A SENSITIVE AND SELECTIVE LCMS/MS METHOD FOR THE IDENTIFICATION AND QUANTIFICATION OF POTENTIAL GENOTOXIC IMPURITIES IN RIBOCICLIB PURE DRUG AND PHARMACEUTICAL FORMULATIONS

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Abstract:
The toxicological valuation genotoxic impurities and assessment of acceptable levels for such impurities in any pure drug and its formulations is a significant as well as very difficult issue. The European Medicines Agency guided that 1.5 µg/day intake of a genotoxic impurity was considered as acceptable for most of the pharmaceutical products. In view of this, the present work was intended to develop a sensitive, simple and accurate LCMS method for the quantification of potential genotoxic impurities (PGIs) such as N-oxide impurity, N-formyl impurity, chloro amide impurity and chloro hydroxy impurity in ribociclib pure drug and its drug product. The method comprises of YMC-Triart C18 (150×4.6mm; 5 µm particle size) column as stationary phase, 0.1 % formic acid in water, methanol and acetonitrile at pH 4.6 in the ratio of 15:45:40 (v/v) as mobile phase at 0.5 mL/min flow rate. The eluents were monitored in multiple reaction monitoring operated in positive ion mode using a mass detector. The method clearly separates the analytes that shows characteristic mass transition at m/z of 435 ⟶ 280 for ribociclib, 451 ⟶ 297 for N-oxide impurity, 463 ⟶ 280 for N-formyl impurity, 293 ⟶ 254 for chloro amide impurity and 252 ⟶ 154 for chloro hydroxy impurity. There are no impurities or un-wanted compounds detected in both chromatograms as well as mass pattern proved that the method was specific. The method was validated for parameters such as linearity, precision, recovery, ruggedness, robustness and reported acceptable results. The method was very sensitive that can detect the analytes up to a very low concentration of 0.020 µg/mL for N-formyl impurity, chloro amide impurity, 0.030 µg/mL for N-oxide and chloro hydroxy impurity. The method can be utilized for assessment of potential genotoxic impurities during the synthesis process of ribociclib as well as manufacturing the pharmaceutical products.

Keywords: Genotoxic Impurities, LCMS analysis, Ribociclib, Method Development, Formulation analysis

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Introduction:

Ribociclib is a medical drug belongs to kinase inhibitors class and an inhibitor of cyclin Cyclin-dependent kinase 4 and 6 used to treat certain types of breast cancer [1]. Ribociclib used for the treatment of advanced or metastatic breast cancer in postmenopausal women and was prescribed in combination with aromatase inhibitor such as letrozole [2]. Ribociclib was used in combination with fulvestrant for the treatment of hormone receptor–positive advanced breast cancer that has spread to other parts of the body as an initial treatment or in people who have not been treated successfully with other treatments in women who have already experienced menopaus [3]. The patients undergoing ribociclib therapy may experience some common side effects such as nausea, fatigue, neutropenia and leukopenia [4].

Pharmaceutical impurities are the unwanted substances that are induced from the process of synthesis of drug namely process related or synthesis impurities and some are generated by degradation of synthetic drug namely degradation impurities. Some impurities can induce mutations in genes and can causes chromosomal breaks or rearrangements and have the potential to cause cancer and called Potential Genotoxic impurities (PGIs) [5,6]. The controlling of impurities in a pharmaceutical product was considered as very essential for producing quality and safe product with minimal side effects. The researchers continuously working to establish various methods and procedures for minimization of trace level impurities. Preliminary conformation and quantification of impurities was considered as initial and very essential step for controlling the potential impurities [7]. The identification and quantification of impurities at trace level required cutting edge analytical techniques such as LCMS [8].

On the personal verification of the available literature for the analysis of ribociclib confirms that HPLC methods reported for the estimation of ribociclib [9] and in combination with palbociclib [10] in pharmaceutical formulations. LCMS methods reported for the estimation of ribociclib in human plasma [11,12] and dried blood spots [13]. Bio-analytical methods reported for the estimation of ribociclib in combination with other drugs such as letrozole [14], oleanolic acid [15], palbociclib [16], palbociclib and letrozole [17], abemaciclib and palbociclib [18], Abemaciclib, Palbociclib, Anastrozole, Letrozole and Fulvestrant [19]. One capillary electrophoresis method was reported for the estimation of ribociclib in combination with abemaciclib and palbociclib in pharmaceutical formulations [20]. No method reported for the separation and quantification of PGIs in ribociclib pure drug and its dosage forms. Hence the present study was aimed to develop a simple and sensitive LCMS method for the detection and quantification of PGIs in ribociclib pure drug and dosage forms. Based on the availability, the PGIs such as N-oxide impurity, N-formyl impurity, chloro amide impurity and chloro hydroxy impurity were studied. The molecular structure of ribociclib and its PGIs in the study were presented in figure 1.

