Establishment of a Rapid and Highly Sensitive Reverse-phase High-performance Liquid Chromatography Based Analytical Assay Method for Duvelisib

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ABSTRACT

Background: Duvelisib is an antineoplastic agent that received global approval from the United States Food and Drug Administration in 2018. An extensive literature search revealed that analytical method for the quantification of duvelisib at nanogram level is not available till date. A highly sensitive analytical method is necessary to analyze diversified samples containing trace levels of the analyte such as dissolution samples of sustained release formulation, cross-contamination study samples etc. **Materials and Methods:** Primary aim of this research was to develop a high throughput, accurate, reproducible, and highly sensitive high performance liquid chromatography method for quantification of duvelisib and validate according to the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use and Association of Official Analytical Collaboration guidelines. **Results:** A linear relationship was confirmed over 70 to 5000 ng/mL concentrations with a limit of detection value of 20 ng/mL. The intra and inter-day precision were found to be 0.97 to 1.87% and 1.25 to 1.99%, respectively. **Conclusion:** This is the first time to report a quantitative assay method for duvelisib, which can quantify the analyte even at the nanogram level. This method will be suitable for sample analysis of quality control, stability, cross-contamination, dissolution study of sustained-release formulation and effluent analysis of duvelisib in pharmaceutical industries and research laboratories.

Key words: Duvelisib, HPLC, Analytical assay method, Development, Validation.

INTRODUCTION

Duvelisib (ABBV-954; COPIKTRA; INK-1197; IPI-145; VS-0145) is a orally used drug having potential immunomodulating and antineoplastic activity.[1] It falls under biopharmaceutical classification system (BCS) class IV category with less solubility and permeability.[2] Pharmacologically, it inhibits phosphoinositide 3-kinases (PI3Ks) specifically δ and γ isoforms (PI3Kδ and PI3Kγ), which helps malignant B-cells to grow and survive.[3,4] PI3Ks are known for their crucial contribution towards maintaining cellular homeostasis. The significant involvement of the PI3K signalling is evidenced for modulation of various basic cellular functionalities of B and T cells. Compared to other classes, class I PI3Ks are found as α, β, γ and δ isoforms.[5] Out of these, γ and δ isoforms are only found in haematopoietic cells whereas, widespread expression of α and β isoforms is found in normal cells.[6,7] It is well known that PI3K-α has a significant role in tumor formation whereas, PI3K-β is involved in thrombotic disorders. The remaining two isoforms (PI3K-γ and δ) have profound involvement in the process of inflammation and immune system regulation.[8] Anomalies in the regulation of PI3K signaling pathway are responsible

for development of cancer in humans. PI3K enzyme inhibition has been proved to be an effective strategy in cancer treatment in recent years. Different PI3K inhibitors, namely idelalisib, duvelisib and copanlisib are commercially available in the treatment of cancer.[1,9] Duvelisib is a newer class of molecule to treat hematological malignancies.[2,10] It was marketed by Verastem Oncology as Copiktra for the oral treatment of cancers.[11] Both δ and γ isoforms of PI3K are inhibited by duvelisib. However, it exhibits high selectivity for PI3K-δ, which is 10-fold higher than that of PI3K-γ.[12] Duvelisib received global approval from the United States Food and Drug Administration (USFDA) in September2018.[13] It is recommended against all types of lymphocytic leukaemia after two initial treatments. It received accelerated approval in the USA to treat follicular lymphoma after two initial treatments. It also got fast-track approval to treat follicular lymphoma owing to its overall response rate.[12] It is recommended to administer duvelisib orally with 25 mg dose two times a day. Chemically, duvelisib is ((S)-3-(1-(9H-purin-6-ylamino)ethyl)-8-chloro-2-phenylisoquinolin-1(2H)-one). Duvelisib is available in the market as 15 mg and 25 mg capsules. The major excipients present in the capsule are microcrystalline cellulose, colloidal silicon dioxide, crospovidone, and magnesium stearate.[14]

