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DEVELOPMENT AND VALIDATION OF AN HPLC METHOD FOR DETERMINATION OF (2R, 3S)-3-((TERT-BUTOXY CARBONYL)AMINO)-2-HYDROXY-3-PHENYLPROPANOIC ACID IN (2A,5B,7B,10B,13A)4-ACETOXY-13({(2R,3S)-3-((TERTBUTOXY CARBONYL)AMINO)-2-HYDROXY-3-PHENYLPROPANOYL}OXY)-1-HYDROXY-7,10-DIMETHOXY-9-OXO-5,20-EPOXYTAX-11-EN-2-YL-BENZOATE,DEHYDRATE(CABAZITAXEL)

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Abstract:

High performance liquid chromatography (HPLC) is an important quantitative technique used for the content evaluation if cabazitaxel[1], generally used for the estimation of pharmaceutical samples. It is the most qualitative, safest and fastest chromatographic technique for the analytical development part of drug components. This article was prepared with an aim to synthesize, characterize and development of by-product with the review different parameters of HPLC technique used such as instrumentation, parameter, quantitative of quantification and detection limit and system applications.

Keywords:

High performance liquid chromatography (HPLC), instrumentation, Retention, Specificity and Parameters, mobile phase.

Introduction:

Literatures survey that cabazitaxel biproduct can be synthesized and validated by HPLC technique by various parameters. Synthesis of new impurity can be used as biproduct of cabazitaxel. The biproduct is part of main product and it's a degradent[2] confirmed based on mass report. This impurity may establish in final drugs substance based on commercial batches of API.

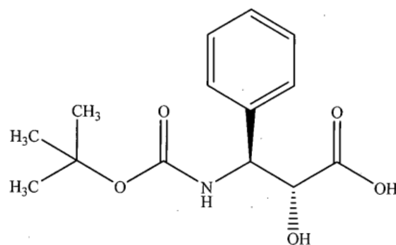

Fig-1: (2r, 3s)-3-((Tert-Butoxy Carbonyl) Amino)-2-Hydroxy-3- Phenylpropanoic Acid.

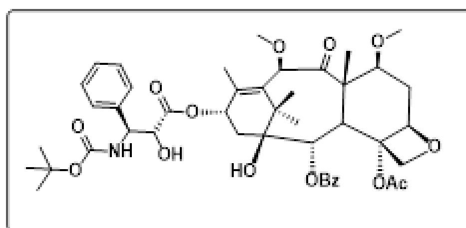

Fig-2: Cabazitaxel.

Material and Methods

Chemicals:

Potassium Dihydrogen orthophosphate : HPLC grade or equivalent

Acetonitrile : HPLC grade-Merck or equivalent

Methanol : HPLC grade

Orthophosphoric acid : HPLC grade-Merck or equivalent

Water : Milli ‘Q’

Chromatographic Conditions:

Column : Zorbax SB C-18, 150 x 4.6 mm, 3.5-□m (or) equivalent

Flow rate : 1.0 mL/minute

Detector Wavelength : 210 nm

Injection volume : 20 µL

Column temperature : 30ºC

Elution mode : Gradient

Run time : 60 minutes

Diluent : Acetonitrile

Preparation of buffer solution: Transfer 1.0mL of ortho phosphoric acid into 1000 mL of water adjust pH to 3.0± 0.05 with dilute potassium hydroxide solution[3].

Preparation of Solvent-A: Buffer is used as Solvent-A

Preparation of Solvent-B: Prepare a mixture of buffer and acetonitrile in the ration of 20:80(v/v).

Gradient Program:

Time (Minutes)	Solvent-A(%v/v)	Solvent-B(%v/v)
0.01	75	25
10	75	25
18	55	45
25	30	70
35	20	80
45	10	90
50	75	25
60	75	25

Preparation of Standard solution:

Weigh accurately and transfer about 5.0 mg of IMPURITY Standard in to a 50 mL volumetric flask, dissolve and dilute to the volume with diluent and mix.

Dilute 1.0 mL of above solution into 100 mL volumetric flask dissolve and dilute with diluent.