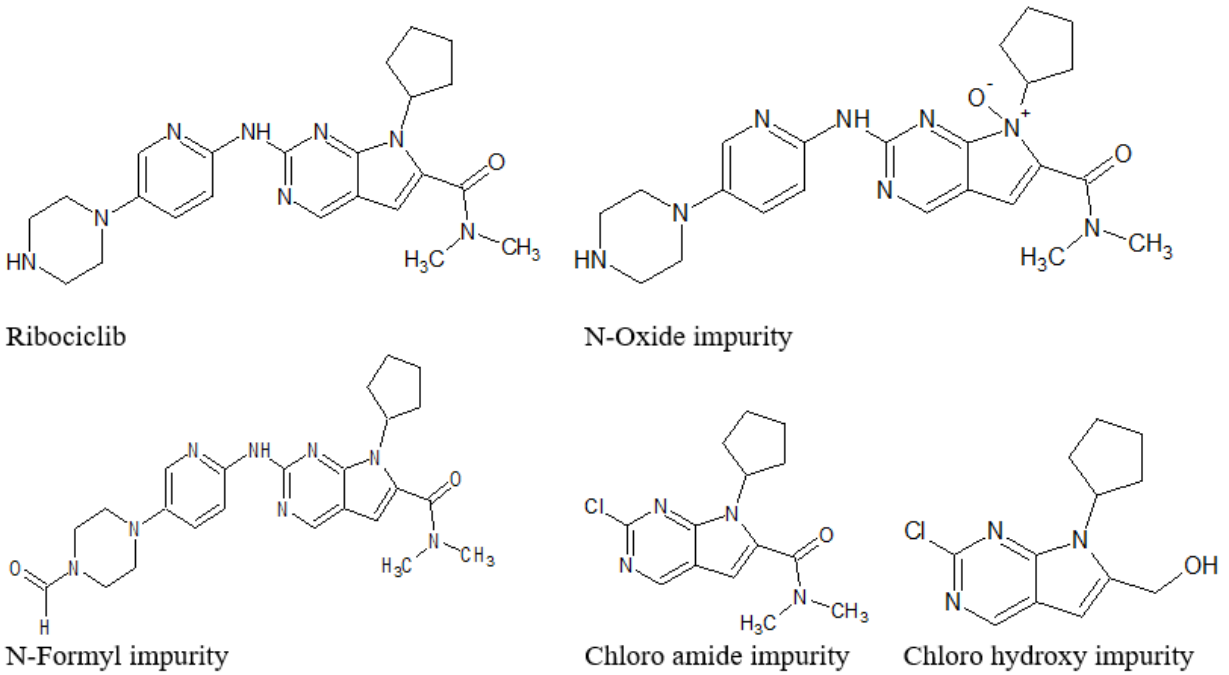


Figure 1: Molecular structure of analytes in the study

Materials and Methods:

Instrumentation:

The analysis was performed on Waters (Japan) alliance 2695 LCMS system connected with triple quadruple mass detector (LAA 1369) and 0.1 – 1500 µL injectable sample injector. The analytes were separated on C18 columns of different configurations and packing materials. The equipment was monitored and detected eluents were monitored using masslynx 4.2 software. The mass detector was operated in positive ion mode that was operated in multiple reaction monitoring (MRM) mode. The analyser was operated with suitable conditions of drying gas flow (10 L/min), nebulizer pressure (30 psi), gas temperature (300 °C), and spray voltage (5500 V).

Chemicals and Reagents:

The active pharmaceutical ingredient of ribociclib (98.69 %), its PGIs in the study namely N-oxide impurity, N-formyl impurity, chloro amide impurity and chloro hydroxy impurity along with its marketed formulation dosage form with brand name Kryxana® - 200 mg was obtained as gift sample from Novartis Pharmaceuticals, Hyderabad, Telangana. LiChropur™ grade chemicals used in the study like formic acid and HPLC quality solvents such as methanol, acetonitrile, water and 0.2 µ membrane filters were obtained Merck chemicals, Mumbai.

Preparation of solutions:

Mobile Phase:

The solvents such as methanol, acetonitrile and different strengths of formic acid in water were mixed in various compositions and pH ranges were used for preparing mobile phase compositions during the method development study. The solvents at selected composition was accurately measured and mixed in a clean and dry reservoir bottles. The ultrasonic batch sonicator was used for uniform mixing and degassing the mobile phase. Then it was filtered through 0.2 micron membrane filter and was preserved in an amber colour bottle whenever necessary.

Standard stock solution:

The ribociclib and its impurities standard stock solution were prepared separately. An accurately weighed 25 mg of ribociclib and its PGIs were taken in a volumetric flask containing 15 mL methanol separately. The volumetric flasks sonicated for 2 min using an ultrasonic bath sonicator to dissolve the analytes in solvent completely. Then the solution was filtered using 0.2 µ membrane filter in to a separate clean and dry volumetric flask and then the final volume was made up to the mark using the same solvent. The stock solution of ribociclib and its PGIs at a concentration of 1000 µg/mL were obtained separately. During the analysis, equal volumes of selected concentration of ribociclib and its PGIs were mixed for obtaining a mixed standard solution.

Formulation solution:

The Kryxana® formulation tablets were powdered to obtain a uniform powder and an amount of tablet powder equivalent to 10 mg of ribociclib standard was weighed and was taken in a clean and dry volumetric flask containing 5 mL of methanol. The content was sonicated for 5 min to dissolve the drug completely in solvent and was filtered through 0.2 µ membrane filter in to a separate clean and dry volumetric flask. Then the final volume was made up to mark to obtain formulation solution containing 1000 µg/mL of ribociclib. Then the solution was diluted to 250 µg/mL using methanol diluent and this solution was used for evaluating the effectiveness of the method for identification and quantification of potential genotoxic impurities in ribociclib. The formulation solution was spiked with known concentration of studied PGIs was also studied for the evaluation of method effectiveness for the separation and quantification of PGIs in the study.

Method development:

The method for the resolution, detection and quantification of PGIs in ribociclib pure drug and formulations was performed by adopting the guidelines issued by ICH [21]. During the method development the standard solution containing 1.0 µg/mL of ribociclib and its impurities was analysed. The standard solution was analysed in various method conditions that were changed progressively and each changed conditions, the peak area response, peak symmetry, system suitability and mass spectral pattern was verified for method conformation. The method parameters such as composition, pH and flow rate of mobile phase, nature, configuration and temperature of stationary phase were optimized during the method. The method condition that produces acceptable results were considered as suitable and were precede for further validation.

Method validation:

The developed method was validated in terms of linearity, precision, ruggedness, robustness, sensitivity as per ICH guidelines [22]. Further, the developed was studied for its applicability for assessing the PGIs in formulations.

Results and discussion:

The work aimed to establish a simple and sensitive LCMS method for the separation, identification and quantification of four PGIs such as N-oxide impurity, N-formyl impurity, chloro amide impurity and chloro hydroxy impurity in ribociclib pure drug and formulations. The method development was aimed such that the method can detect and analyse the PGIs impurities at trace level. During the development of method, various configurations and manufactures of columns such as C8 and C18 were studied and the column that resolves the analytes with acceptable symmetry was considered as suitable for the analysis. The mobile phase was optimized by varying different compositions of solvents such as methanol, acetonitrile and formic acid in water in different pH levels and flow rates was studied. The column temperature and mass operating conditions were also optimized such that the conditions that resolve and analyse the PGIs at trace level.