An extensive literature search confirmed unavailability of analytical techniques for quantitation of duvelisib atsub microgram level. The method reported by Srujani *et al.* for quantification of duvelisib can only quantify the analyte upto a lower limit of 6.25 µg/mL with a linear response.[15] However, the method is not useful to analyze duvelisib at a low concentration. The method we have developed is about 90 times more sensitive than the previous method. Another method reported by Siddesh *et al.* is to simultaneously quantify copanlisib, duvelisib and idelalisib in rat plasma samples.[16] The method can only be used to quantify the drugs in biological samples. Total run time of the method to quantify the analytes is 15 min. However, the method we have reported here is much faster and the total run time (6 min) is less than half of the method reported previously. Similarly, an ultra-performance liquid chromatography- tandem mass spectrometry (UPLC-MS/MS) method reported by Shao *et al.* is also not useful for quantification of duvelisib in analytical samples. The method is to quantitate duvelisib in beagle dog's plasma samples originated from pharmacokinetic studies.[17] Therefore, there is an obvious need for establishment of a sensitive analytical method for duvelisib, which should be available in the public domain. The scope of this study was mainly inclined towards the development

and validation of an analytical method, which can be useful to quantitate duvelisib as a drug substance or pharmaceutical dosage forms.[18] Scientific community keeps on updating analytical methods to make them more simple, reliable, cost-effective, reproducible with a high level of accuracy and precision.[19,20] Primary aim of this study was to develop and validate an accurate, precise, robust, rapid, selective, and highly sensitive high performance liquid chromatography (HPLC) method for quantification of duvelisib at very low concentration. Nanogram level detection is essential to analyze stability and cross-contamination samples in industry. The developed method achieved higher sensitivity against previously reported methods and was fully validated as per the guidelines recommended by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH),[21] and the Association of Official Analytical Collaboration (AOAC).[22]

MATERIALS AND METHODS

Chemicals and reagents

Duvelisib (purity, 99.45%) (Figure 1) was purchased from Renwik Bioinnovations, New Delhi (Batch No: CSN13707-002). HPLC grade acetonitrile (ACN) and methanol (MeOH) were purchased from Thermo Fischer Scientific India Pvt Ltd. Ultra-purified water was collected from Millipore Milli-Q system (Synergy UV) in our laboratory and used throughout the study.

HPLC instrumentation and chromatographic conditions

The reversed phase (RP)-HPLC **(**Agilent 1260 Infinity series) system was assembled with a quaternary pump (DEADP18979), degasser, autosampler (DEADA00334), column oven, and a diode array detector (DEAAX08589). The system was operated by OpenLab software for
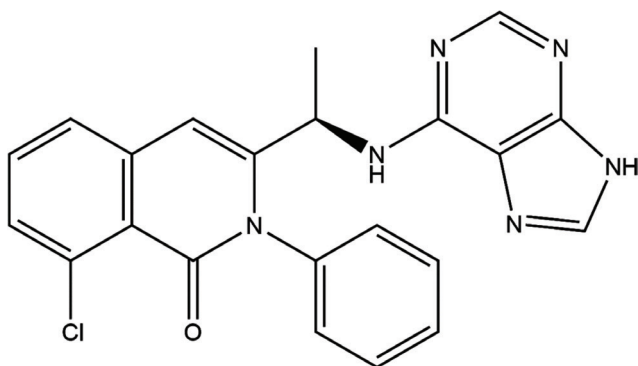

Figure 1: Molecular structure of duvelisib.

controlling the instrument parameters. Separations were carried out on an C_{18} column (250 × 4.6 mm, 5 μm) (Agilent Zorbax Eclipse Plus). The column was kept at 25°C. A mobile phase mixture of 0.1% formic acid in water:ACN (60:40, %v/v) was used at a flow rate of 1 mL/min. The injection volume was 10 μL and ultra violet (UV) detection was performed at 286 nm wavelength. The total run time was 6 min. All standard solutions were prepared by weighing the analyte using a calibrated weighing balance (XS3DU, Mettler Toledo, Switzerland). Identification of duvelisib peak was ensured by comparing the retention time (RT) with standard injections.

