Scientific Research Publishing

American Journal of Analytical Chemistry, 2024, 15, 333-346
https://www.scirp.org/journal/ajac
ISSN Online: 2156-8278
ISSN Print: 2156-8251

Development and Validation of a UPLC Method for the Determination of Docetaxel and Its Related Substances in Pharmaceutical Dosage Forms, an Antineoplastic Agent

Srinivasulu Kasa[1*], Sreenivas Pippalla[2], Mopidevi Narasimha Naidu[3], Dipak Goyal[4]

[1]Department of Chemistry, Osmania University, Hyderabad, India
[2]Department of Chemistry, Sikkim Professional University, Gangtok, India
[3]Dr. Reddy's Laboratories Ltd., Hyderabad, India
[4]Department of Chemistry, University of Massachusetts, Massachusetts, USA
Email: *kasas82003@gmail.com

How to cite this paper: Kasa, S., Pippalla, S., Naidu, M.N. and Goyal, D. (2024) Development and Validation of a UPLC Method for the Determination of Docetaxel and Its Related Substances in Pharmaceutical Dosage Forms, an Antineoplastic Agent. *American Journal of Analytical Chemistry*, **15**, 333-346.
https://doi.org/10.4236/ajac.2024.1510021

Received: September 26, 2024
Accepted: October 27, 2024
Published: October 30, 2024

Abstract

A novel, simple, and sensitive Ultra Performance Liquid Chromatography (UPLC) method was developed and validated for the quantification of process-related impurities and degradants, as well as the assay of Docetaxel. The stability-indicating capability of the method was demonstrated through forced degradation studies and a comprehensive mass balance evaluation. Chromatographic separation was achieved using an ACQUITY UPLC BEH C18 column (100 × 2.1 mm, 1.7 µm), with gradient elution. The mobile phase A comprised a mixture of water, methanol, and acetonitrile (500:300:200, v/v/v), while mobile phase B was acetonitrile and water (800:200, v/v). The flow rate was set at 0.4 mL/min, with detection at 232 nm using a photodiode array detector. The method exhibited excellent performance, with a tailing factor of 1.10 for Docetaxel. The method was rigorously validated for precision, accuracy, linearity, LOD, LOQ, ruggedness, specificity, and robustness. Forced degradation studies confirmed the method's suitability for stability analysis. Stability testing on the drug substance was conducted following ICH guidelines.

Keywords

UPLC, Method Development, Validation, Docetaxel, Impurities, ICH Guidelines

1. Introduction

Docetaxel is a chemotherapeutic agent belonging to the taxane class, a semi-

synthetic analogue of paclitaxel (Taxol), originally derived from the bark of the rare Pacific yew tree (*Taxus brevifolia*) [1]. Due to the limited availability of paclitaxel, extensive research led to the development of docetaxel—a derivative synthesized from 10-deacetyl baccatin III, which is readily extracted from the renewable European yew tree. Structurally, docetaxel differs from paclitaxel in two positions. It possesses a hydroxyl group at carbon 10, while paclitaxel has an acetate ester, and it contains a tert-butyl carbamate ester on the phenylpropionate side chain instead of paclitaxel's benzyl amide. These structural variations make docetaxel more water-soluble than paclitaxel [1].

Docetaxel is an antineoplastic agent from the taxoid family, semisynthetically derived from the renewable needle biomass of yew plants. TAXOTERE (docetaxel) Injection Concentrate is a sterile, non-pyrogenic, clear yellow to brownish-yellow viscous solution available in single-dose vials containing either 20 mg (0.5 mL) or 80 mg (2 mL) docetaxel (anhydrous). Each mL contains 40 mg docetaxel (anhydrous) and 1040 mg polysorbate 80. Prior to administration, TAXOTERE must be diluted with a supplied diluent that contains 13% ethanol in water for injection.