Preparation of Test solution:

Weigh accurately about 25.0 mg of test sample into a 25 mL volumetric flask, dissolve and dilute to the volume with diluent. Prepare in duplicate.

Procedure:

Equilibrate the column for at least 60 minutes.

Inject diluent as blank solution into the system and record the chromatogram.

Program the data processor to inhibit the peaks due to blank solution and perform blank correction if necessary.

Inject Standard solution into the system and record the chromatogram.

Check for the system suitability acceptance criteria, if met the requirements, proceed further[4].

System suitability acceptance criteria:

The theoretical plates for the Cabazitaxel peak obtained from standard solution should be **not less than 3000.**

Calculate the % of IMPURITY by using area normalization method.

Sample Information:

S.No	Sample	~ RT (minutes)	~RRT
1.	IMPURITY	17.8	0.56
2.	CAB	31.4	1.00

VALIDATION PARAMETERS & PROCEDURE:

The following parameters should be considered for the validation.

System suitability/System precision:

Preparation of Standard stock solution: Weigh accurately about each 5.0 mg of IMPURITY standard into 100 mL volumetric flask, dissolve and dilute to the volume with diluent and mix.

Preparation of Standard solution (0.10%): Dilute 0.50 mL of standard stock solution to 25 mL with diluent and mix.

Procedure: Inject diluent as blank solution and record the chromatogram. Inject Standard solution into the system for six replicates and record the chromatograms.

Acceptance Criteria: The % RSD for the peak area of IMPURITY obtained from six replicate injections of standard solution should be not more than 10.0

The tailing factor for IMPURITY obtained from standard solution should be not more than 2.0.

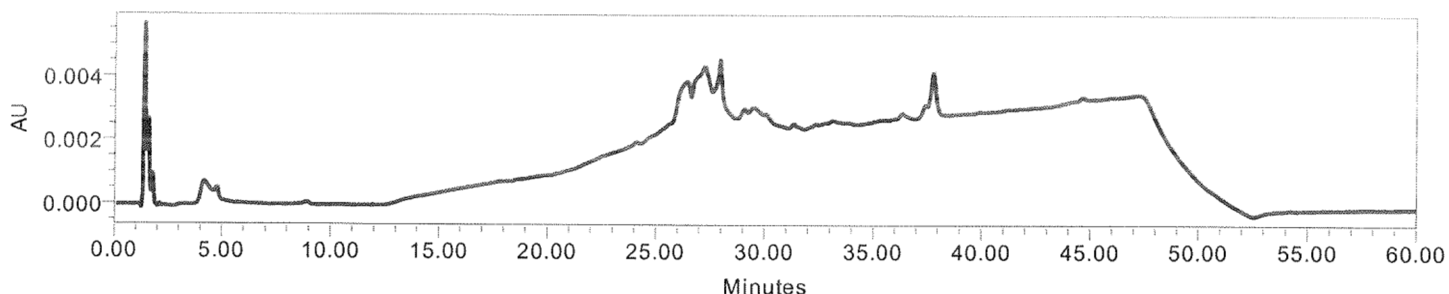

Fig-3: Blank.

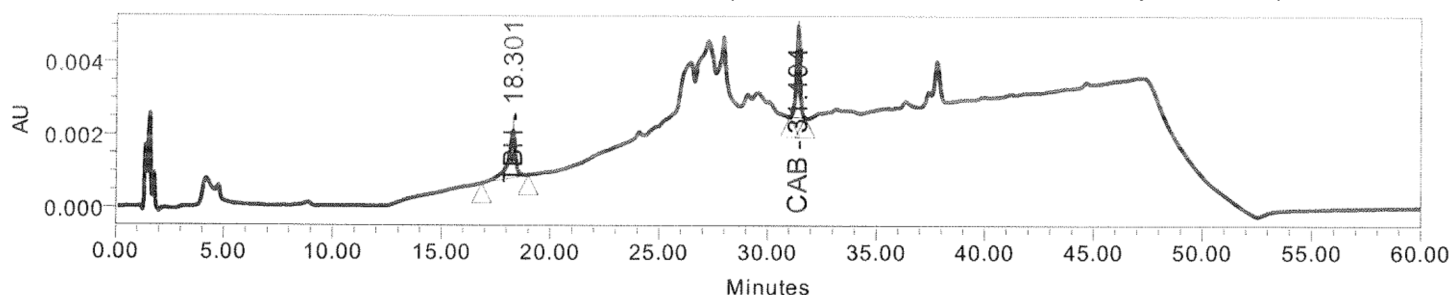

Fig-4: System Suitability Test.