Preparation of standard and working solution

Owing to the insolubility of duvelisib in water, ACN and MeOH were utilized as cosolvents. Different ratios of water and organic solvents were prepared and solubility was examined. Duvelisib was found to be less soluble in ACN and ACN-water mixture. However, it was freely soluble in MeOH-water mixture (50:50,%v/v) and it was used as diluent. Finally, the standard solution of duvelisib was prepared in diluent in a 10 mL volumetric flask to obtain a 1000 μg/mL concentration. Working standard solution was generated after dilution of 1 mL of the above solution upto 10 mL to obtain 100 μg/mL concentration. The working solution was suitably diluted with diluent to get the final concentrations of 0.05, 0.07, 0.1, 0.2, 0.5, 1, 2 and 5 μg/mL.

Method validation by RP-HPLC

The developed RP- HPLC method was subjected to validdtion for the different parameters such as limit of detection (LOD), limit of quantitation (LOQ), linearity, accuracy, precision (intra-day and inter day), robustness and specificity.[23] ICH Q2 R1 and AOAC guidelines were followed for setting the acceptance criteria of the validation study.[24]

System suitability

The chromatographic system should be checked for its suitability prior to the analysis to nullify the possibility of any variation caused by instrumental conditions. To ensure this, system suitability test was performed for the chromatographic system. The system suitability was evaluated by injecting six replicates of duvelisib (1 μg/mL). Various system suitability attributes like retention time, theoretical plate, asymmetry and tailing factor were evaluated.

Sensitivity

Sensitivity of the quantification method was established through the prediction of LOD and LOQ. LOD and LOQ are the minimum concentration that can be detected and quantitated, respectively. As per the ICH guideline, LOD and LOQ can be determined by three methods, namely visual evaluation, signal to noise ratio and standard deviation of responses and slope.[21] Here, we have used the standard deviation of responses and slope method to determine the LOD and LOQ.

The linearity levels were selected from 0.05 to 5.0 μg/mL for LOD and LOQ prediction. Seven different linearity concentrations including 0.05, 0.1, 0.2, 0.5, 1.0, 2.0 and 5.0 μg/mL were generated from the working standard solution (100 μg/mL) by serial dilution. A volume of 1 mL was diluted up to 10 mL with diluent (MeOH: water (50:50 %v/v)) mixture to achieve the concentration of 10 μg/mL. Similarly, from 10 μg/mL solution, 5 mL and 2 mL were diluted up to 10 mL to have 5μg/mL and 2 μg/mL levels, respectively. From 10 μg/mL solution, 1 mL was diluted up to 10 mL to get 1μg/mL solution. Similarly, 0.05 μg/mL, 0.1 μg/mL, 0.2 μg/mL and 0.5 μg/mL samples were prepared from 1 μg/mL working standard solution. These solutions were analyzed by the HPLC system and peak areas were noted. From the peak area, standard error, slope, correlation coefficient and intercept were calculated. The LOD and LOQ were predicted from this calculated standard error and slope by using the following formula.[25]

$$LOD = 3.3 \times (\text{standard error of responses/slope})$$

$$LOQ = 10 \times (\text{standard error of responses/slope})$$

To confirm the predicted values, six replicates of 20 ng/mL and 70 ng/mL were injected individually as LOD and LOQ, respectively. From the peak area of the replicate injections, % RSD was calculated.

Linearity

Linearity range was established by preparing seven different solutions of 0.07 to 5 μg/mL of duvelisib covering 7% (0.07 μg/mL), 10% (0.1 μg/mL), 20% (0.2 μg/mL), 50% (0.5 μg/mL), 100% (1.0 μg/mL), 200% (2.0 μg/mL) and 500% (5.0 μg/mL) of the target concentration. The graph was plotted between peak area on Y-axis versus their respective concentration (μg/mL) on the X-axis. The regression equation was calculated from the plot.