Several analytical methods have been reported for the quantification of docetaxel in both bulk drug and formulated products. These methods include chromatographic techniques with using UV detector [2]. Other reported methods of analysis are reverse phase [3] [4], ion pair [5] HPLC methods, injection dosage forms [6] [7] and spectrophotometric method for the determination of Docetaxel in pharmaceutical dosage forms [8]. Isolation and characterization of some process related impurities [9] and degradation impurities of docetaxel are also published [10]. A stability indicating HPLC assay method for Docetaxel has been reported [11]. A method for estimation of related substances of Docetaxel trihydrate drug substance is also published in Pharmeuropa [12]. The methods for the determination of Docetaxel in human plasma and HPLC are available in literature [13]-[17]. Evaluation of the Pharmaceutical Quality of Docetaxel Injection Using New Stability Indicating Chromatographic Methods for Assay and Impurities is aviable in literature [18]. One UPLC-MS/MS method is available for determination of total docetaxel from a lipid microsphere formulation in human plasma [19].

Despite the available literature, no existing analytical methods effectively separate all known related compounds and degradation impurities of docetaxel. Additionally, current methods involving LC-MS/MS and LC-MS are often expensive and require intricate procedures, making them less viable for routine quality control. Ultra-performance liquid chromatography (UPLC) offers a cost-effective and time-efficient alternative for such analyses.

Therefore, the objective of this research was to develop a novel, selective, and stability indicating UPLC method for the determination of docetaxel and its impurities pharmaceutical products. The method was validated in accordance with USP <1225> "Validation of Compendial Procedures" and the International Conference on Harmonization (ICH) guidelines Q2 (R1) for the validation of analytical

procedures [20] [21]. This method aims to be a more time- and cost-efficient alternative to the existing techniques, offering better reproducibility and suitability for routine quality control analyses.

2. Experimental

2.1. Materials

Acetonitrile (HPLC grade; Merck, India), Methanol (HPLC grade; Merck, India) and highly pure water were from a Milli-Q water purification system from Millipore (Billerica, MA). Docetaxel and its nine impurities (10-DAB-III, N-Formyl, 2'3'-Epi Docetaxel, 2'-Docetaxel, Imp-A, Imp-B, Imp-C, Imp-D, and 10-Dec Docetaxel) were provided by the Process Research Department at Dr. Reddy's Laboratories, Hyderabad, India. Sodium hydroxide, hydrochloric acid, and hydrogen peroxide were also sourced from Merck.

2.2. Equipment

The analysis was carried out using Waters Acquity UPLC system (Waters, Milford, MA, USA), equipped with a quaternary gradient pump, autosampler, column oven, and photodiode array detector. Data processing was done using Empower software (Waters). The UPLC column used for method development was Acquity UPLC BEH-C18 (2.1 mm × 100 mm, 1.7 µm).

Stability studies were performed in humidity chambers (75% RH, 40˚C, and 65% RH, 25˚C) from Thermolab (India), and photo stability studies in a chamber from Sanyo (UK). Thermal stability tests were conducted in a dry air oven from Mack pharmatech (Hyderabad, India).

LC-MS/MS analysis was done in positive ionization mode with capillary and cone voltages set to 3.5 kV and 25 V.

2.3. Chromatographic Conditions

A new gradient method was developed for separating process impurities of Docetaxel from its degradation peaks, thus proving the method to be stability indicating. The chromatographic method employed mobile phase A, comprised a mixture of water, methanol, and acetonitrile (500:300:200, v/v/v), while mobile phase B was acetonitrile and water (800:200, v/v). The method employed the gradient programs listed in **Table 1** for the analysis of Assay and impurities. The method was developed by using an Acquity UPLC BEH-C18 (2.1 mm × 100 mm, 1.7 µm). The flow rate of the mobile phase was 0.4 mL/min. The column temperature was maintained at 25˚C, and the detection wavelength was monitored at 232 nm. The injection volume was 5 µL. A mixture of acetonitrile-water (1:1, v/v) was used as diluent.

2.4. Sample Preparations for Assay and Impurities

A stock solution of Docetaxel standard was prepared at 0.5 mg/mL for analysis of

related substances and assay determination. A stock solution of impurities (mixture of 10-DAB-III, N-Formyl, 2'3'-Epi Docetaxel, 2'-Docetaxel, Imp-A, Imp-B, Imp-C, Imp-D and 10-Dec Docetaxel) at 0.1 mg/mL was also prepared in the diluent. A solution of Docetaxel test sample was prepared at 500 µg/mL for analysis of related substances and for assay determination.

Table 1. UPLC gradient program for analysis of assay and impurities.