Specificity:

Preparation of IMPURITY stock solution: Weigh accurately about 5.0 mg of IMPURITY standard into 100 mL volumetric flask, dissolve and dilute to the volume with diluent and mix.

Preparation of IMPURITY solution (0.10): Dilute 0.50 mL of IMPURITY stock solution to 25 mL with diluent and mix.

Preparation of Blend solution: Weigh about 25.0 mg of the test sample into 25 mL volumetric flask, add 0.50 mL of standard stock solution, dissolve and dilute to the volume with diluent and mix.

Procedure: Inject diluent as a blank and conclude the interference due to blank at the retention time of carryover impurities. Inject individual solution of IMPURITY impurity solution and blend solution into the system and record the chromatograms[5]. Establish retention time (RT) for IMPURITY obtained from individual solution and blend solution.

Acceptance Criteria: There should be no interference due to blank and possible known impurities at the retention time of corresponding carryover impurities.

Impurity should resolve from each other and possible known impurities.

The elution order and retention time (RT) of IMPURITY obtained from individual solution and the blend solution should be comparable. (± 10% Variation for retention time)

Test Solution Stability: Perform the analysis of test sample spiked with IMPURITY at limit level and carryout solution stability up to 24 hours with two different intervals.

Procedure:

Prepare and inject spiked test sample solution (Blend solution) containing IMPURITY at specification level and Palbociclib at test conc. level record the chromatogram. Inject each interval stability sample solution and record

the chromatograms. Compare the % variation of IMPURITY obtained from initial sample and each time interval of solution stability sample and calculate together. Report the solution stability in hours.

Note: After finding the time interval of solution at which is not stable no need to evaluate solution stability for the samples of remaining time intervals. The time intervals can be reduced and altered based on the intended application.

Acceptance criteria: The variation content (%) of impurity obtained from solution stability study and initial result (fresh sample) should be meet the below criteria.

If initial result is below 0.10%; variation should be within ±0.03 for impurity.

If initial result is above 0.10%; variation should be within ±0.05 for impurity.

Detection Limit (DL): This is the measurement of lowest concentration of analyte that can be detected but not to be quantified.

Preparation of DL solution: Prepare DL solution containing carryover impurities with respect to test concentration by diluting each individual standard stock solution, so as to get S/N ratio is about 3:1 to 5:1

Procedure: Inject diluent as blank solution and record the chromatogram.

Inject DL solution and record the chromatogram. Calculate the S/N ratio using the software.

Acceptance criteria: The S/N ratio should be about 3:1 to 5:1

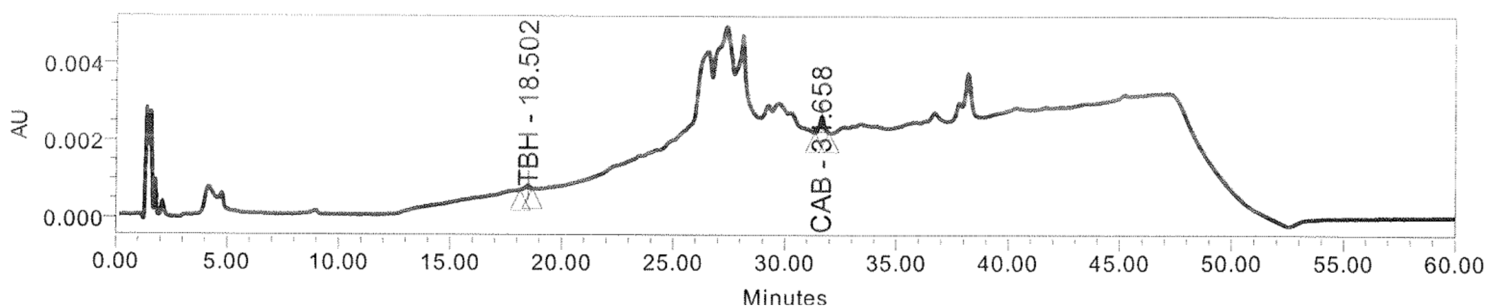

Fig-5: Detection Limit Solution.