In each trail condition, the standard solution containing 1.0 µg/mL concentration of ribociclib and its PGIs was analysed. The peak symmetry, resolution, chromatographic response of each analyte was summarized and the conditions that produces best results was considered as suitable conditions for the analysis of ribociclib and its PGIs in the study. The optimized separation was achieved on YMC-Triart C18 (150×4.6mm; 5 µm particle size) column, 0.1 % formic acid in water, methanol and acetonitrile at pH 4.6 in the ratio of 15:45:40 (v/v) at a flow rate of 0.5 mL/min. The column eluents were monitored using mass detector operated in multiple reaction monitoring mode and the mass operating conditions were presented in table 1. In the developed condition, the standard solution containing selected and known concentration of ribociclib was analysed at a sample volume of 10 µL. The chromatographic response was recorded in each sample analysed in the developed method.

S No	Analyte	Precursor ion (m/z)	Product ion (m/z)	Fragmentor (V)	Collision energy (eV)	Electron Multiplier Voltage (V)	MS1 RES
1	Ribociclib	435	280	160	30	600	Wide
2	N-oxide impurity	451	297	145	35	600	Wide
3	N-formyl impurity	463	280	155	40	600	Wide
4	Chloro amide impurity	293	254	140	30	600	Wide
5	Chloro hydroxy impurity	252	154	150	35	600	Wide

Table 1: Mass detector conditions for the analysis of ribociclib and its genotoxic impurities

The specificity of the optimized method was evaluated by analysing the standard solution containing 0.5 µg/mL concentration of ribociclib and PGIs in the study as well as the diluent used for the preparation of standard solutions as blank.

The chromatogram observed for blank as shown in figure 2 doesn't show any peak throughout the run time whereas the standard chromatogram show well resolved and retained peaks corresponds to the ribociclib and its PGIs in the study.

