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Research

A novel rp-hplc method for the estimation of palbociclib in bulk drug & pharmaceutical dosage form

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Abstract

A novel, specific, accurate, rugged, precise reversed-phase high performance liquid chromatography (RP-HPLC) method has been developed for the quantitative determination of Palbociclib in active pharmaceutical ingredients and in its Pharmaceutical dosage form by using Phenomenex Luna C18 (4.6mm x 150mm, 5µm) column with a mobile phase containing a mixture of Acetonitrile and Potassium dihydrogen phosphate buffer adjusted to pH-2.8 with ortho phosphoric acid in the ratio of 25:75%v/v. The flow rate was 1.0 ml/min and effluent were monitored at 220 nm and a peak eluted at 3.174 min and column oven temperature was maintained ambient. Calibration curve was plotted with a range from 10- 30 µg/ml. The LOD and LOQ values of Palbociclib were found to be 1.3µg/ml and 3.9 µg/ml respectively. The percentage recovery of the Palbociclib was found to be within the limits. The developed RP-HPLC method was validated according to the current International Conference on Harmonization (ICH) guidelines for specificity, LOD, LOQ, linearity, accuracy, precision, intermediate precision and robustness. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of Palbociclib in bulk drug and in its pharmaceutical dosage form. The proposed method was applied for the analysis of tablet formulations, to improve QC and assure therapeutic efficacy.

Keywords: Palbociclib, RP-HPLC, Accuracy, Validation, ICH Guidelines.

INTRODUCTION

Chromatography Quality can be defined as the character, which defines the grade of excellence. A good quality drug is something, which will meet the established product specifications, can be safely bought and confidently used for the purpose for which it is intended.1 To get a good quality drug ,the manufacturing for making a drug should have quality built into it. Analytical chemistry is the science that seeks ever improved means of measuring the chemical composition of natural and artificial materials. Analytical chemistry is a sub- discipline

of chemistry that has the broad mission of understanding the chemical composition of all matter and developing the tools to elucidate such compositions.[2]

Analytical methods: Spectrophotometry andcolorimetry; UV-visible spectroscopy; Chromatography and Electrophoresis.

Chromatography

Chromatography is a method used for separating organic and inorganic compounds so that they can be analysed and studied. Chromatography is a great physical method for observing mixtures and solvents. The word chromatography means colour separation where chroma means colour and graphy means separation. Chromatography is based on different migration. Solutes with a greater affinity for the mobile phase will spend more time in this phase than solutes that prefer the stationary phase. As the solutes move through the stationary phase the different components are going to be absorbed and are going to stop moving with mobile phase .Thus they are separated. This is called as chromatographic development.

High performance liquid chromatography

HPLC is able to separate macromolecules and ionic species labile natural products, polymeric materials, and a wide variety of other high –molecular weight poly functional group. HPLC is the fastest growing analytical technique for the analysis of the drugs. It's simplicity, high specificity, and wide range of sensitivity makes it ideal for the analysis of many drugs in both dosage forms and biological fluids .In this ,the separation is about 100 times faster than the conventional liquid chromatography due to packing of particles in the range of 3-10µm.Modern LC uses very small particles for packing. The small particle size results in more rapid approach to the distribution equilibrium and consequently smaller plate height, so that a given length of column includes large number of plates which makes the column efficient and the peak narrow. But close packing of these small particles reduces the flow rate of the mobile phase through the packed bed (the packing said to develop high back pressure) and in order to achieve a reasonable flow rate it is necessary to apply pressure to the mobile phase. So the designation, put forth as high pressure liquid chromatography. Thus HPLC is having advantages of improved resolution, faster separation, improved accuracy, precision and sensitivity.

HPLC

High performance liquid chromatography (HPLC) is a separation technique utilizing differences in distribution of compounds to two phases, called stationary phase and mobile phase. The stationary phase designates a thin layer created on the surface of fine particles and the mobile phase designates the liquid flowing over the particles. Under a certain dynamic condition, each component in a sample has difference distribution equilibrium depending on the solubility in the phase and or molecule size. As a result, components move at different speed over the stationary phase and thereby separated from each other. The column is a stainless steel or resin tube, which is packed with spherical solid particles. Mobile phase is constantly fed into the column inlet at a constant rate by a liquid pump. A sample is injected from the sample injector, located near the column inlet. The injected sample enters the column with mobile phase and the components in the sample migrates through it, passing between the stationary and mobile phase. Compound move in the column only when it is in mobile phase. Compounds that tend to be distributed in the stationary phase migrate slower. In this way,each component is separated on the column and sequentially elute from the outlet. Each component eluting from the column is detected by a detector to the outlet of the column. When the separation process is monitored by the recorders starting at the time of sample is injected, a graph is obtained. This graph is called chromatogram. The time required for a compound to elute (called retention time) and the relationship between compound concentration (amount) and peak area depend on the characteristic of the compound. Retention is therefore used as an index for qualitative determination and peak surface area as index for quantitative determination. There are two modes of elution process

MATERIALS AND METHODS

Palbociclib-Sura labs, Water and Methanol for HPLC-LICHROSOLV (MERCK), Acetonitrile for HPLC-Merck, Potassium Dihydrogen Phosphate-Finar Chemicals.