Quantitation Limit (QL): This is the measurement of lowest concentration of analyte that can be quantified with acceptable precision.

Preparation of QL solution: Based on the S/N ratio obtained from DL solution, derive QL concentration of IMPURITY so as to get S/N ratio is about 10:1

Procedure: Inject QL solution and record the chromatogram.

Calculate the S/N ratio by using the software.

Acceptance Criteria: The S/N ratio should be about 10:1

Precision at QL:

Procedure: Inject QL solution for six replicates (six times) and record chromatograms.

Calculate % RSD for the peak area of IMPURITY obtained from six replicate injections of QL level.

Acceptance criteria: The % RSD for the peak area of IMPURITY obtained from six replicate injections of QL level should be not more than 15.0.[6]

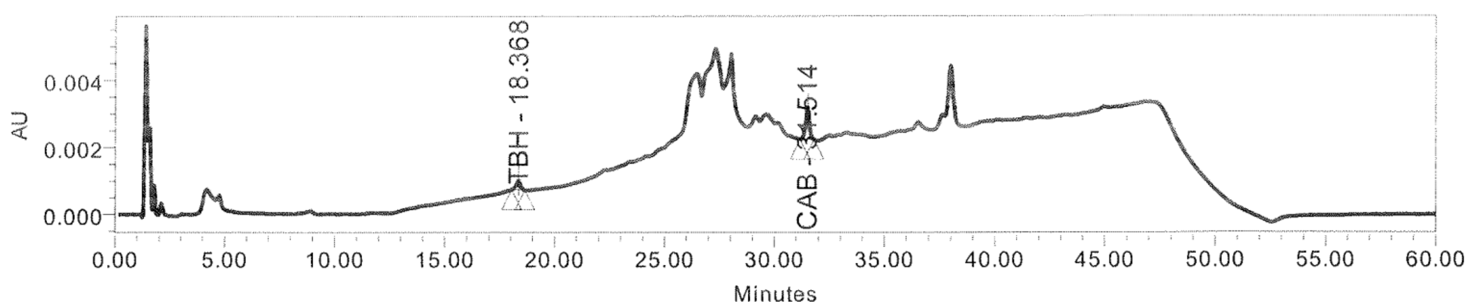

Fig-6: Quantification Limit Solution.

Accuracy at QL:

Preparation of Test solution: Weigh accurately about 25.0 mg of test sample into 25 mL volumetric flask, dissolve and dilute to the volume with diluent and mix.

Preparation of Accuracy at QL solution: Prepare the solution having 100% sample and spike the IMPURITY at QL level.

Note: Should prepare in triplicates.

Procedure: Prepare and inject test solution in single, Accuracy at QL level in triplicate and record the chromatograms. Calculate the % recovery of IMPURITY.

Acceptance criteria: The % recovery should be between 70 and 130 at QL level.

Linearity: Perform linearity with different concentrations of IMPURITY by analyzing a minimum six concentrations i.e. QL, 50%, 75%, 100%, 125% and 150% w.r.to limit level.

Preparation of Linearity stock solution: Weigh accurately about each 5.0 mg of IMPURITY standard into

100 mL volumetric flask, dissolve and dilute to the volume with diluent and mix.

Preparation of Linearity level -1 solution (QL): Prepare and inject QL solution in dupilcate. (or) Consider first two injections of Precision at QL.

Preparation of Linearity level -2 solution: Take 0.50 mL of linearity stock solution into 50 mL volumetric flask, dilute to the volume with diluent and mix.

Preparation of Linearity level -3 solution: Take 0.75 mL of linearity stock solution into 50 mL volumetric flask, dilute to the volume with diluent and mix.

