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Simultaneous Estimation of Ribociclib and Palbociclib in Bulk Samples by Reverse Phase High Performance Liquid Chromatography

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Abstract

This work concerns with the development and validation o f a simple, specific and cost effective RP-HPLC method for simultaneous estimation of Ribociclib and Palbociclib in bulk samples. Chromatography was carried on Kromasil C18 column with mobile phase comprising of 20mM Phosphate buffer (pH-5): Methanol: Acetonitrile (40:30:30, v/v/v). The flow rate was adjusted to 1ml/min with PDA detection at 260 nm. The retention times of Ribociclib and Palbociclib were found to be 3.47min and 4.48 min, respectively and other replicate standard system suitability parameters are within the limit and uniform. The different analytical parameters such as accuracy, precision, linearity, robustness, limit of detection (LOD), limit of quantification (LOQ) were determined according to the International Conference on Harmonization (ICH) Q2B guidelines. The detector response was linear in the range of 5-25 mcg/ml. and 1-5 mcg/ml for Ribociclib and Palbociclib respectively.

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INTRODUCTION

Breast cancer is the most common female tumor type and accounts for the leading cancer mortality in women worldwide. In spite of the great achievement in diagnosis and treatment, breast cancer remains a significant global burden. Among the emerging therapies, cyclin-dependent kinase 4/6 (CDK4/6) inhibitors are the most attractive findings [1-2].

CDK4/6 coordinates the cell cycle progression by reversible combination with cyclin D, and the bipartite complex of these elements phosphorylates pivotal tumor suppressors and transcription factors, contributing to cell cycle progression [3-5].

The essential roles of CDK4/6 in cell cycle regulation make them effective targets for cancer therapeutic intervention, especially in breast cancer. The orally highly selective inhibitors of CDK4/6 are currently under active investigation, including palbociclib, ribociclib, and abemaciclib.

Among these, palbociclib and ribociclib remarkably prolonged the progression-free survival (PFS) in combination with letrozole for patients with ER-positive/HER2-negative advanced breast cancer, and have gained accelerated approval from Food and Drug Administration (FDA) as initial endocrine based therapy for these patients [6-7].

Palbociclib (Fig.1.0) is a drug for the treatment of ER-positive and HER2 - negative breast cancer developed by Pfizer.

It is a selective inhibitor of the cyclin-dependent kinases CDK4 and CDK6. The drug was reviewed and approved under the Food and Drug Administration's (FDA) accelerated Priority Review and Breakthrough Therapy designation programs on February 3, 2015 as a treatment (in combination with letrozole) for patients with estrogen receptor positive advanced breast cancer. This was an accelerated approval. [4] In March 2017, the FDA granted regular approval to palbociclib for HER2 negative breast cancer, in combination with an aromatase inhibitor [8-10].

The molecular formula for palbociclib is C24H29N7O2. The molecular weight is 447.54 daltons. The chemical name is 6-acetyl-8-cyclopentyl-5-methyl-2-{[5-(piperazin-1-yl) pyridin-2 yl] amino} pyrido [2, 3-d] pyrimidin-7(8H)-one. Palbociclib is a yellow to orange powder with pKa of 7.4 (the secondary piperazine nitrogen) and 3.9 (the pyridine nitrogen). At or below pH 4, palbociclib behaves as a high-solubility compound. Above pH 4, the solubility of the drug substance reduces significantly [11].

Ribociclib (Fig.1.0) is an inhibitor of cyclin D1/CDK4 and CDK6, and is being studied as a treatment for drug-resistant cancers. It was developed by Novartis and Astex Pharmaceuticals. It was approved by the US FDA in March 2017 for use in combination with an aromatase inhibitor to treat some metastatic breast cancers [12-14]. The chemical name of ribociclib succinate is: Butanedioic acid—7-cyclopentyl-N, N-dimethyl-2-{[5-(piperazin-1-yl) pyridin-2-yl] amino}-7H-pyrrolo [2, 3-d] pyrimidine-6-carboxamide (1/1). Ribociclib succinate is a light yellow to yellowish brown crystalline powder. The molecular formula for ribociclib succinate is C23H30N8O·C4H6O4 and the molecular weight is 552.64 g/mol [15-18].