Time (min)	Flow rate (mL/min)	Mobile phase A (%)	Mobile phase B (%)
0.01	0.4	90	10
2.0	0.4	90	10
10.0	0.4	70	30
15.0	0.4	40	60
15.1	0.4	90	10
18.0	0.4	90	10

3. Method Development and Optimization

3.1. Forced Degradation Studies and Method Development

Forced degradation studies were conducted to develop a stability indicating UPLC method for quantifying and assessing the purity of Docetaxel. Samples from forced degradation, along with nine impurities (10-DAB-III, N-Formyl, 2'3'-Epi Docetaxel, 2'-Docetaxel, Imp-A, Imp-B, Imp-C, Imp-D, and 10-Dec Docetaxel are shown in Figure 1), were used for the LC method development (Table 2).

Impurities A and C were identified as key process-related impurities in the Docetaxel samples. The goal of the method was to achieve effective separation of 2'3'-Epi Docetaxel, 2'-Docetaxel, Imp-A, and other impurities from Docetaxel. Once a satisfactory separation was achieved, forced degradation studies ensured the method's stability-indicating power. The method was verified on different stationary phases for robustness.

3.2. Detector Selection and Initial Wavelength

The UV spectra of Docetaxel and its nine impurities were scanned at 100 ppm concentrations in methanol. All compounds showed maximum absorbance around 232 nm, making this the chosen detection wavelength for method development (see Figure 2).

Comparison of in-House vs. USP/Ph. Eur Methods: The in-house method developed for Docetaxel impurity analysis offers advantages over USP and Ph. Eur methods, which have poor selectivity and fail to separate key impurities effectively. The UPLC method, with optimized mobile phase composition, gradient program, and column temperature, successfully separated all process-related impurities, carryover analogs, epimers, and degradation products.