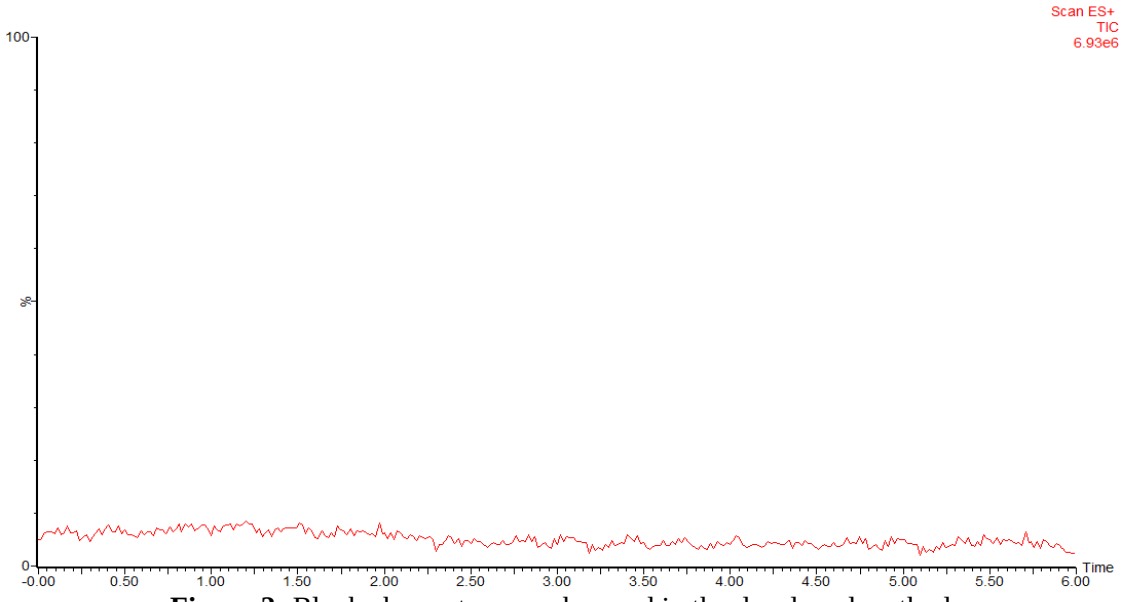


Figure 2: Blank chromatogram observed in the developed method

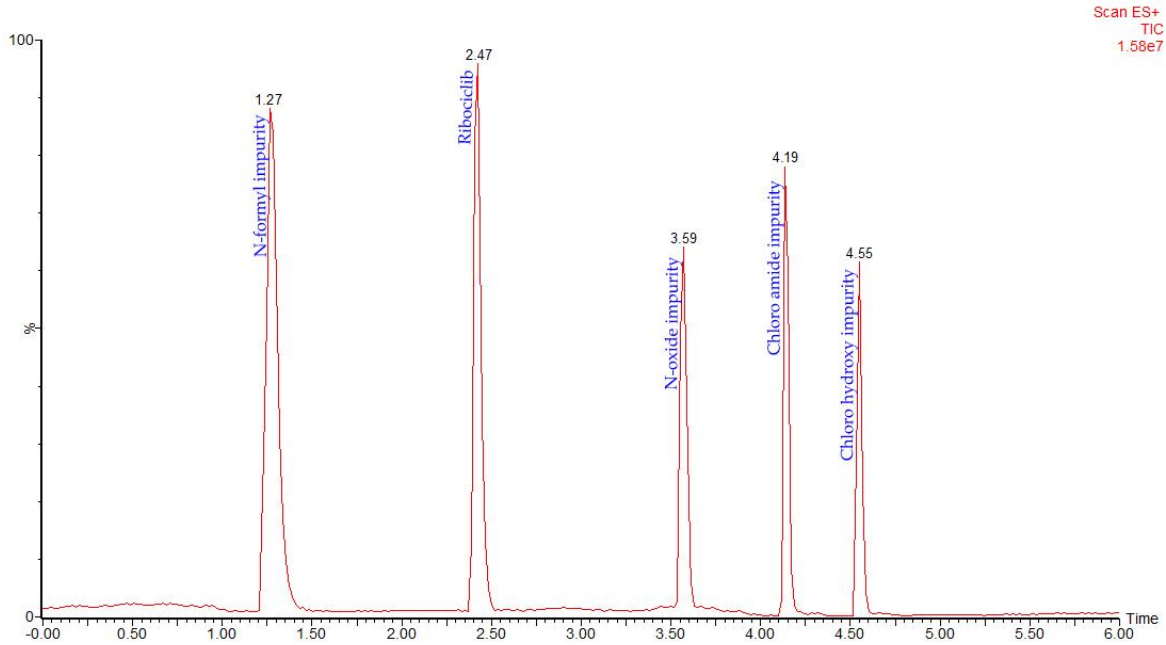
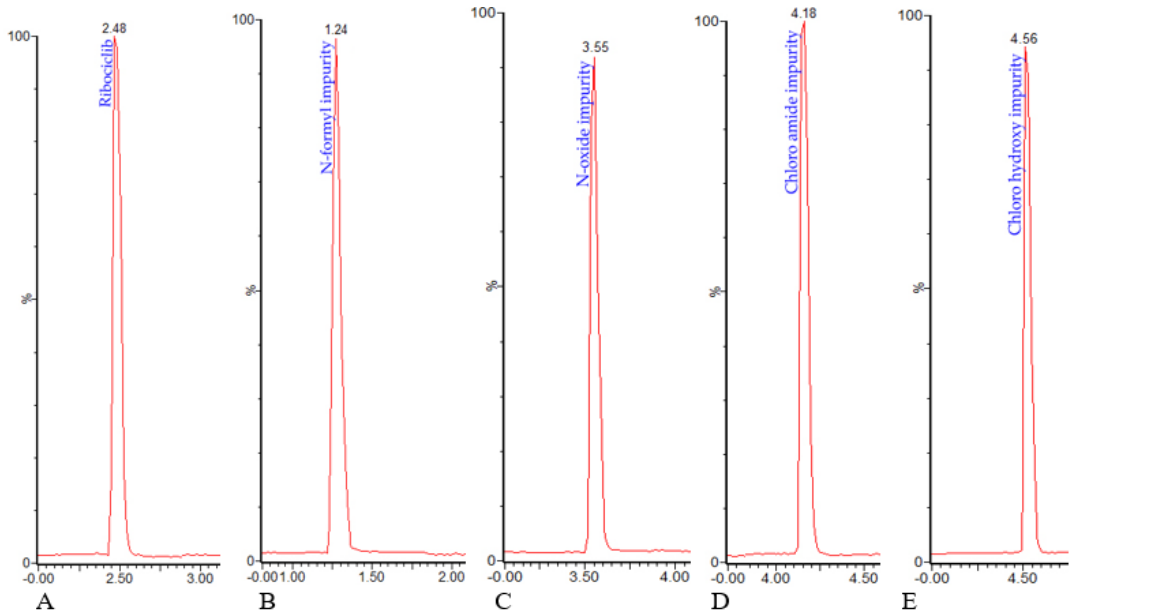


Figure 3: Standard chromatogram observed in the developed method

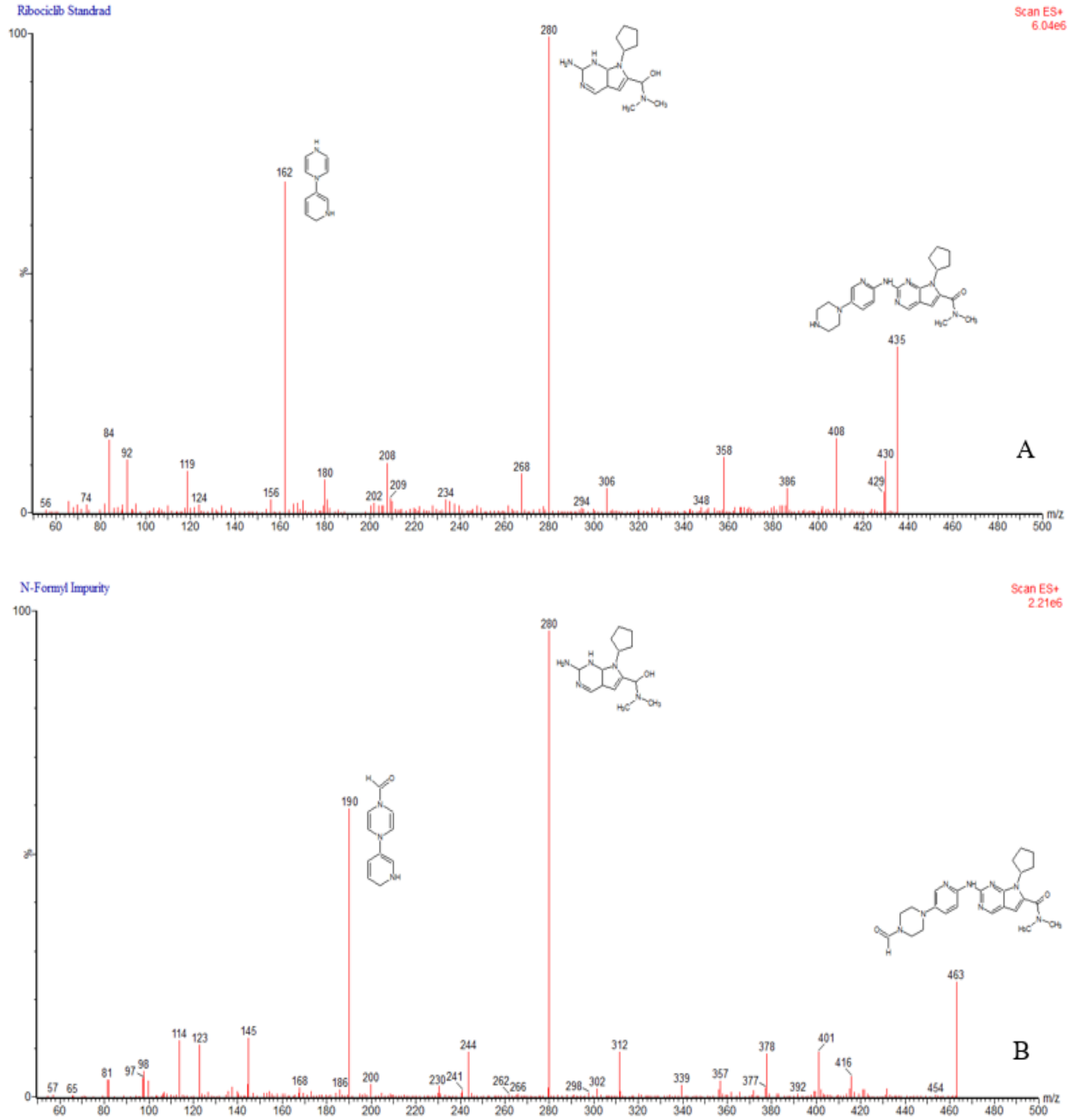
The retention time of the peak observed in the standard solution was compared with the individual analysis of ribociclib and its genotoxic impurities. The compounds eluted at a retention time (t_R) of 2.4 min for ribociclib, 3.59 for N-oxide impurity, 1.2 min for N-formyl impurity, 4.1 min for chloro amide impurity and 4.5 min for chloro hydroxy impurity. The t_R of analytes identified in the combined standard solution was same as the retention time identified for individual analysis ribociclib and its genotoxic impurities. The chromatograms observed for the individual standard analysis of ribociclib and its genotoxic impurities was shown in figure 4. There is no detection of additional compounds or the impurities were detected throughout the run time of combined standard, individual standard solutions as well as the blank analysis confirms that the method was specific for the analysis of ribociclib and its genotoxic impurities.