As per literature survey, no analytical method has been reported for simultaneous estimation of Ribociclib and Palbociclib by RP-HPLC. The aim of present research work to develop and validate a novel, simple, accurate, sensitive, reproducible, economical analytical method to estimate Ribociclib and Palbociclib in bulk samples by RP-HPLC.

MATERIALS AND METHODS

Chemicals Reagents

Working standards of pharmaceutical grade Ribociclib (RC) and Palbociclib (PC) were obtained as generous gifts from Dr.Reddy's laboratories (Hyderabad, AP, India) used as such without further purification. The pharmaceutical dosage form used in the study was Zifi-AZ. Methanol (HPLC grade), OPA (AR grade) purchased from Merck specialities Pvt.ltd (Mumbai, India) and double distilled water used for analysis.

Instrumentation and chromatographic condition

Chromatography was carried out on kromasil C18 column with mobile phase containing of 20mM Phosphate buffer (pH-5): Methanol: Acetonitrile (40:30:30, v/v/v). The flow rate was adjusted to 1ml / min with PDA detection at 260 nm.

Preparation of standard solution

Standard stock solutions of pure drugs were prepared separately by dissolving 10 mg of Ribociclib in 10ml Methanol and 8mg of Palbociclib in 10ml Methanol to get concentrations 1mg/ml and 0.8 mg /ml respectively.

METHOD VALIDATION

The developed method was validated with different analytical parameters such as accuracy, precision, linearity, limit of detection, limit of quantification and robustness according to the international conference on harmonization (ICH) Q2B guidelines [19].

Specificity

The selectivity of an analytical method is its ability to measure accurately and specifically the analyte of interest in the presence of components that may be expected to be present in the sample matrix.

If an analytical procedure is able to separate and resolve the various components of a mixture and detect the analyte qualitatively the method is called selective. It has been observed that there were no peaks of diluents and placebo at main peaks.

Hence, the chromatographic system used for the estimation of Ribociclib and Palbociclib was very selective and specific. Specificity studies indicating that the excipients did not interfere with the analysis. The standard solution shown symmetric peak with retention times of 3.47 min for Ribociclib and 4.48 min for Palbociclib. The results were depicted in Fig.2.0.

System suitability

System suitability tests were carried out on freshly prepared standard stock solution of Ribociclib and Palbociclib. Equal volume of Standard concentration was mixed well. From the prepared solution 20 µL of the sample was injected into HPLC system and the results obtained were used to express the system suitability of the developed method. The results were depicted in Table.1.0 and Fig.3.0.

Linearity & Range

A series of standard concentrations were prepared from 50 % to 150 % of the target concentration of RC and PC. Linearity was assessed by performing single

measurement at several analytes concentration varying quantities of stock standard solution diluted with the mobile phase to give a concentration of 5, 10, 15, 20, 25 µg/mL of RC and 1, 2, 3, 4, 5 µg/mL of PC. Injection was made at intervals of 10.0 min. Linearity of RC was found to be exist between 5-25 µg/mL and for PC was 1-5 µg/mL. The chromatograms were recorded and linearity graph was plotted by using peak area of drug against respective concentrations to obtain the linearity range. The results were depicted in Table.2.0 and Fig.4.0 to 5.0.

Precision

Precision of these methods was checked by analyzing the samples at three different time intervals of the same day (intraday precision) as well as on different days (interday precision). Results were shown in Table-3.0 & 4.0.

LOD and LOQ

Limit of Detection (LOD) and Limit of quantification (LOQ) were calculated by using the values of slopes and intercepts of the calibration curves for both the drugs. LOD and LOQ values for RC were found to be 0.89 and 2.99 mcg/ml and for PC 0.71 and 2.39 mcg/ml.

Robustness

Method robustness was determined by the small changes in chromatographic conditions like as ±0.2ml flow rate and ±5ºc temperature and inject the sample observe the result there were no marked changes compare to other analysis. Results of the Robustness were shown in Table-5.0.