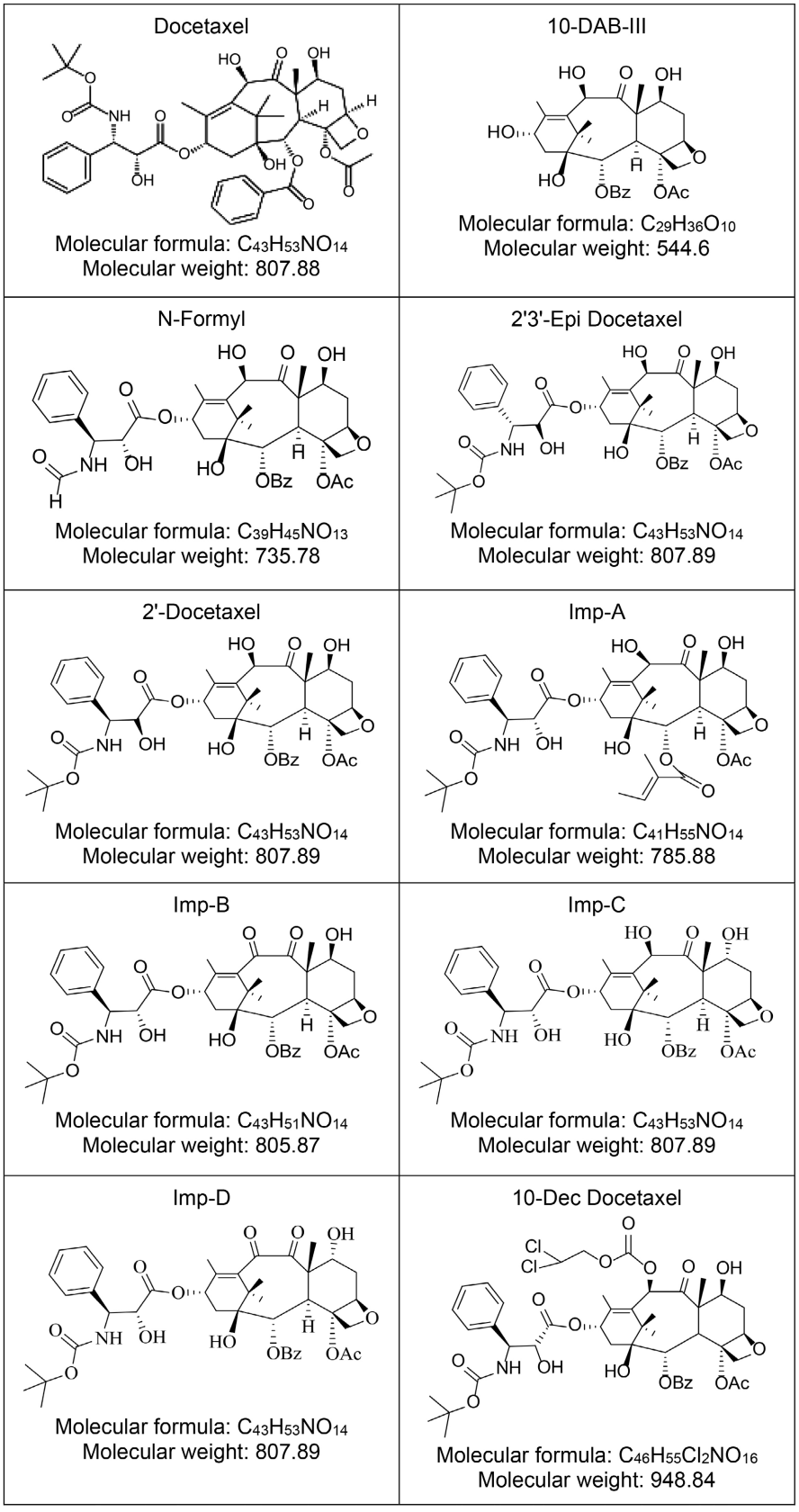

Figure 1. Structures of Docetaxel and its nine impurities.

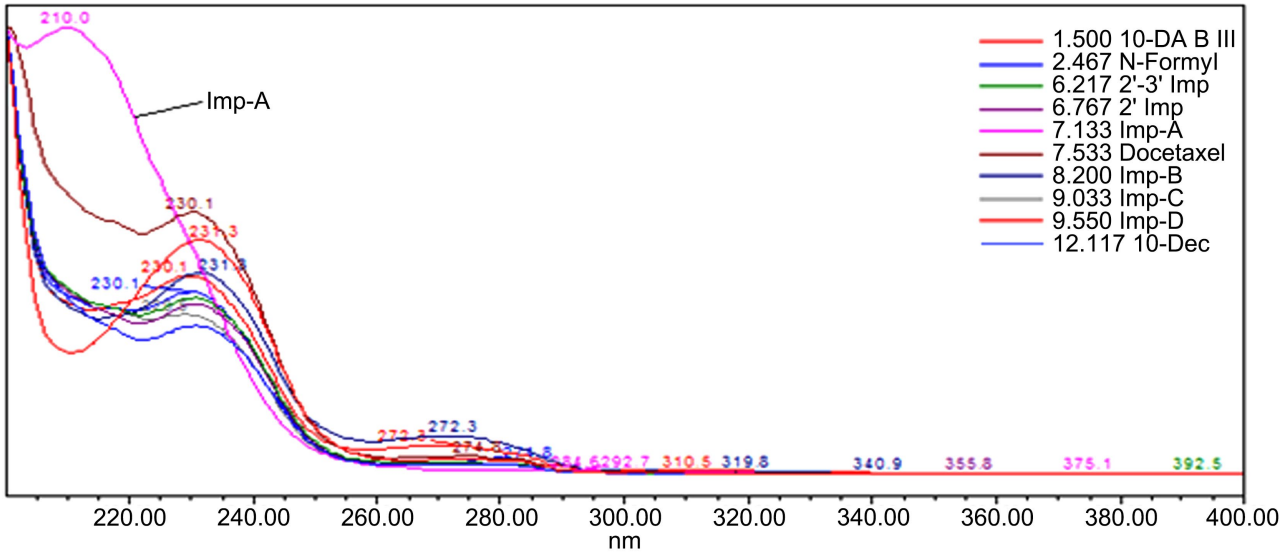

Figure 2. UV spectral overlay data of all impurities and active.