HPLC method development

Preparation of Standard Solution: Accurately weigh and transfer 10 mg of Palbociclib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 2ml of the above Palbociclib stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure: Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.
Mobile Phase Optimization: Initially the mobile phase tried was methanol: Water and ACN: Water with varying proportions. Finally, the mobile phase was optimized to Acetonitrile: Phosphate Buffer (25:75% v/v) respectively.
Optimization of Column: The method was performed with various C18 columns like Symmetry, Zodiac and Xterra. Phenomenex Luna C18 (4.6mm x 150mm, 5µm) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Optimized chromatographic conditions:

Instrument used	:	Waters HPLC with auto sampler and PDA 996 detector model.
Temperature	:	Ambient
Column	:	Phenomenex Luna C18 (4.6mm x 150mm, 5µm)
Mobile phase	:	Acetonitrile: Phosphate Buffer (pH-2.8) (25:75% v/v)
Flow rate	:	1.0mL/min
Wavelength	:	220 nm
Injection volume	:	10 µl
Run time	:	8 minutes

Preparation of mobile phase
Preparation of mobile phase: Accurately measured 250 ml of Acetonitrile (25%) and 750 ml (75%) Phosphate Buffer (pH-2.8) were mixed and degassed in a digital ultra sonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.
Diluent Preparation: The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION

Optimized Chromatogram (Standard)

Column	:	Phenomenex Luna C18 (4.6mm x 150mm, 5µm)
Column temperature	:	Ambient
Wavelength	:	220 nm
Mobile phase ratio	:	Acetonitrile: Phosphate Buffer (Ph-2.8) (25:75% v/v)
Flow rate	:	1.0mL/min
Injection volume	:	10 µl
Run time	:	8 minutes

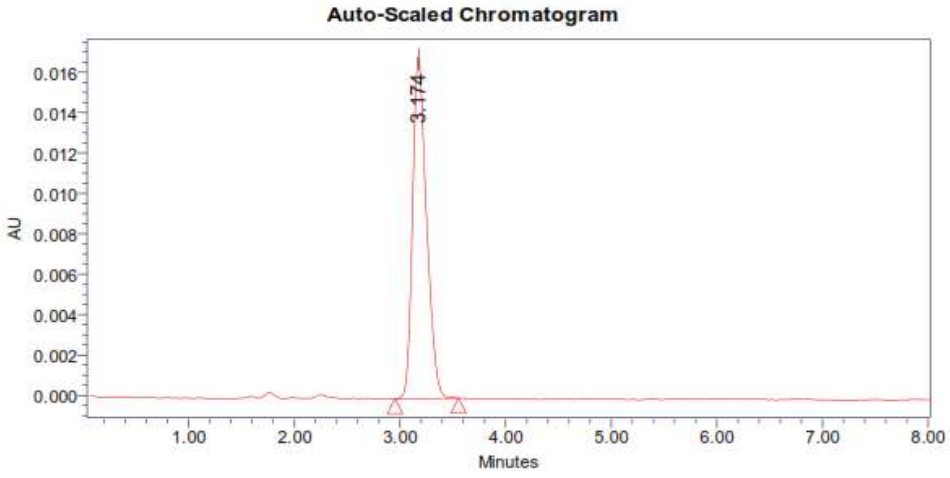

Fig 1: Optimized Chromatogram (Standard)

Table 1: Peak results for Optimized Chromatogram (Standard)

S.No.	Peak name	R_t	Area	Height	USP Tailing	USP plate count
1	Palbociclib	3.174	856985	69854	1.25	8547

This trial shows proper plate count, peak and baseline in the chromatogram. It's Pass the all system suitability parameters. So, it's optimized chromatogram.