Accuracy

Accuracy (closeness to true values) of the quantification method was determined from % recovery of duvelisib at three different levels of 80%, 100% and 120% of target

concentration by injecting in triplicates. The mean percent recovery was calculated using the following formula.[26]

$$Recovery\ (\%) = (Recovered\ concentration/Injected\ concentration) \times 100$$

Precision

The method precision (closeness of agreements measured by changing the different instances) was examined through intra-day and inter-day analysis of samples.[27] For intra-day analysis, three replicates of the samples (n = 3) at each level of 80%, 100% and 120% of target concentration (1 µg/mL) were analysed on the same day. Whereas for inter-day analysis, samples were analysed on the second day by using the same chromatographic conditions and same sample concentrations that are freshly prepared. Peak area, % recovery and % RSD were calculated to determine method precision.

Robustness

Robustness or ability of the entire methodology to remain unaltered by a small and intentional change in different parameters was checked after a change in mobile phase composition of ACN:0.1% formic acid from 40:60 % to 38:62 % and 42:58 % v/v, and flow rate of 1 to 0.9 and 1.1 mL/min. Further, the effect on the accuracy of the result after varying the method conditions was analyzed. At each variation, 80%, 100% and 120% of target concentration solution was injected in triplicates (n = 3). The robustness of the method was determined from the % RSD of peak area and mean % accuracy.

Specificity

Specificity of the technique was determined to examine capability of the developed analytical method in separating the analyte from a complex mixture. It was determined by evaluating excipients interference in duvelisib quantification. Duvelisib is available in capsule dosage form under the brand name COPIKTRA. This capsule contains duvelisib along with its excipients.

In the specificity study, a comparative analysis between blank, blank with excipients, blank with excipients spiked with drug and only drug chromatograms was conducted. The diluent (MeOH: water, 50:50, %v/v) mixture was used as blank. The blank with excipients solution was prepared by dissolving 25 mg of each of the above-mentioned excipients in 10 mL of diluent. The solution of drug spiked in a blank with excipients

was prepared by adding 10 mg of duvelisib and 25 mg of each excipient in a volumetric flask and dissolving in 10 mL of diluent. These two solutions were sonicated for 10 min for complete solubilization and then centrifuged at 25°C at 10,000 rpm for 10 min. After centrifugation, supernatant was withdrawn and further diluted to have 1 µg/mL solution of duvelisib. A separate 1 µg/mL sample solution of duvelisib was prepared after suitable serial dilution from the stock solution. All four sample solutions (blank, blank with excipients, blank with excipients spiked with drug and only drug) were injected into the HPLC system at the same chromatographic conditions. The chromatograms were analysed and compared to verify any possible interference due to the excipients in drug quantification.

RESULTS AND DISCUSSION

Optimization of chromatographic condition

Composition of the mobile phases was varied to achieve an optimum chromatographic method. Different types of modifiers including formic acid, ammonium acetate and ammonium formate were employed for optimizing the chromatographic behavior of the analyte. However, elution of duvelisib with a good peak shape was obtained with 0.1% formic acid (FA) in water.[28] We optimized the method with 0.1% FA in water as it was easy to prepare without complex weighing and pH adjustment requirements. Amongst the various ACN:0.1% FA in water ratios (15:85, 30:70 and 40:60), 40:60 % ratio showed a good peak shape of duvelisib. Isocratic elution at 1 mL/min flow was shown to have optimum retention of duvelisib in the stationary phase used in this method. The detection wavelength selected for the estimation of duvelisib was 286 nm. The optimum chromatographic condition with good peak separation was achieved using C_{18} column (250 × 4.6 mm, 5 µm) (Agilent Zorbax Eclipse Plus). The sample was injected at a volume of 10 µL for each run. The RT for duvelisib was observed at 4.16 min.
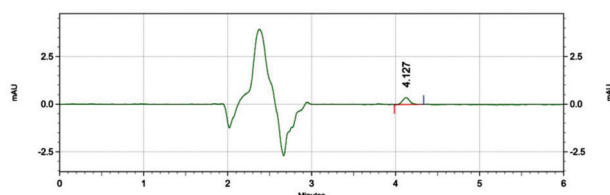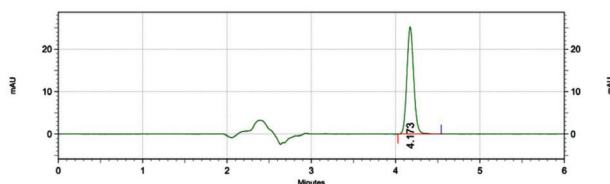
Method validation

System suitability

To determine the system suitability, various chromatographic attributes were taken into consideration before the analysis. There was no significant variation observed in any of the system suitability parameters of retention time, theoretical plate, asymmetry and tailing factor.