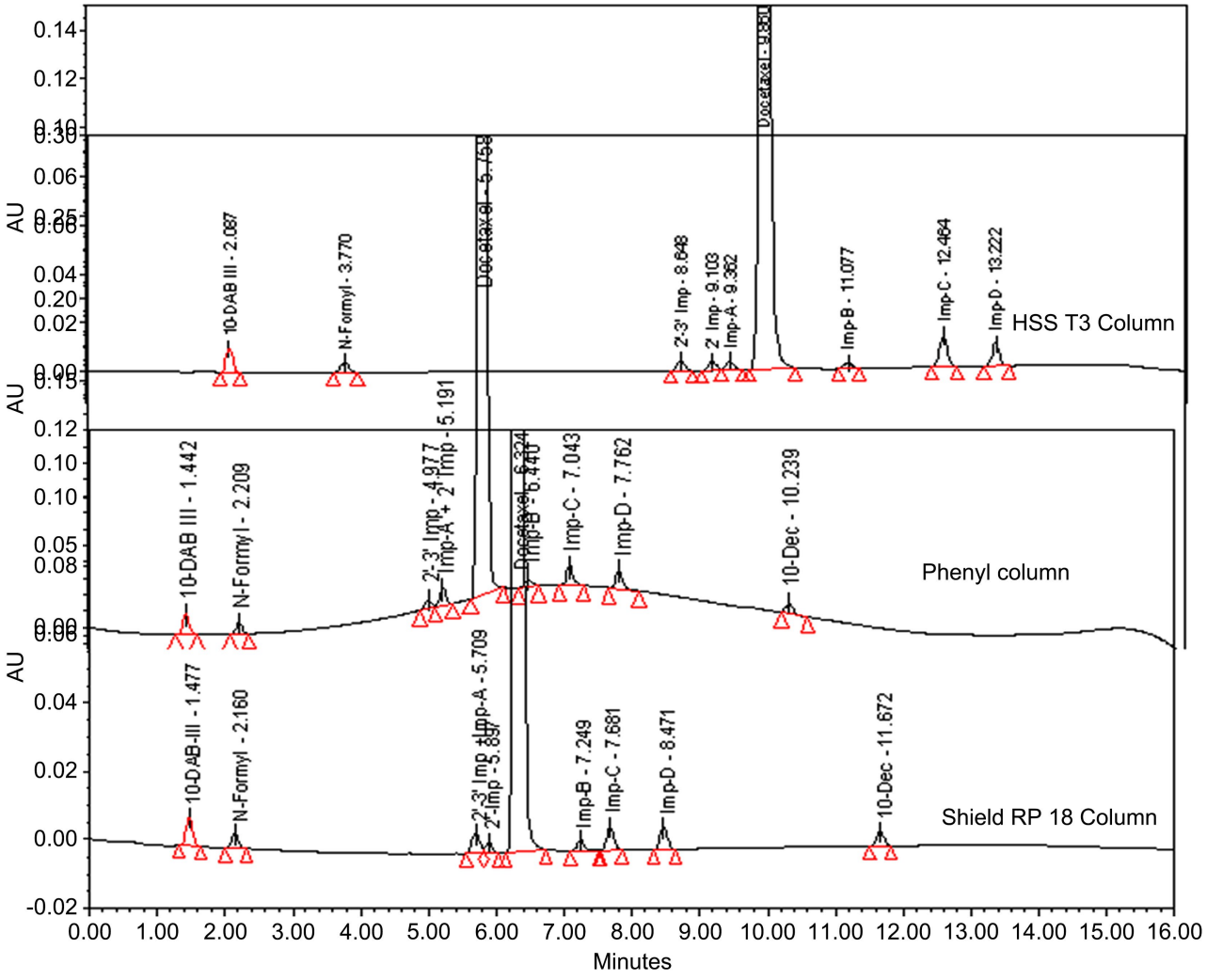

Figure 3. Effect of column chemistry on separation of Docetaxel and its impurities.

Buffer, pH, and Mobile Phase Selection: A variety of mobile phases, including mixtures of acetonitrile, methanol, and water, were tested for selectivity and resolution. The final mobile phase A comprised water, acetonitrile, and methanol (50:30:20, %v/v/v), while mobile phase B was acetonitrile and water (80:20, v/v). This combination provided optimal separation.

Column Selection and Gradient Program: Various columns were evaluated, including Waters BEH Shield RP18, Waters BEH Phenyl, and Waters HSS T3. However, only the Waters BEH C18 column (100 × 2.1 mm, 1.7 µm) provided satisfactory resolution (see Figure 3). The gradient program was optimized to achieve separation within 18 minutes, improving speed and cost-efficiency compared to the 50-minute run time in the pharmacopoeia.

System Suitability: The system suitability criteria were defined by the resolution between Impurity-A and Docetaxel (see Figure 4), with a requirement for a resolution greater than 2.5 and a tailing factor of less than 1.5 for the Docetaxel peak.