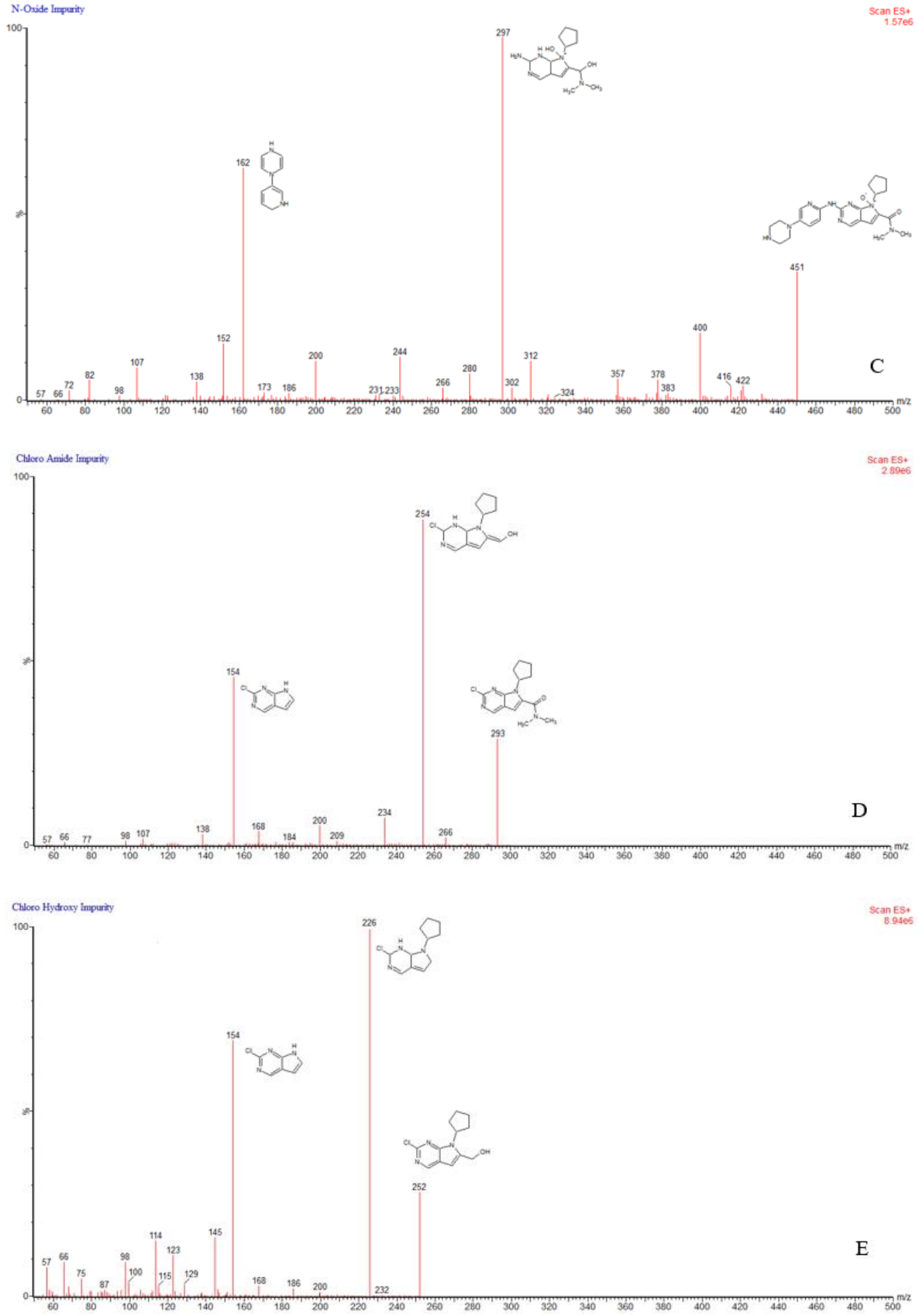


ribociclib; B) N-formyl impurity; C) N-oxide impurity; D) Chloroamide impurity; E) Chlorohydroxy impurity

Figure 4: Chromatograms observed for individual analysis of ribociclib and its genotoxic impurities in the study

The mass spectral characterization of individual analyte peak was monitored in positive ion mode using multiple reaction monitoring method. The peak corresponds to ribociclib identified at t_R of 2.4 min shows parent ion at m/z of 435 (m+1). The mass spectra shows daughter fragments at m/z of 280 and 162. The mass spectra at the t_R of PGIs in the study were evaluated and the parent and daughter ion fragments identified at m/z of 451 and 297 for N-oxide impurity, 463 and 280 min for N-formyl impurity, 293 and 254 for chloro amide impurity and 252 and 154 for chloro hydroxy impurity respectively. The mass fragmentation pattern of the individual analysis of the analystes was in correlation with the results observed for the combined analysis of analytes. There is no additional detection or fragments correspond to impurities or the un-identified fragments detected in the mass patter of the analytes. This proves that the method provides specific analysis results corresponds to ribociclib and its studied genotoxic impurities. The % abundance of the daughter fragments were noticed to be significantly higher than the other ions detected in the spectrum. Hence the method was specific and suitable for the analysis of ribociclib and its studied genotoxic impurities. The mass fragmentation results were represented in figure 5.