Preparation of Linearity level -4 solution: Take 1.0 mL of linearity stock solution into 50 mL volumetric flask, dilute to the volume with diluent and mix.

Preparation of Linearity level -5 solution: Take 1.25 mL of linearity stock solution into 50 mL volumetric flask, dilute to the volume with diluent and mix.

Preparation of Linearity level -6 solution: Take 1.5 mL of linearity stock solution into 50 mL volumetric flask, dilute to the volume with diluent and mix.

Procedure: Inject QL to level-6 solution in duplicate and record the chromatograms. Plot linearity graph between average peak areas of duplicate injections against concentration of IMPURITY determine the correlation coefficient value for IMPURITY.

Acceptance Criteria: Correlation coefficient value should be not less than 0.99,

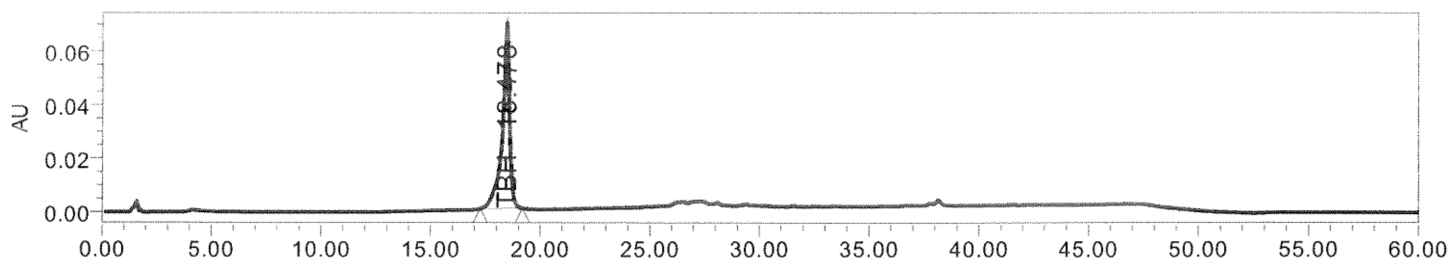

Fig-7: Linearity Solution.

Accuracy:

Preparation of Accuracy stock solution: Weigh accurately about each 5.0 mg of IMPURITY standard into 100 mL volumetric flask, dissolve and dilute to the volume with diluent and mix.

Preparation of Test solution: Weigh accurately about 50.0 mg of test sample into 50 mL volumetric flask, dissolve and dilute to the volume with diluent and mix.

Preparation of Accuracy at 50% level solution: Weigh accurately about 50.0 mg of test sample into 50 mL volumetric flask, add 0.50 mL of accuracy stock solution, dissolve and dilute to the volume with diluent. (This solution contains 0.05 IMPURITY with respect to test solution concentration which covers the 50% of specification level).[7]

Preparation of Accuracy at 100% level solution: Weigh accurately about 50.0 mg of test sample into 50 mL volumetric flask, add 1.0 mL of accuracy stock solution, dissolve and dilute to the volume with diluent.

(This solution contains 0.10% of IMPURITY with respect to test solution concentration which covers the 100% of specification level)

Preparation of Accuracy at 150% level solution: Weigh accurately about 50.0 mg of test sample into 50 mL volumetric flask, add 1.5 mL of accuracy stock solution, dissolve and dilute to the volume with diluent.

(This solution contains 0.15% of IMPURITY with respect to test solution concentration which covers the 150% of specification level)

Note: Should prepare Accuracy at 50% and 150% levels in triplicate preparations and Accuracy at 100% level in six preparations. Use first three preparations of Accuracy at 100% level for accuracy study and total six preparations for method precision study.