Optimized Chromatogram (Sample)

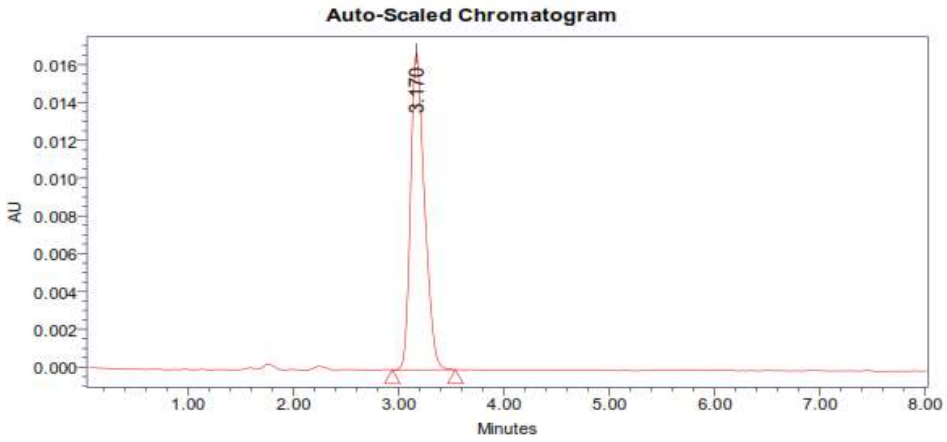

Fig 2: Optimized Chromatogram (Sample)

Table 2: Results of Optimized Chromatogram (Sample)

S.No.	Name	Retention time (min)	Area (µV sec)	Height (µV)	USP tailing	USP plate count
1	Palbociclib	3.170	865845	69857	1.26	8659

- *Theoretical plates must be not less than 2000.*
- *Tailing factor must be not less than 0.9 and not more than 2.*
- *It was found from above data that all the system suitability parameters for developed method were within the limit.*

System suitability

Table 3: Results of system suitability for Palbociclib

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Palbociclib	3.146	856985	69854	8569	1.26
2	Palbociclib	3.123	856857	68954	8547	1.25
3	Palbociclib	3.192	857894	68975	8596	1.25
4	Palbociclib	3.164	857468	69854	8541	1.26
5	Palbociclib	3.181	854785	69856	8616	1.25
Mean			856797.8			
Std. Dev.			1197.992			
% RSD			0.139822			

- *%RSD of five different sample solutions should not more than 2.*
- *The %RSD obtained is within the limit, hence the method is suitable.*

Assay (Standard)

Table 4: Results of Assay (Standard) for Palbociclib

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Palbociclib	3.170	866854	70152	8659	1.26
2	Palbociclib	3.174	868478	69987	8657	1.27
3	Palbociclib	3.170	865987	70154	8654	1.26

4	Palbociclib	3.157	865896	69985	8659	1.27
5	Palbociclib	3.153	859864	69587	8674	1.27
Mean			865415.8			
Std. Dev.			3272.034			
% RSD			0.378088			

- *%RSD of five different sample solutions should not more than 2.*
- *The %RSD obtained is within the limit, hence the method is suitable.*

Assay (Sample)

Table 5: Peak results for Assay sample

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Palbociclib	3.155	875845	70025	1.28	8659	1
2	Palbociclib	3.155	876584	70066	1.27	8696	2
3	Palbociclib	3.155	874598	69989	1.28	8785	3

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Palbociclib in pharmaceutical dosage form was found to be 99.87%.

Linearity

Chromatographic data for linearity study

Data for Linearity of Palbociclib

Concentration µg/ml	Average Peak Area
10	442986
15	652547
20	856985
25	1063654
30	1268475

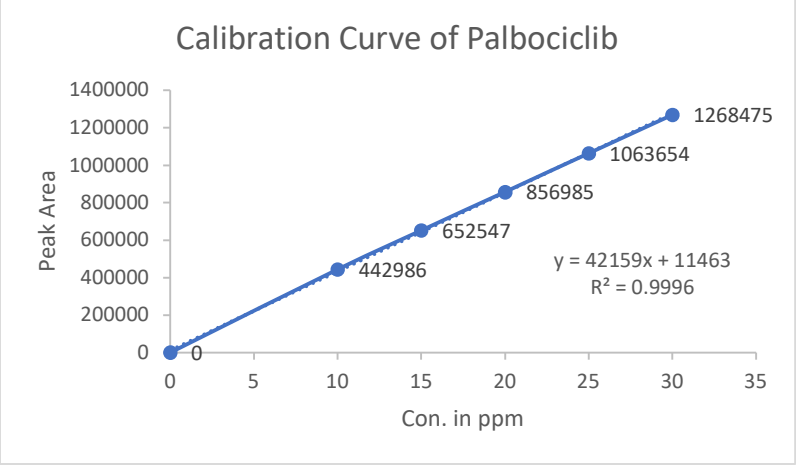

Fig 3: Calibration Curve of Palbociclib

Precision
Repeatability

Table 6: Results of method precision for Palbociclib:

S. No.	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Palbociclib	3.165	856985	69856	8569	1.26
2	Palbociclib	3.163	856898	69845	8597	1.25
3	Palbociclib	3.158	856789	69865	8589	1.26
4	Palbociclib	3.167	859854	69874	8569	1.25
5	Palbociclib	3.171	854789	69798	8564	1.26
6	Palbociclib	3.167	856978	69859	8599	1.25
Mean			857048.8			
Std.dev			1617.106			
%RSD			0.188683			

- *%RSD for sample should be NMT 2.*
- *The %RSD for the standard solution is below 1, which is within the limits hence method is precise.*

Intermediate precision
Analyst 1

Table 7: Results of ruggedness for Palbociclib

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Palbociclib	3.158	865845	70023	8659	1.27
2	Palbociclib	3.163	864356	70015	8667	1.27
3	Palbociclib	3.167	867584	69989	8654	1.28
4	Palbociclib	3.165	865987	70114	8645	1.28
5	Palbociclib	3.171	865975	69985	8635	1.27
6	Palbociclib	3.171	865982	69998	8695	1.28
Mean			865954.8			
Std. Dev.			1022.223			
% RSD			0.118046			

- *%RSD of Six different sample solutions should not more than 2.*

Analyst 2

Table 8: Results of Intermediate precision Analyst 2 for Palbociclib

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Palbociclib	3.173	878548	70254	8758	1.26
2	Palbociclib	3.134	874598	70265	8798	1.27
3	Palbociclib	3.161	874589	69989	8742	1.26
4	Palbociclib	3.174	875984	70145	8759	1.26
5	Palbociclib	3.199	875981	70158	8746	1.27
6	Palbociclib	3.199	875984	69998	8796	1.27
Mean			875947.3			
Std. Dev.			1444.511			
% RSD			0.164908			

- %RSD of Six different sample solutions should not more than 2.

Accuracy

Table 9: The accuracy results for Palbociclib

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	429549.7	10	9.916	99.16%	99.68%
100%	856189.3	20	20.036	100.18%	

150%	1272534	30	29.912	99.706%

- *The percentage recovery was found to be within the limit (98-102%).*

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Robustness

Table 10: Results for robustness

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	856985	3.174	8547	1.25
Less Flow rate of 0.9 mL/min	841542	3.488	8256	1.23
More Flow rate of 1.1 mL/min	812546	2.877	8146	1.20
Less organic phase	802654	4.705	8365	1.16
More organic phase	826549	2.090	8154	1.14

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

CONCLUSION

Hence the proposed method was found to be rapid, accurate, precise, specific, robust and economical. The mobile phase is simple to prepare and economical. The method shows non-interference of formulation excipients in the estimation. This method is also having an advantage that the retention time of the Palbociclib is below 5 min and the drug can be assayed with the short time. Thus, the method is not time consuming and can be used in laboratories for the routine analysis of single and combination drugs.

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REFERENCES

1. Merit.W, Dean.S, Instrumental method of analysis, 7th edition, 75-83.
2. Sharma.B.K, Instrumental method of chemical analysis, 1994, 18th edition, 3-12.
3. Gaten.W.E, Instrumental method of chemical analysis, 5th edition, 33-52.
4. Beckett.A.H, Stenlake J.B, Practical pharmaceutical chemistry, 4th edition, 249-255.
5. Gorog.S, Ultraviolet- visible Spectrophotometry in pharma analysis, 135-149.
6. Kalsi P.S, Spectroscopy of organic compounds, 16-24.
7. Srivastava A.K, Jain P.C, Chemical analysis- An instrumental approach, 204- 215.
8. Parimoo.P, Pharmaceutical analysis, 145-170, 294-301.
9. Clarke.S, Analysis of drugs and poisons, 3rd edition, 500-510.
10. Ohannesian.L, Streeter J.A, Drugs and pharmaceutical sciences, Hand book of pharmaceutical analysis, 130- 149.
11. Pharmacopoeia of India, appendix-4, vol.2, controller of publications, New Delhi,1996, A-66.
12. Sethi P.P, Quantitative analysis of drugs in pharmaceutical formulation 2nd edition33-37.
13. Sethi P.D, Qualitative analysis of drugs in pharmaceutical formulations, 3rd edition,182-184.
14. Pharmainfo. Net, 2005-2008.
15. www.Pharmaarticles. Net/exclusive/technical/Basic-principles- of- hptlc.html.