Table 1: Prediction of DL and QL.	
Concentration (µg/mL)	**Area**
0.05	3142
0.1	6038
0.2	11047
0.5	28492
1.0	56074
2.0	110575
5.0	277349
Standard error	394.57
Slope	55211.84
Correlation coefficient	0.999
Intercept	389.15
Predicted DL(µg/mL)	0.02
Predicted QL(µg/mL)	0.07

Table 2: Results of accuracy study.				
Level	**Back calculated conc. (µg/mL)**	**SD**	**RSD (%)**	**Recovery (%)**
80 % (0.8 µg/mL)	0.76	1.94	1.99	95
100 % (1.0 µg/mL)	0.95	1.78	1.85	95.16
120 % (1.2 µg/mL)	1.16	1.88	1.24	97.38

Table 3: Intra-day precision study results.						
Conc. (µg/mL)	**Area**	**Back calculated conc. (µg/mL)**	**Accuracy (%)**	**SD**	**Mean accuracy (%)**	**RSD (%)**
0.80	51040	0.85	105.40	1.83	103.52	1.77
	49268	0.82	101.75			
	50078	0.83	103.42			
1.00	59348	0.98	98.05	0.96	99.05	0.97
	60506	1.00	99.96			
	60010	0.99	99.14			
1.20	70856	1.17	97.55	1.88	99.63	1.87
	73521	1.22	101.22			
	72727	1.20	100.13			

Figure 2: Representative chromatograms of duvelisib at low level

Figure 3: Representative chromatogram of duvelisib at its upper limit of quantification.

Sensitivity

Sensitivity was established through LOD and LOQ determination. The LOD and LOQ for duvelisib were predicted which was confirmed through their replicate injections and calculating the relative standard deviation (RSD) (Table 1). The LOD and LOQ values for the method were observed as 20 ng/mL and 70 ng/mL, respectively. A representative chromatogram at the LOQ level is shown in Figure 2.

Linearity

A seven point calibration curve was plotted for duvelisib to establish the linearity. The linearity was established within a range from 0.07 to 5 µg/mL which dictate that the method is linear. The regression coefficient (r^2) for duvelisib was 0.999. A representative chromatogram at upper limit of quantification (ULOQ) level is shown in Figure 3.

Accuracy

Accuracy was evaluated by percentage recovery at three different concentrations of 80%, 100% and 120% of the target concentration. Percentage recovery of duvelisib in the developed method was obtained within 95.00 to 97.38%. Results for accuracy of the method are shown in Table 2.

Precision

Intra-day and inter-day precision results were examined through RSD (%) of the results. Intra-day precision was evaluated by analysing three different samples of different concentrations of 80 %, 100 % and 120 % of target concentration on same day and experimental conditions. The RSD for duvelisib was found to be within 0.97 to 1.87 % (Table 3). Inter-day precision was determined by injecting the samples of same three different levels on different days and RSD was found to be within 1.25 to 1.99 % (Table 4). The experimental values (% RSD) were found to be within the acceptable limit ensuring the desired precision of the developed method.[22]

Table 4: Inter day precision results.						
Conc. (µg/mL)	Area	Back calculated conc. (µg/mL)	Accuracy (%)	SD	Mean accuracy (%)	RSD (%)
0.80	45330	0.77	95.78	1.94	97.63	1.99
	46125	0.78	97.46			
	47162	0.80	99.65			
1.00	58081	0.98	98.18	1.79	96.57	1.85
	55990	0.95	94.64			
	57316	0.97	96.89			
1.20	69954	1.19	98.54	1.23	98.5	1.25
	70785	1.20	99.71			
	69042	1.17	97.26			