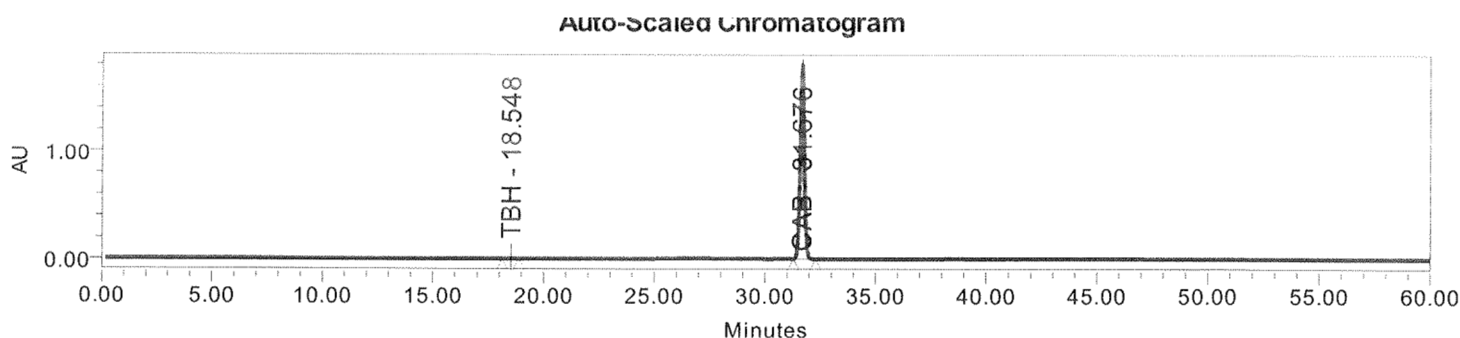

Fig-8: 100% spike solution.

Procedure:

Inject test solution in single, each accuracy at 50% and 150% levels in triplicate preparations and Accuracy at 100% level in six preparations. Calculate the % recovery of carryover impurity.

Acceptance criteria: The % recovery should be between 80 and 120 for 50%, 100% & 150% level.

Calculation for % Recovery:

$$\text{Impurity Calculation (\%)} = \frac{AU}{AS} \times \frac{CS}{CU} \times \text{Potency (\%)}$$

$$\text{\% Recovery} = \frac{\text{Content in spiked sample (\%)} - \text{Content in test sample (\%)}}{\text{Concentration added (\%)}} \times 100$$

Where,

AU = Peak area of IMPURITY in spiked test solution

AS = Average area of IMPURITY in standard solution

CU = Concentration of spiked test solution (mg/mL)

CS = Concentration of IMPURITY in standard solution (mg/mL)

P = Purity / Potency of IMPURITY standard (%)

Method Precision: Perform the analysis by spiking the test sample with IMPURITY at specification level in six different preparations and determine the method precision.[8]

Note: The data from the Accuracy at 100% level six preparations can be used for method precision.

Procedure: Prepare and inject spiked test sample in six different preparations into the system and record the chromatograms. (Consider the Accuracy at 100% level in six preparations) Calculate the % of IMPURITY in each preparation as per the method. Calculate the % RSD for the results obtained from the method precision study.

Acceptance criteria: The % RSD for the results obtained from method precision study should be not more than 10.0

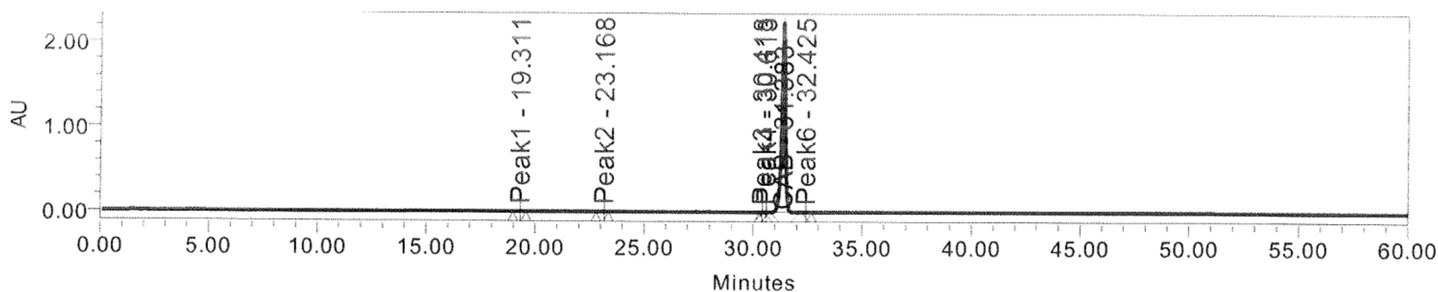

Fig-9: Cabazitaxel API Sample.