Ruggedness

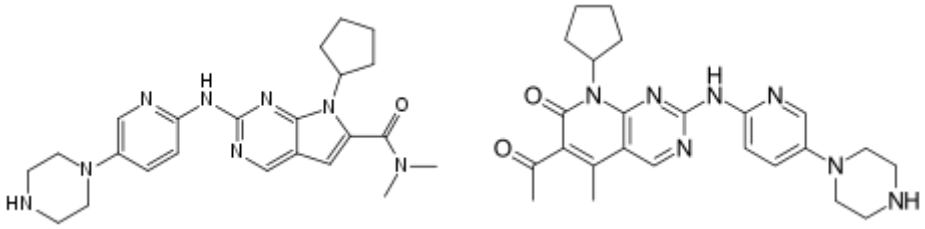
This is to prove the lack of influence of operational and environmental variables of the test results by using the method. Ruggedness is a measure of reproducibility of test results under the variation in conditions normally expected from system to system and from analyst to analyst. It was carried out by using a test sample assay method with six replicates using different analyst, column and system. The results were depicted in Table. No- 6.0.

Solution Stability

Solution stability was assed using standard and test stock solutions. These stocks were prepared and stored at room temperature and refrigerated conditions (2-8°C) for 36 h and % differences was calculated. The results were depicted in Table. No.7.0 to 10.0.

Filter validation

A study was conducted to determine the effect of filter on the assay, dissolution and impurities. Test solution was prepared as per the test method. Some portion of the above solution was filtered through three different filters namely 0.45µm PVDF filter, 0.45µm PTFE and 0.45µm Nylon filter and some portion was centrifuged and injected into the HPLC system. The % difference values between centrifuged and filtered sample were calculated. The results were depicted in Table. No.11.0.



A **B**

Fig.1.0: Chemical structure of A) Ribociclib (RC) and B) Palbociclib (PC)

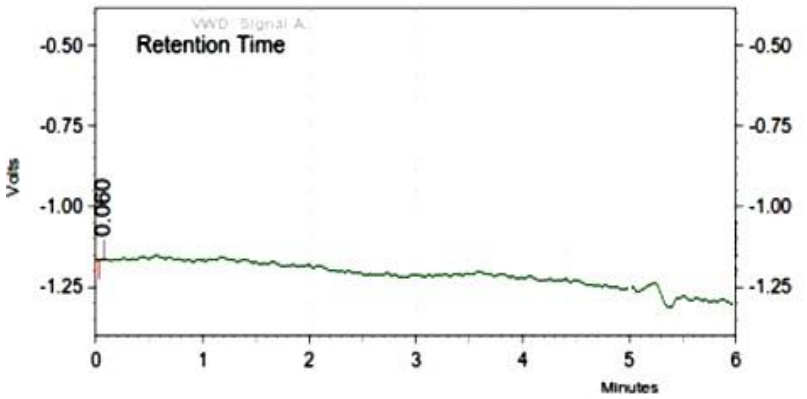


Fig.2.0: Blank chromatogram

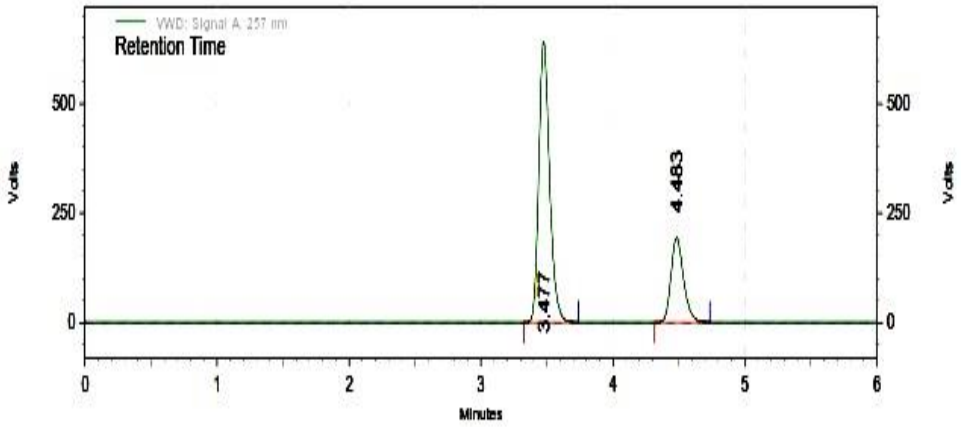


Fig.3.0: Standard chromatogram of Ribociclib (RC) and Palbociclib (PC)