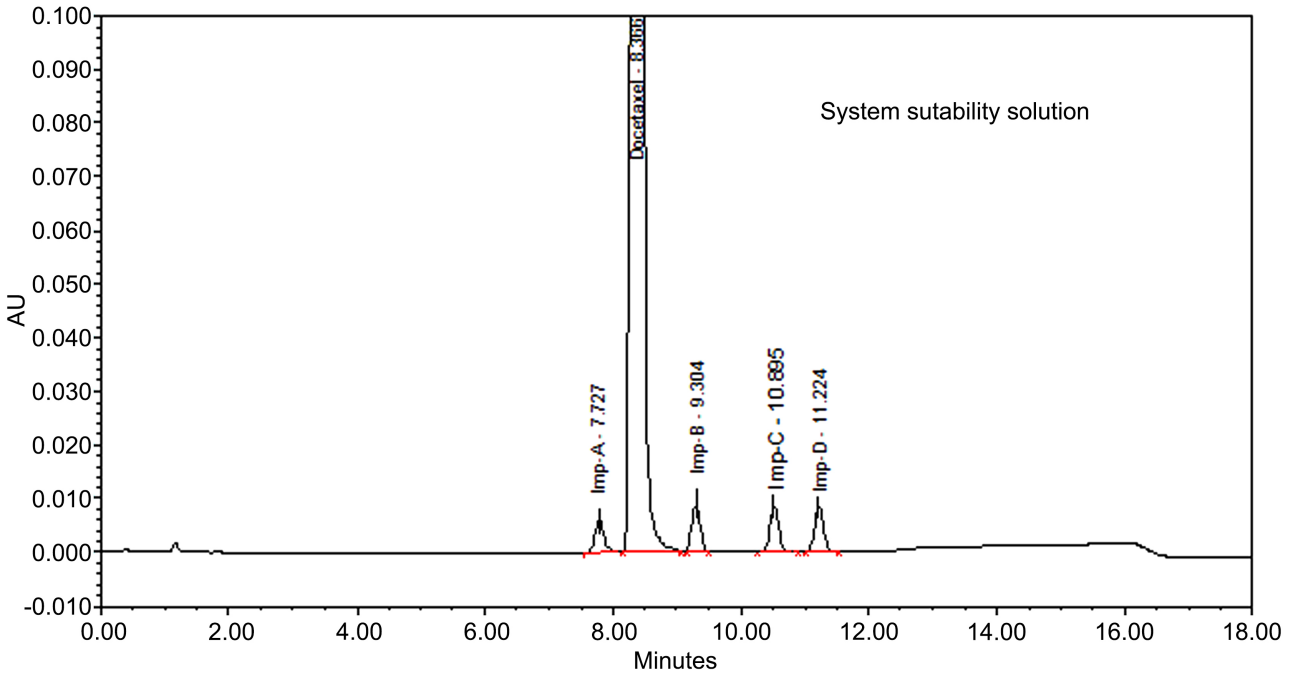

Figure 4. System suitability chromatogram of (control strategy of method) UPLC method.

4. Discussion

Based on the final results, optimal separation was achieved using the ACQUITY UPLC BEH C18 column (100 × 2.1 mm, 1.7 µm particle size) with mobile phase A consisting of water, methanol, and acetonitrile in the ratio of 50:30:20 (%v/v/v), and mobile phase B consisting of water and acetonitrile in the ratio of 20:80 (v/v). A gradient program was employed as follows: Time (t)/% Solvent B: 0.01/10, 2.0/10, 10.0/30, 15.0/60, 15.1/10, and 18.0/10. The analyte detection was performed at a wavelength of 232 nm, ensuring successful separation of Docetaxel from its impurities and degradation products (see Figure 5).