Mass fragmentation spectra observed at the retention of 2.4 min for ribociclib (A), 1.24 min for N-formyl impurity (B), 3.55 min for N-oxide impurity (C), 4.1 min for chloro amide impurity (D) and 4.5 min for chloro hydroxy impurity

Figure 5: Mass fragmentation spectra obtained at the retention time of ribociclib and its PGIs in the developed method

Method Validation:

In the developed method, various concentrations of ribociclib and its genotoxic impurities was analysed. The calibration curve was constructed by using peak area response against analyte strength. The accurate fit with high correlate calibration curve was obtained in the concentration levels of 0.03 µg/mL to 6.0 µg/mL. The least-squares linear regression analysis was performed for the evaluation of calibration curve parameters such as regression equation, intercept and slope. The calibration equation was obtained as y = 249466x + 46199 (R^2 = 0.9996), y = 149908x + 21168 (R^2 = 0.9992), y = 206397x + 39751 (R^2 = 0.9993), y = 177573x + 24418 (R^2 = 0.9994) and y = 153567x + 25715 (R^2 = 0.9993) respectively for ribociclib, N-oxide impurity, N-formyl impurity, chloro amide impurity and chloro hydroxy impurity respectively. The results of the linearity were represented in table 1.

S No	Concentration in µg/mL	Peak are response obtained				
		Ribociclib	N-oxide impurity	N-formyl impurity	Chloro amide impurity	Chloro hydroxy impurity
1	0.03	49281.5	21021.9	40054.8	28516.7	24689.5
2	0.1	68614.8	42563.2	74982.3	46582.5	43200.7
3	0.5	155168.7	94747.3	147132.5	125143.9	99152.4
4	1	311527.9	184847.9	251476.8	201326.4	195468.2
5	2	554757.1	304798.2	427487.2	361915.8	328490.3
6	4	1049105.3	617492.5	865154.8	729510.3	627971.9
7	6	1535157.8	925951.7	1285154.7	1098257.1	954150.2

Table 1: Linearity results

The recovery experiment was conducted in the concentration of 1.0 µg/mL, 2.0 µg/mL and 4.0 µg/mL in the linearity level for ribociclib and its PGIs in the study. The recovery level solution was analysed in triplicate in the optimized method and the peak area response of the each analyte was compared with the corresponding peak area response on the calibration level. The % recovery of each analyte in each analysis and the % RSD in each spiked level was calculated. The % recovery of 98-102 % and the % RSD of less than 2 was considered as acceptable as per the guidelines. The % RSD in each spiked level was observed to less than 2 for ribociclib and its PGIs in the study. The results observed to be under the acceptable level confirms that the method was recoverable and accurate. The results attained in recovery study of the method optimized for the analysis of ribociclib and its PGIs was represented in table 2.

S. No	Compound	Level	Concentration in µg/mL	Recovered in µg/mL Mean±SD	% Recovery	% RSD
1	Ribociclib	50%	1	0.985±0.002	98.540±0.217	0.220
2		100%	2	1.979±0.006	98.970±0.308	0.311
3		200%	4	3.940±0.017	98.507±0.422	0.428
4	N-oxide impurity	50%	1	0.987±0.006	98.688±0.585	0.593
5		100%	2	1.989±0.005	99.440±0.240	0.242
6		200%	4	3.989±0.021	99.713±0.521	0.522
7	N-formyl impurity	50%	1	0.989±0.003	98.870±0.305	0.309
8		100%	2	1.987±0.009	99.340±0.443	0.446
9		200%	4	3.988±0.026	99.710±0.646	0.647
10	Chloro amide impurity	50%	1	0.993±0.004	99.257±0.350	0.353
11		100%	2	1.980±0.010	98.983±0.511	0.516
12		200%	4	4.030±0.031	100.740±0.766	0.761
13	Chloro hydroxy impurity	50%	1	0.993±0.005	99.307±0.545	0.549
14		100%	2	1.967±0.006	98.370±0.288	0.293
15		200%	4	4.024±0.012	100.600±0.296	0.294

Table 2: Accuracy results

The standard solution having 1.0 µg/mL concentration of ribociclib and its PGIs was utilized for the evaluation of the repeatability and reproducibility of the developed method. The solution was analysed six times in one day for intraday precision, six times in three consecutive

days for interday precision. The same solution was analysed six times in one day by three different analysts for evaluation of the ruggedness of the method. The peak response of the ribociclib and its PGIs was summarized in each study and the % RSD of each analyte in each study was calculated. The % RSD of less than 2 was considered as acceptable as per the guidelines and based on the results as summarized in table 3, the results were achieved were under the acceptable level confirms that the method was precise and reproducible.

The minor variations in the optimized method conditions such as composition of mobile phase with no change in pH, change in pH and change in flow rate of mobile phase was made intentionally for the evaluation of the robustness of the method. The mobile phase composition of 0.1 % formic acid in water, methanol and acetonitrile in 15:45:40 (MP change 1) and 15:45:40 (MP change 2) at pH 4.5 (pH change 1) and 4.7 (pH change 2) was studied at a flow rate of 0.45 mL/min (FR change 1) and 0.55 mL/min (FR change 2). In each changed condition, the standard solution containing 1.0 µg/mL of ribociclib and its PGIs in the study was analysed. The % change in the peak area response of the individual analyte was calculated by comparing the results noted in each changed condition with its corresponding standard calibration curve. The % change of less than 2 was considered as acceptable and as summarized in table 3, the results noticed were under the acceptable for ribociclib and its PGIs studied. Based on the results, it was confirmed that there is no significant changes was observed while change in the minor variations in the developed method and hence the method was rugged.