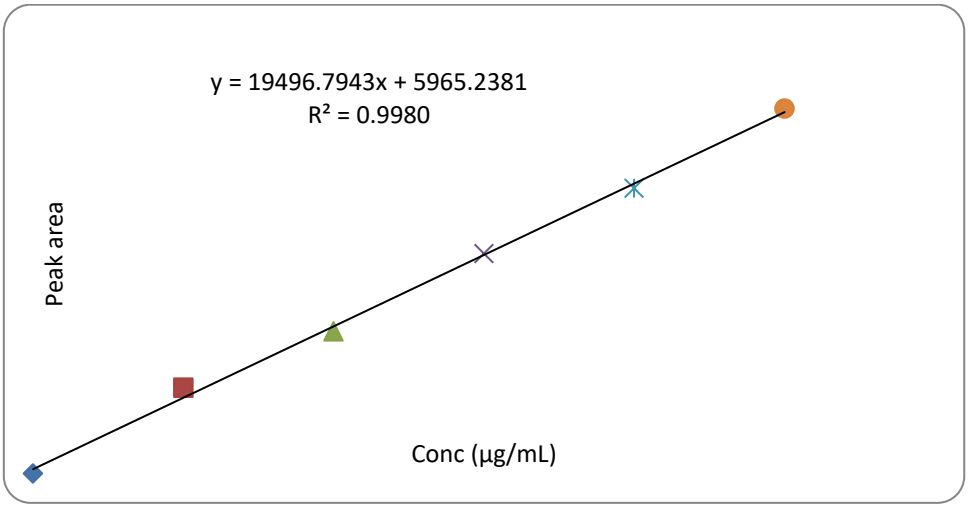


Fig.4.0: Linearity of Ribociclib (RC)

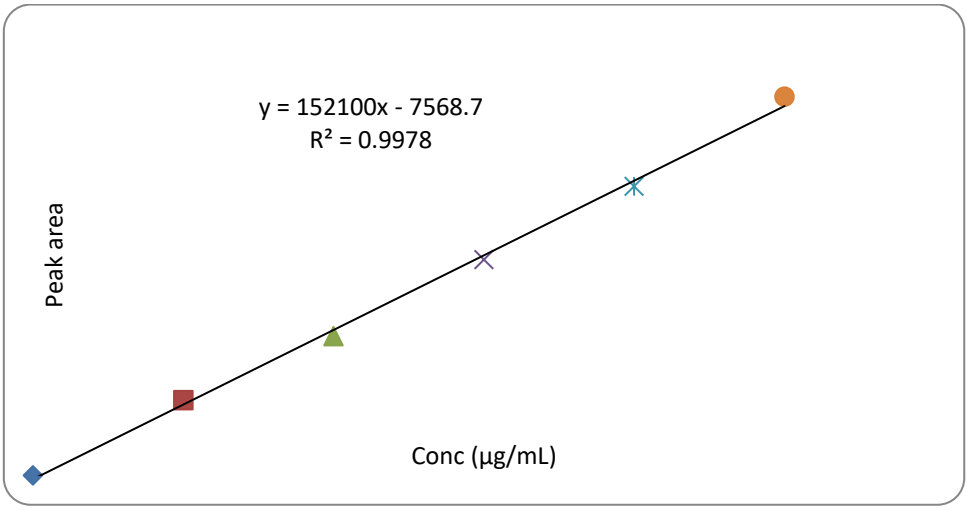


Fig.5.0: Linearity of Palbociclib (PC)

Table. 1.0: System suitability results of Ribociclib (RC) and Palbociclib (PC)

Retention Time	Ribociclib	3.47 min
	Palbociclib	4.48 min
Peak Area	Ribociclib	439283
	Palbociclib	299938
Theoretical plates	Ribociclib	85697
	Palbociclib	95771
Tailing Factor	Ribociclib	1.21
	Palbociclib	1.42
Resolution	Ribociclib	-
	Palbociclib	3.9

Table. 2.0: Linearity data of Ribociclib (RC) and Palbociclib (PC)

S.NO	Concentration µg/mL	Area of PC	Concentration µg/mL	Area of RC
1	1	152806	5	117048
2	2	283276	10	194016
3	3	439273	15	299946
4	4	588873	20	389026
5	5	771861	25	498015
Concentration range		1-5µg/mL	5-25µg/mL	
Slope (m)		152100	19496	
Correlation coefficient		0.9978	0.9980	