Table 5: Results of robustness study.						
Level	Mobile phase ratio 42:58 % v/v			Mobile phase ratio 38:62 % v/v		
	Average conc. (µg/mL)	RSD (%)	Accuracy (%)	Average conc. (µg/mL)	RSD (%)	Accuracy (%)
80 % (0.8 µg/mL)	0.77 + 1.09	1.14	95.89	0.78 + 0.79	0.82	96.65
100 % (1.0 µg/mL)	0.97 + 2.28	2.37	96.15	0.97 + 2.10	2.18	96.52
120 % (1.2 µg/mL)	1.20 + 1.64	1.65	99.06	1.19 + 0.80	0.81	98.54
	Flow rate 0.9 mL/min			Flow rate 1.1 mL/min		
80 % (0.8 µg/mL)	0.84 + 1.82	1.74	104.53	0.85 + 3.41	3.24	105.45
100 % (1.0 µg/mL)	0.99 + 1.15	1.16	98.72	0.99 + 0.85	0.86	99.03
120 % (1.2 µg/mL)	1.20 + 2.32	2.34	99.49	1.20 + 1.20	2.01	99.22

Robustness

Minor changes such as alteration in mobile phase composition and flow rate were included in the experiment to check robustness of the developed method. The results indicate that there were no major changes in RT and peak area of the drug. Furthermore, RSD was found to be within 1.14 to 2.37 % for mobile phase ratio 42:58 % (ACN:0.1 % FA in water) and 0.81 to 2.18 % for mobile phase ratio 38:62 % (ACN:0.1 % FA in water). Similarly, RSD was found to be within 1.16 to 2.34 % for flow rate 0.9 mL/min and 0.86 to 3.24 % for flow rate 1.1 mL/min (Table 5). Robustness study results revealed that the developed method was robust and analytical results remain unaffected due to minor changes in chromatographic conditions.

Specificity

Specificity was established by evaluating whether there was any interference of formulation excipients at the RT of the drug. Overlay of chromatograms of different samples containing excipients with blank and spiked drug is shown in Figure 4. The study revealed that there was an absence of any interfering peak of excipients at the RT of duvelisib as depicted in the Figure. The specificity was confirmed from the absence of any interference in the quantitative determination of duvelisib.

CONCLUSION

In this study, a simple, rapid, sensitive and cost-effective HPLC method for quantification of duvelisib has been established after required validation as per the ICH and
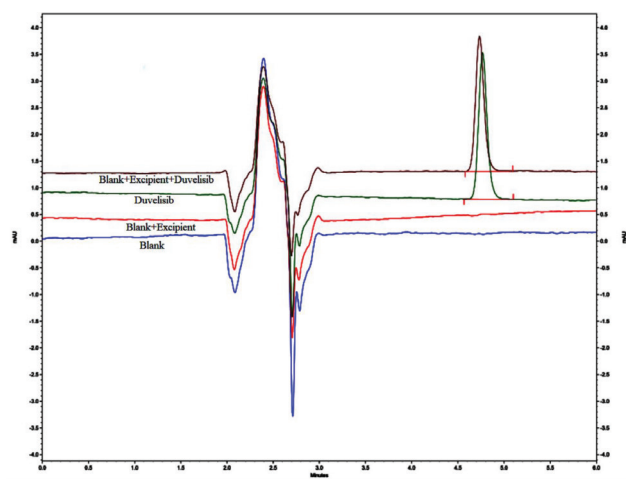
Figure 4: Overlay of chromatograms of different samples containing excipients with blank and spiked drug to signify the interference caused by the excipients (specificity study).