The method sensitivity was established by evaluation the detection limit (LOD) and quantification limit by adopting signal (n) to noise (n) ratio. The s/n of 10 and 3 were considered as LOQ and LOD for the analytes in the developed method. The LOD was obtained as 0.010 µg/mL for ribociclib, 0.020 µg/mL for N-formyl impurity, chloro amide impurity, 0.030 µg/mL for N-oxide and chloro hydroxy impurity. The LOQ was calculated as 0.033 µg/mL for ribociclib, 0.066 µg/mL for N-formyl impurity, chloro amide impurity and 0.099 µg/mL for N-oxide impurity and chloro hydroxy impurity. The sensitivity results confirm that the method can detect the analytes up to a very nominal concentration of 0.001 µg/mL and hence proved that the method was very sensitive.

The stability of the solution prepared for the analysis of ribociclib and its PGIs was evaluated by incubating the standard solution in an auto-sampler for 48 h at 25 ^{o}C. The incubated solution was analysed in every 6 h in the developed method. The peak area response of the individual analyte was summarized in each time interval and the % stability was calculated by comparing the peak area response with its corresponding standard calibration curve. The % assay was observed to be more than 99 % for analytes up to 24 h and a very high % assay was observed up to 48 h. This proved that the solutions prepared were stable up to 48 h.

The developed method was applied for the identification and quantification of potential genotoxic impurities in formulations. The formulation solution at a concentration of 200 µg/mL prepared using Kryxana® formulation of ribociclib was analysed in the developed method. The formulation solution spiked with known concentration of genotoxic impurities was also analysed in the developed method. The chromatogram observed for the formulation solution spiked with impurities clearly shows the peaks corresponds to impurities along with ribociclib. Whereas the chromatogram observed for the un-spiked formulation sample doesn't show any peak at the retention time of PGIs in the study. This proved that the method can effectively identify and quantify the impurities in formulation. Hence this method can successfully be utilized for the quantification of PGIs in ribociclib bulk drug as well as formulation dosages. The summary results of the method validation were presented in table 3.

S No	Parameter	Results observed				
		Ribociclib	**N-oxide impurity**	**N-formyl impurity**	**Chloro amide impurity**	**Chloro hydroxy impurity**
1	Linearity range	0.03-6.0 µg/mL	0.03-6.0 µg/mL	0.03-6.0 µg/mL	0.03-6.0 µg/mL	0.03-6.0 µg/mL
2	% RSD Intraday Precision (n=6)	0.20	0.59	0.43	0.46	0.32
3	% RSD Interday Precision (n=6)	0.31	0.76	0.96	0.84	0.64
4	% RSD Ruggedness (n=6)	0.89	0.74	0.66	0.88	0.70
5	% Accuracy in 50% spiked level (n=3)	98.540	98.688	98.870	99.257	99.307
6	% Accuracy in 100% spiked level (n=3)	98.970	99.440	99.340	98.983	98.370

7	% Accuracy in 150% spiked level (n=3)	98.507	99.713	99.710	100.740	100.600
8	% Change Robustness					
	MP change 1	0.11	0.90	0.15	0.23	0.86
	MP change 2	0.78	0.46	0.18	0.70	0.17
	pH change 1	0.92	0.55	0.50	0.93	0.56
	pH change 2	0.08	0.48	0.83	0.55	0.43
	FR change 1	0.73	0.99	0.56	0.47	0.71
	FR change 2	0.97	0.84	0.33	0.84	0.30
9	% stability at 48 h (n=6)	98.15	97.95	97.28	98.31	98.05
10	LOD in µg/mL	0.01	0.03	0.02	0.02	0.030
11	LOQ in µg/mL	0.033	0.099	0.066	0.066	0.099
12	% assay in formulation	98.85	Not detected	Not detected	Not detected	Not detected

Table 3: Summary results obtained in the study

Conclusion:

In this investigation, a simple and sensitive analytical LCMS approach was developed and successfully validated for identification and quantification PGIs such as N-oxide impurity, N-formyl impurity, chloro amide impurity and chloro hydroxy impurity in ribociclib bulk drug and formulations. The ESI source was protected and favourable analytical conditions was proved by diverting the entry of the mobile phase in to the mass detector using a switch valve. The method shows calibration curve linear in the concentration range of 0.03 µg/mL to 6.0 µg/mL and proved to be precise, accurate and specific in the assessed concentration range. The detection levels was observed to be 0.010 µg/mL for ribociclib, 0.020 µg/mL for N-formyl impurity, chloro amide impurity, 0.030 µg/mL for N-oxide and chloro hydroxy impurity proved that the method was sensitivity. The method can identify and quantify the PGIs in bulk drug and formulations. The method can also be applicable to in-process monitoring of studied impurities during the process of synthesis of ribociclib. Based on the achieved results it can be concluded that the study will ensure the safe use of ribociclib during production of formulations.