Table. 3.0: Intraday precision data for Ribociclib (RC) and Palbociclib (PC)

Sample. No	Area of RC	Area of PC
1.	452283	293846
2.	449150	293546
3.	449624	293357
4.	459578	294621
5.	449634	293107
6.	449458	283762
Mean	451621	292040
SD	4061.35	4088.75
%RSD	0.90	1.40

Table. 4.0: Interday precision data for Ribociclib (RC) and Palbociclib (PC)

Sample. No	Area of RC	Area of PC
1.	453273	293846
2.	456537	294776
3.	459273	288446
4.	456537	292446
5.	453724	292776
6.	451724	293846
Mean	455178	292689
SD	2761.74	2240.65
%RSD	0.61	0.77

Table.5.0. Robustness of Ribociclib (RC) and Palbociclib (PC)

S. No	Parameter	Condition	RC Area (n=3)	RC % change	PC Area (n=3)	PC % change
1	Standard	Standard conditions	4392730.000		2999460.000	
2	Mobile Phase composition (±2%)	20mM Phosphate buffer (pH-5): Methanol: Acetonitrile (44:28:28, v/v/v)	4332731.366		2997260.073	
		20mM Phosphate buffer (pH-5): Methanol: Acetonitrile (36:32:32, v/v/v)	439172	-1.361	299826	-0.033
3	Mobile phase pH (±0.2units)	4.8	439270	-0.022	2979350.631	
		5.2	4392610.002		2949221.011	
4	Wavelength (nm) (±2%)	258	4372620.455		2949210.000	
		262	4326211.061		296346	-0.483
5	Flow rate (mL) ± 0.2 mL	1.2	438262	-1.304	2949460.472	
		0.8	4332421.145		295642	-0.236

Table.6.0.Ruggedness of Ribociclib (RC) and Palbociclib (PC)

Sr. No.	RC (%Accuracy)			PC (%Accuracy)		
	SET I	SET II	SET III	SET I	SET II	SET III
1	99.50	101.60	101.76	99.70	98.60	98.40
2	101.90	101.40	99.60	98.70	98.20	99.70
3	99.60	99.50	101.90	98.10	99.77	99.88
4	100.89	100.60	101.40	98.10	99.24	99.60
5	101.40	99.90	101.60	99.20	99.65	99.20
6	101.60	98.92	99.50	99.60	98.54	98.00
Average	100.82	100.32	100.96	98.90	99.00	99.13
SD	1.03	1.07	1.11	0.71	0.64	0.76
% RSD	1.03	1.06	1.09	0.72	0.65	0.77
Overall Average	100.70			99.01		
Overall % RSD	1.06			0.71		

SET – I: Variability due to HPLC system
SET – II: Variability due to HPLC column
SET – III: Variability due to analyst

Table.7.0. Solution Stability of Ribociclib (RC) at room temperature

Time	Standard stock			Test stock		
	Fresh	Stability Stock	% Diff.	Fresh	Stability Stock	% Diff.
Initial	439273	439272	NA	427182	427180	NA
6h	437263	434273	0.684	427182	421410	1.351
12h	435252	435273	-0.005	437154	437150	0.001
20h	439273	439273	0.000	443551	437150	1.443
26h	439273	446253	-1.589	427182	423230	0.925
30h	432733	439252	-1.506	427182	427150	0.007
36h	439233	436263	0.676	407182	412210	-1.235

Table.8.0. Solution Stability of Palbociclib (PC) at room temperature

Time	Standard stock			Test stock		
	Fresh	Stability Stock	% Diff.	Fresh	Stability Stock	% Diff.
Initial	243846	243842	NA	226755	226752	NA
6h	243241	243212	0.012	246755	246747	0.003
12h	231354	231322	0.014	246643	245747	0.363
20h	223843	223442	0.179	246721	245268	0.589
26h	243821	243211	0.250	246743	242322	1.792
30h	243846	243242	0.248	246421	245442	0.397
36h	243456	240222	1.328	246721	245762	0.389

Table.9.0. Solution Stability of Ribociclib (RC) at refrigerated temperature

Time	Standard stock			Test stock		
	Fresh	Stability Stock	% Diff.	Fresh	Stability Stock	% Diff.
Initial	435123	435122	NA	424182	424181	NA
6h	433163	431243	0.443	497182	491410	1.161
12h	441132	445225	-0.928	432154	435150	-0.693
20h	438273	439243	-0.221	446551	444150	0.538
26h	436273	444253	-1.829	420182	420230	-0.011
30h	432723	438242	-1.275	427142	427150	-0.002
36h	437233	436243	0.226	405182	411210	-1.488

