	1.34	1.00
11	Unknown	25.51	759898	0.03	1.77	1.00
12	Unknown	26.93	803403	0.03	1.87	1.00
Totals			2969246864	100.00		

Fig. 2c. Chromatogram for thermal degradation



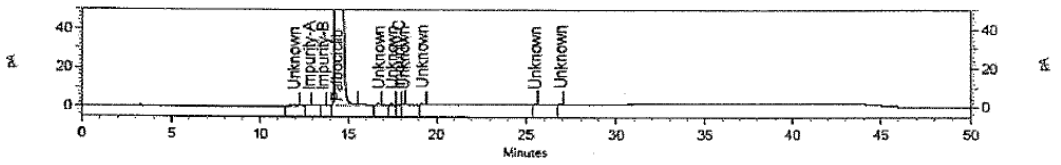
NORMALIZED CHROMATOGRAM



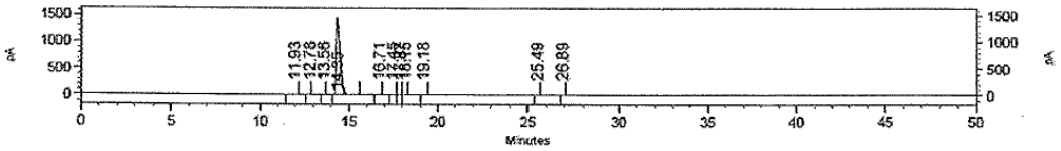
DAD: Signal A, 230.0 nm/Bw:4.0 nm Results

S.No	Name	RT	Area	Area Percent	Relative RT	Peak purity
1	Unknown	11.97	875170	0.03	0.83	1.00
2	Impurity-A	12.81	185561	0.01	0.89	1.00
3	Impurity-B	13.57	210473	0.01	0.94	1.00
4	Palbociclib	14.37	2941131593	99.82	1.00	1.00
5	Unknown	16.72	761649	0.03	1.16	1.00
6	Unknown	17.47	563560	0.02	1.22	1.00
7	Impurity-C	17.91	278251	0.01	1.25	1.00
8	Unknown	19.20	1441118	0.05	1.34	1.00
9	Unknown	25.51	294582	0.01	1.78	1.00
10	Unknown	26.92	669956	0.02	1.87	1.00
Totals			2946411913	100.00		

Fig. 2d. Chromatogram for humidity degradation



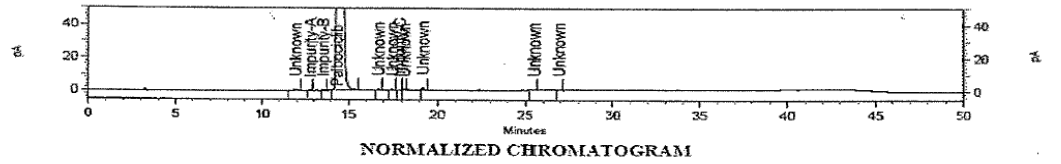
NORMALIZED CHROMATOGRAM



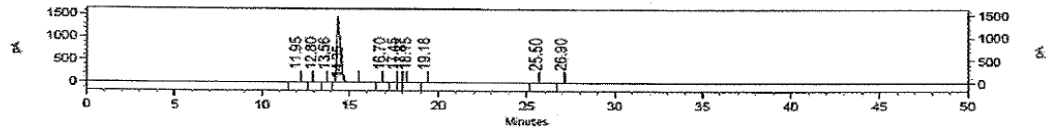
DAD: Signal A, 230.0 nm/Bw:4.0 nm Results

S.No	Name	RT	Area	Area Percent	Relative RT	Peak purity
1	Unknown	11.93	796956	0.03	0.83	1.00
2	Impurity-A	12.78	187456	0.01	0.89	1.00
3	Impurity-B	13.56	244216	0.01	0.95	1.00
4	Palbociclib	14.35	2972512452	99.79	1.00	1.00
5	Unknown	16.71	901605	0.03	1.16	1.00
6	Unknown	17.45	966434	0.03	1.22	1.00
7	Impurity-C	17.87	297684	0.01	1.25	1.00
8	Unknown	18.15	551874	0.02	1.26	1.00
9	Unknown	19.18	1346054	0.05	1.34	1.00
10	Unknown	25.49	279103	0.01	1.78	1.00
11	Unknown	26.89	660022	0.02	1.87	1.00
Totals			2978743856	100.00		