Intermediate Precision:

Carry out the precision study on a different day, with different instrument, different analyst and different column using with fresh preparations.

Procedure: Establish the system suitability as per the method. Prepare and inject standard solution in six replicates and record the chromatograms. Prepare and inject spiked test sample solution in six preparations (which is analyzed under method precision study) and record the chromatograms. Calculate the % of IMPURITY in each preparation as per the method. Calculate the % RSD for the results obtained from the intermediate precision study. Calculate the cumulative % RSD for the results obtained from method precision study and intermediate precision study.[9]

Acceptance criteria: System suitability should comply as per the method. The % RSD for the results obtained from intermediate precision study should be not more than 10.0. The cumulative % RSD for the results obtained from method precision study and intermediate precision study should be not more than 15.0.[10]

Conclusion:

In the present research, a fast, simple, accurate, precise, and linear stability-indicating HPLC method has been developed and validated for the content in final active pharmaceutical ingredient of cabazitaxel, and hence it can be employed for routine quality control analysis. The analytical method conditions and the mobile phase solvents provided good resolution for impurity sample and final sample. In addition, the main features of the developed method robust enough to reproduce accurate and precise results under different chromatographic conditions based on ICH guidelines[11]. Conclude from the above studies whether the method is valid and suitable for the determination of byproduct of Cabazitaxel by HPLC.

References:

1. Galsky MD, Dritselis A, Kirkpatrick P, Oh WK: Cabazitaxel. Nat Rev Drug Discov. 2010 Sep;9(9):677-8.
2. Kort A, Hillebrand MJ, Cirkel GA, Voest EE, Schinkel AH, Rosing H, Schellens JH, Beijnen JH: Quantification of cabazitaxel, its metabolite docetaxel and the determination of the demethylated metabolites RPR112698 and RPR123142 as docetaxel equivalents in human plasma by liquid chromatography-tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci. 2013 Apr 15; 925:117-23.

3. Nightingale G, Ryu J: Cabazitaxel (jevtana): a novel agent for metastatic castration-resistant prostate cancer. P T. 2012 Aug;37(8):440-8.
4. Kommineni, Nagavendra, Shaheen Mahira, Abraham J. Domb, and Wahid Khan. 2019. "Cabazitaxel-Loaded Nanocarriers for Cancer Therapy with Reduced Side Effects" *Pharmaceutics* 11, no. 3: 141.
5. Summers N, Vanderpuye-Orgle J, Reinhart M, Gallagher M, Sartor O. Efficacy and safety of post-docetaxel therapies in metastatic castrate-resistant prostate cancer: A systematic review of the literature. Current Medical Research and Opinion. 2017;33(11):1995–2008. pmid:28604117.
6. Petrylak DP, Tangen CM, Hussain MH, et al: Docetaxel and estramustine compared with mitoxantrone and prednisone for advanced refractory prostate cancer. N Engl J Med 351:1513-1520, 2004.
7. Vrignaud P, Sémiond D, Lejeune P, et al: Preclinical antitumor activity of cabazitaxel, a semisynthetic taxane active in taxane-resistant tumors. Clin Cancer Res 19:2973-2983, 2013.
8. Cabazitaxel Versus Docetaxel As First-Line Therapy for Patients With Metastatic Castration-Resistant Prostate Cancer: A Randomized Phase III Trial—FIRSTANA, Stéphane Oudard et al., J Clin Oncol, 2017.
9. Reduced-Dose Cabazitaxel After Docetaxel in Metastatic Castration-Resistant Prostate Cancer , By Matthew Stenger et al., Prostate Cancer, 2017.
10. Center for Drug Evaluation and Research: NDA 20-1023 review: Cabazitaxel. Updated 2016.
11. Continuing cabazitaxel beyond 10 cycles for metastatic castrate-resistant prostate cancer: is there a benefit? Loma AL-Mansouri et al., Eur J Hosp Pharm Sci Pract, 2021.

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