Table.10. Solution Stability of Palbociclib (PC) at refrigerated temperature

Time	Standard stock			Test stock		
	Fresh	Stability Stock	% Diff.	Fresh	Stability Stock	% Diff.
Initial	233744	233743	NA	226755	226751	NA
6h	242231	243111	-0.363	246723	246712	0.004
12h	231323	231311	0.005	246643	245612	0.418
20h	223821	223423	0.178	246711	245247	0.593
26h	243821	243211	0.250	245345	242145	1.304
30h	243836	243242	0.244	246410	245264	0.465
36h	243441	240231	1.319	246252	245756	0.201

Table.11.0. Filter Interference Results for Ribociclib (RC) and Palbociclib (PC)

RC				
Filtration Method	**Centrifuged**	**Nylon**	**PTFE**	**PVDF**
Area (Inj. 1)	435273	439092	428262	438173
Area (Inj. 2)	436936	434242	435273	435253
Avg. Area	436104	436667	431768	436713
% Difference		-0.129	1.122	-1.145
PC				
Filtration Method	**Centrifuged**	**Nylon**	**PTFE**	**PVDF**
Area (Inj. 1)	297962	297833	298635	299546
Area (Inj. 2)	299946	297846	299835	288925
Avg. Area	298954	297839.5	299235	294236
% Difference		0.373	-0.469	1.671

RESULTS AND DISCUSSIONS

In this RP-HPLC method, the conditions were optimized to obtain an adequate separation of eluted compounds. Initially, various mobile phase compositions were tried, to separate analytes. The mobile phase and flow rate selection was based on peak parameters (height, tailing, theoretical plates, capacity or symmetry factor) run time and resolution.

The system with 20mM Phosphate buffer (pH-5): Methanol: Acetonitrile (40:30:30, v/v/v) at flow rate of 1.0 mL/min was found to be robust method and the developed method was validated as per the ICH guidelines [19].

A suitability test was applied to various system suitability parameters and the results obtained were within acceptable limits of tailing factor ≤ 2.0 and theoretical plates > 2000. The calibration curve was constructed with series of concentration in the range of 5-25µg/mL and 1-5 µg/mL for Ribociclib and Palbociclib. This concluded that the method was linear throughout the range selected.

Specificity was studied for the quantification of excipients in the tablet dosage form of Ribociclib and Palbociclib. From the results it was indicated that none of impurities were interfere at analytes retention time. Hence the developed method was specific.

The precision of the method was measured in terms of repeatability, which was determined by sufficient number of aliquots of a homogenous sample with in the day (intraday) and next consequent three days for inter day precision. For each cases % RSD was calculated and results were the acceptable limits. The low values of RSD indicate that the method was precise.

Robustness test was carried out by small variation in the chromatographic conditions and % change was calculated. The % change in the results was calculated and it was found robust as % change was below 2.0 %.

A signal-to-noise ratio 2:1 is generally considered acceptable for estimating the detection limit. LOD is found to be 0.898 µg/mL for RC and 0.71 µg/mL for PC and LOQ is found to be 2.99 µg/mL for RC and 2.39 µg/mL for PC.

Sample and standard solution are stable at 5°C for 36 h as the % difference in the area was found to be less than 2.0%. Filter interference was done on three types of 0.45µ filters (Nylon, PVDF, PTFE) and the %difference was found to be below 2.0 % for sample solutions and standard solutions calculated against centrifuged samples and standard.

CONCLUSION

The new HPLC method developed and validated for simultaneous estimation of Ribociclib and Palbociclib in bulk samples and assured the satisfactory precision and accuracy and also determining lower concentration of each drug in its solid combined dosage form by RP-HPLC method. The method was found to be simple, accurate, precise, economical and rapid and they can be applied for routine analysis in laboratories and is suitable for the quality control of the raw materials, formulations, dissolution studies and can be employed for bioequivalence studies for the same formulation.

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CONFLICT OF INTEREST

Authors declare that, there is no conflict of interest.

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