Fig. 2e. Chromatogram for photolytic degradation



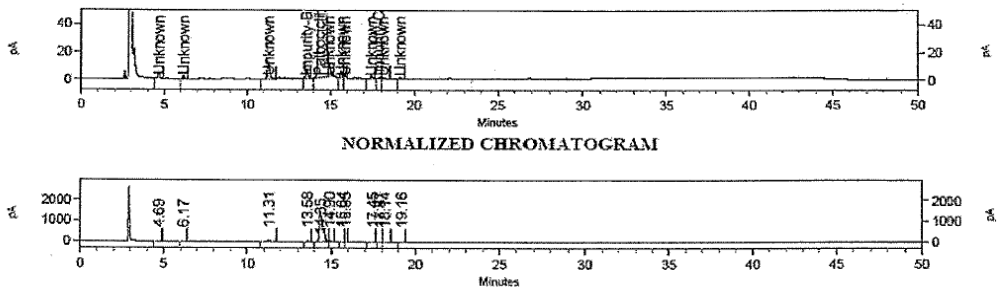
NORMALIZED CHROMATOGRAM



DAD: Signal A, 230.0 nm/Bw:4.0 nm Results

S.No	Name	RT	Area	Area Percent	Relative RT	Peak purity
1	Unknown	11.95	882508	0.03	0.83	1.00
2	Impurity-A	12.80	193440	0.01	0.89	1.00
3	Impurity-B	13.56	251318	0.01	0.94	1.00
4	Palbociclib	14.35	2965393407	99.82	1.00	1.00
5	Unknown	16.70	719069	0.02	1.16	1.00
6	Unknown	17.45	643548	0.02	1.22	1.00
7	Impurity-C	17.87	277960	0.01	1.24	1.00
8	Unknown	18.15	198454	0.01	1.26	1.00
9	Unknown	19.18	1368643	0.05	1.34	1.00
10	Unknown	25.50	287012	0.01	1.78	1.00
11	Unknown	26.90	626958	0.02	1.87	1.00
Totals			2970842317	100.00		

Fig. 2f. Chromatogram for UV degradation



DAD: Signal A, 230.0 nm/Bw:4.0 nm Results

S.No	Name	RT	Area	Area Percent	Relative RT	Peak purity
1	Unknown Impurity	4.69	4912230	0.16	0.33	1.00
2	Unknown Impurity	6.17	2941870	0.10	0.43	1.00
3	Unknown Impurity	11.31	14978415	0.50	0.79	1.00
4	Impurity-B	13.58	8595045	0.29	0.95	1.00
5	Palbociclib	14.35	2948975698	98.29	1.00	1.00
6	Unknown Impurity	14.90	8400726	0.28	1.04	1.00
7	Unknown Impurity	15.64	3807722	0.13	1.09	1.00
8	Unknown Impurity	15.85	1124264	0.04	1.11	1.00
9	Unknown Impurity	17.45	2666560	0.09	1.22	1.00
10	Impurity-C	17.87	865179	0.03	1.25	1.00
11	Unknown Impurity	18.14	1602707	0.05	1.26	1.00
12	Unknown Impurity	19.16	1451583	0.05	1.34	1.00
Totals			3000321999	100.00		

Fig. 2g. Chromatogram for peroxide degradation

Fig. 2a – 2g. Forced degradation study chromatograms of Palbociclib

Table 6. Precision study for Palbociclib and its impurities

Name	Method precision	Intermediate precision
	% RSD (n=6)	
Palbociclib	0.4	0.5
Impurity-A	0.4	0.7
Impurity-B	0.8	0.5
Impurity-C	1.1	0.5
Impurity-D	0.3	1.0

Table 7. Results for Solution stability of test solution

Duration	Imp. A	Imp. B	Imp. C	Imp. D	Single maximum unknown impurity	Total impurities
	Impurities content (% w/w)					
Initial	0.01	0.003	0.01	ND	0.04	0.11
24 hours	0.01	0.004	0.01	ND	0.04	0.11
48 hours	0.01	0.005	0.01	ND	0.04	0.12