Reference:

1. Syed YY, Ribociclib: First Global Approval, Drugs, 2017, 77(7): 799-807 https://doi.org/10.1007/s40265-017-0742-0
2. Neelima V, Hope SR, Cyclin-Dependent Kinase 4/6 Inhibitors for the Treatment of Breast Cancer: A Review of Preclinical and Clinical Data, Clinical Breast Cancer, 2016, 16(1): 8-17 https://doi.org/10.1016/j.clbc.2015.07.005
3. Fasching PA, Beck JT, Chan A, De Laurentiis M, *et al*., Ribociclib plus fulvestrant for advanced breast cancer: Health-related quality-of-life analyses from the MONALEESA-3 study, Breast, 2020, 54:148-154 https://doi.org/10.1016%2Fj.breast.2020.09.008
4. Giuseppe Curigliano, Carmen Criscitiello, Angela Esposito, Mattia Intra & Saverio Minucci, Pharmacokinetic drug evaluation of ribociclib for the treatment of metastatic, hormone-positive breast cancer, Expert Opinion on Drug Metabolism & Toxicology, 2017, 13(5): 575-581 https://doi.org/10.1080/17425255.2017.1318848
5. David QL and Alireza SK, Analytical challenges in stability testing for genotoxic impurities, TrAC Trends in Analytical Chemistry, 2013, 49: 108-117 https://doi.org/10.1016/j.trac.2013.06.004
6. David JS and Sean DM, Guidelines and pharmacopoeial standards for pharmaceutical impurities: Overview and critical assessment, Regulatory Toxicology and Pharmacology, 2012, 63(2): 298-312 https://doi.org/10.1016/j.yrtph.2012.03.016
7. Raman NV, Prasad AV and Ratnakar Reddy K, Strategies for the identification, control and determination of genotoxic impurities in drug substances: a pharmaceutical industry perspective, J Pharm Biomed Anal, 2011, 55(4): 662-667 https://doi.org/10.1016/j.jpba.2010.11.039
8. Derek IR, Control of Genotoxic Impurities in Active Pharmaceutical Ingredients: A Review and Perspective, Org Process Res Dev, 2010, 14(4): 946-959 https://doi.org/10.1021/op900341a
9. Siddhesh Kamble, Vijay Munipalli, Harshada Talapadatur, Raman Mohan Singh, Sayali Warde, Smita Nayak and Bhaskar Vaidhun Development and Validation of Novel HPLC Method for Analytical Evaluation of Ribociclib, Ijppr Human, 2021, 22(1): 510-525

10. Sreelakshmi M, Sasidhara RLC and Raviteja B, Simultaneous Estimation of Ribociclib and Palbociclib in Bulk Samples by Reverse Phase High Performance Liquid Chromatography, International Journal of Pharmacy and Biological Sciences, 2019, 9(2): 413-421 https://doi.org/10.21276/ijpbs.2019.9.2.54
11. Vamseekrishna Gorijavolu, Ajay Kumar Gupta, Chowdary YA and Raviteja B, Estimation of Ribociclib in Human Plasma Samples by LC-ESI-MS/MS, Journal of Chemical and Pharmaceutical Research, 2017, 9(11):61-66
12. Bao X, Wu J, Sanai N, Li J, Determination of total and unbound ribociclib in human plasma and brain tumor tissues using liquid chromatography coupled with tandem mass spectrometry, J Pharm Biomed Anal, 2019, 166:197-204 https://doi.org/10.1016%2Fj.jpba.2019.01.017
13. Louwrens Braal C, Mei HL, Tineke Rienks, Claudia JT *et al*., Quantification of ribociclib in dried blood spots by LC–MS/MS: Method development and clinical validation, Journal of Pharmaceutical and Biomedical Analysis, 2021, 201: 114118 https://doi.org/10.1016/j.jpba.2021.114118
14. Prasanthi S, Himabindu G and Kumar YV, Bioanalytical method for simultaneous estimation of ribociclib and letrozole and its application to pharmacokinetic studies using ultra-performance liquid chromatography, International Journal of Applied Pharmaceutics, 2022, 14(3): 95–102 https://doi.org/10.22159/ijap.2022v14i3.44440
15. Amit Kumar Sahu, Tarang Jadav, Niraj Rajput, Manish Kumar Sharma and Pinaki Sengupta, Bioanalysis by LC-MS/MS and preclinical pharmacokinetic interaction study of ribociclib and oleanolic acid, Bioanalysis, 2022, 14(15) https://doi.org/10.4155/bio-2022-0123
16. Fanny Leenhardt, Matthieu Gracia, Catherine Perrin, Claudia Muracciole-Bich, *et al*., Liquid chromatography–tandem mass spectrometric assay for the quantification of CDK4/6 inhibitors in human plasma in a clinical context of drug-drug interaction, Journal of Pharmaceutical and Biomedical Analysis, 2020, 188: 113438 https://doi.org/10.1016/j.jpba.2020.113438
17. Posocco B, Buzzo M, Poetto AS, Orleni M, Gagno S, Zanchetta M, *et al*., Simultaneous quantification of palbociclib, ribociclib and letrozole in human plasma by a new LC-MS/MS method for clinical application, PLoS ONE, 2020, 15(2): e0228822 https://doi.org/10.1371/journal.pone.0228822
18. Martínez-Chávez A, Rosing H, Hillebrand M, *et al*., Development and validation of a bioanalytical method for the quantification of the CDK4/6 inhibitors abemaciclib, palbociclib, and ribociclib in human and mouse matrices using liquid chromatography-tandem mass spectrometry, Anal Bioanal Chem, 2019, 411: 5331-5345 https://doi.org/10.1007/s00216-019-01932-w
19. Turković L, Bočkor L, Ekpenyong O, Silovski T, Lovrić M, Crnković S, Nigović B and Sertić M, Development and Validation of a Novel LC-MS/MS Method for the Simultaneous Determination of Abemaciclib, Palbociclib, Ribociclib, Anastrozole, Letrozole, and Fulvestrant in Plasma Samples: A Prerequisite for Personalized Breast Cancer Treatment, Pharmaceuticals, 2022, 15(5):614 https://www.mdpi.com/1424-8247/15/5/614#
20. Mlinarić Z, Turković L, Begović I, Nigović B and Sertić M, Rapid Capillary Electrophoresis Method for Simultaneous Determination of Abemaciclib, Ribociclib, and Palbociclib in Pharmaceutical Dosage Forms: A Green Approach, Molecules, 2022, 27(21):7603 https://doi.org/10.3390/molecules27217603
21. International council for harmonisation of technical requirements for pharmaceuticals for human use, ICH harmonised guideline, analytical procedure development, Q14, 2022
22. International council for harmonisation of technical requirements for pharmaceuticals for human use, ICH harmonised guideline, validation of analytical procedures, Q2(R2), 2022