AOAC guideline. Parameters like specificity, linearity, accuracy, precision, specificity were evaluated during validation of the developed method. All validation results were within the acceptable limit as specified in the regulatory guidelines. The established quantitative analysis technique was confirmed to be sensitive, accurate, and precise in the validation experiment. The chromatographic run time was very short which is highly suitable for rapid analysis of routine quality control and stability samples of duvelisib. The method we have developed is about 90 times more sensitive than the previous method. Comparison of this method with existing methods is shown in Table 6. Specificity analysis confirmed that the method is selective for duvelisib in presence of commonly used excipients for its pharmaceutical formulation. This is the first time to report a quantitative assay method for duvelisib, which can quantify the analyte even at the nanogram level. The method will be suitable for sample analysis of quality control, stability, cross-contamination, dissolution study of sustained-release formulation and effluent analysis of duvelisib in pharmaceutical industries and research laboratories.

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Table 6: Comparision of our method with existing methods.

Method	Stationary phase	Mobile phase	FR (ml/min)	RT (min)	range (µg/ml)	Application	Advantages	Disadvanatges
Our method	Agilent Zorbax Eclipse C$_{18}$ column (250 x 4.6 mm, 5 µm)	0.1% formic acid in water: ACN (60:40, %v/v)	1	4.16	0.07-5	Can be applied to diversified sample analysis like sustain release formulation, cross contamination, effluent analysis.	Most sensitive UV-based method, can be mass compatible	Not applicable for bioanalytical purpose
Existing method (15)	Agilent Zorbax Eclipse C$_{18}$ (150×4.6 mm, 5 µm)	0.1 % orthophosphoric acid (46.3%): Acetonitrile (53.7%)	0.91	2.85	6.25-37.5	Forced degradation study	QBD based method	Relatively less sensitivity. Only useful for biological sample analysis
Existing method (16)	Hypersil Gold C$_{18}$ column (250 x 4.0 mm, 5 µ; Thermo Scientific, USA)	10 mM ammonium formate, pH: 4.2 (adjusted with formic acid) and acetonitrile	1	12.6	0.05-5	Pharmacokinetic analysis	Suitable for bioanalysis	Only useful for biological sample analysis
Existing method (17)	Acquity BEH C$_{18}$ column (2.1 mm × 50 mm, 1.7 µm)	Acetonitrile and 0.1 % formic acid in water	0.4	1.26	0.0005-3	Pharmacokinetic analysis	Most sensitive bioanalytical method	Only useful for biological sample analysis

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

BCS: Biopharmaceutical classification system; **PI3Ks:** Phosphoinositide 3-kinases; **USFDA:** United States Food and Drug Administrartion; **UPLC-MS/MS:** Ultra performance liquid chromatography- tandem mass spectrometry; **HPLC:** High performance liquid chromatography; **ICH:** International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use; **AOAC:** Association of Official Analytical Collaboration; **ACN:** Acetonitrile; **MeOH:** Methanol; **RP:** Reversed phase; **UV:** Ultraviolet; **LOD:** Limit of detection; **LOQ:** Limit of quantitation; **RSD:** Relative standard deviation; **ULOQ:** Upper limit of quantification; **FA:** Formic acid; **RT:** Retention time.

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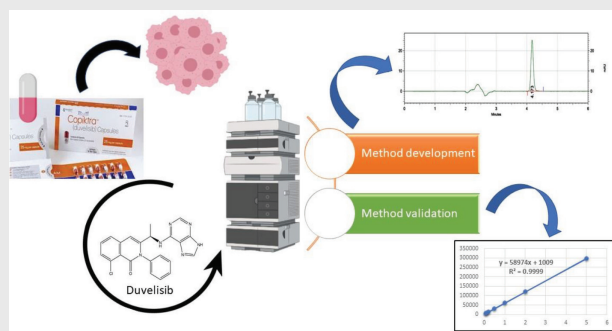
SUMMARY

A simple, rapid, and highly sensitive HPLC method for quantification of duvelisib has been developed and validated in this study. This is the first time to report a quantitative assay method for duvelisib, which can quantify the analyte even at the nanogram level. This method will be suitable for sample analysis of quality control, stability, cross-contamination, dissolution study of sustained-release formulation and effluent analysis of duvelisib in pharmaceutical industries and research laboratories.

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