Table 8. Results for forced degradation study

Stress condition	Peak purity	Purity (% area)
Acid degradation	1.000	99.68
Alkali degradation	1.000	87.78
Thermal degradation	1.000	99.56
Humidity degradation	1.000	99.83
Photolytic degradation	1.000	99.79
UV degradation	1.000	99.82
Peroxide degradation	1.000	98.29

Table 9. Comparison table for the existing methods with the present method

Mobile phase (v/v)	Stationary phase	Linearity (µg/mL)	Comments	Ref.
Acetonitrile: Sodium acetate buffer (30:30, 0.5% TEA)	Kromasil C18 (250 mm × 4.6 mm, 5 µm)	4.04 – 20.19	Method for content in capsules (HPLC)	6
Sodium dihydrogen phosphate buffer (pH 5.5): Acetonitrile: methanol (80:10:10)	Intersil C8 (4.6 mm × 250 mm, 5µm)	5 - 50	Simultaneous estimation in capsules (HPLC)	7
Ammoniumacetate: Acetonitrile (32:68)	Inertsil ODS- 3V (4.6 mm × 250 mm, 5 µm)	5 - 1000	Stability indicating Assay method (HPLC)	8
Acetonitrile: 0.1% TEA (pH 3.3; adjusted with 50% OPA, 70:30)	Agilent Zorbax C18 (150 mm × 4.6 mm, 5 µm)	0.1 -3.0	Bioanalytical method (HPLC)	9
Ammonium bicarbonate in water (eluent A) and ammonium bicarbonate in water-methanol (1:9 *v*/*v*, eluent B), Gradient elution	Gemini C18 (50 mm× 2.0 mm, 5 µm)	0.002 – 0.2	Bioanalytical method(LC-MS/MS)	10
Methanol: Water (0.1% acetic acid, 55:45	Acquity UPLC BEH C18 (1.7 µm,50mm × 2.1 mm)	0.0005 – 0.6	Bioanalytical method (UHPLC-MS/MS)	11
Water with 0.1% formic acid (phase A) and Methanol/isopropanol (9:1, v/v) with 0.1% formic acid (phase B)	Luna Omega Polar C18 (3 µm, 100 Å, 50 x 2.1 mm)	0.0003 – 0.25	Bioanalytical method (LC-MS/MS)	12
Water (70 % Perchloric acid) : Acetonitrile, Gradient elution	Inertsustain swift C18 (4.6 x 250 mm, 5µ)	0.18 – 2.50	Method for process related impurities (HPLC)	Present method

TEA: Triethyl amine; OPA: Ortho phosphoric acid

Palbociclib sample was subjected to a series of stress degradations and the drug was found to be stable in all the exposed conditions except under alkali stress where a major degradation was observed (about 12 %). A major degradant peak (9.45%) was observed under the optimized conditions at 2.93 min. which was well separated from the other peaks in alkali treated sample. The peaks for Palbociclib in all the forced degradation samples were found to be spectrally homogenous and passed the peak purity (> 0.99). The separation and peak purity results showed that the RP-HPLC method for quantification of Palbociclib and its process related impurities is specific for determination of all the degradation impurities and can be deployed for stability studies of Drug Suabstance. The forced degradation chromatograms are given in Figs. 2a – 2g and the data is given in Table 8.

4. CONCLUSION

A new gradient RP-HPLC method was developed for the separation and determination of process related impurities in Palbociclib and validated as per ICH guidelines. The method was found to be simple, sensitive, precise, robust and accurate as observed from the statistical data. The present method could achieve separation of all the impurities in the presence of the drug and its degradation products in a variety of stress conditions and can be concluded as a selective and stability indicating method for the determination of impurities in Palbociclib. Therefore, this method can be used for routine quality control testing and also stability analysis of Palbociclib drug substance.

DISCLAIMER

The products used in this research are commonly and predominantly used in this area of research. There is no conflict of interest between the authors and producers of the products as they are used only for the advancement of Knowledge. This work was only funded by the personal efforts of the authors and was not funded by external sources.

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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