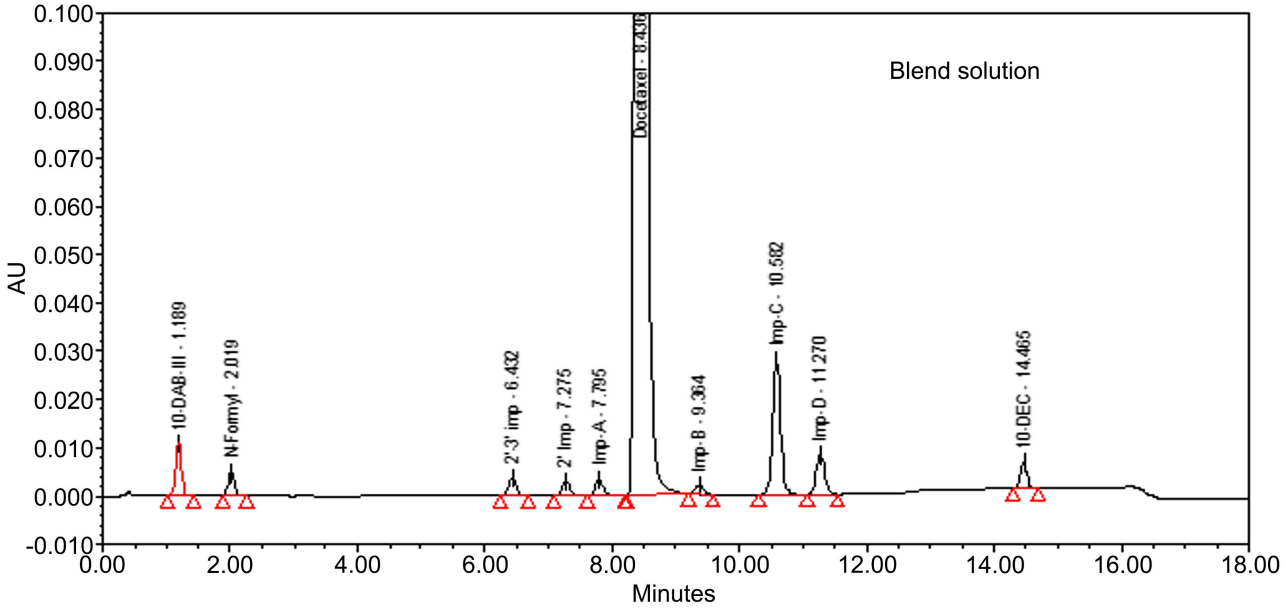

Figure 5. Typical chromatogram of impurity spiked solution.

The system suitability test (SST) results demonstrated the following:

- USP resolution between Impurity-A and the Docetaxel peak exceeded 2.5.
- The tailing factor for the Docetaxel peak was no more than 1.5.

Figure 4 and Figure 5 in the UPLC chromatograms show satisfactory separation of all components.

Forced degradation studies were conducted to confirm the method's stability-indicating capability.

Table 2. Forced degradation conditions.

S.No.	Stressed Condition	Description
1	Water Degradation	Drug solution in water was maintained at 90˚C for 3 days.
2	Oxidation (30% H_2O_2)	Drug solution in 30% v/v H_2O_2 was stirred at room temperature for 2 days.
3	Acid Hydrolysis (0.1N HCl)	Drug solution in 0.1N HCl was maintained at room temperature for 16 hours.
4	Base Hydrolysis (0.001N NaOH)	Drug solution in 0.001N NaOH was tested immediately.
5	Photo Degradation (Without "Al" Foil & With "Al" Foil)	Photo degradation was carried out by exposing the drug substance in solid state to light, providing an overall illumination of no less than 1.2 million lux hours and integrated near-UV energy of no less than 200 W h/m^2, over a period of 10 - 11 days in a photostability chamber.
6	Thermal Degradation	The drug substance was subjected to dry heat at 105˚C for 10 days.

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities [22]. One lot of Docetaxel product was selected for stress testing. As per the ICH stability guidelines (Q1AR2): "Stress testing is likely to be carried out on a single batch of material (uniform material throughout the study)." Various stress conditions—heat (based on the melting point, *i.e.*, 90˚C), acid (HCl), base (NaOH), oxidative (hydrogen peroxide), and light (sunlight/UV 254 nm)—were employed according to the guidance provided in the ICH stability guidelines. The details of the stress conditions applied are as follows.

5. Method Validation, Results and Discussion

The UPLC method was validated following ICH guidelines.

Degradation Conditions and Mass Balance of Docetaxel

Degradation Condition	Time	Assay (%w/w on anhydrous basis)	(Total impurities %w/w)	Mass Balance
Thermal treatment (105˚C)	10 days	98.3	2.1	100.4
Photo degradation (Open stress)	As per ICH Q1B	87.2	12.2	99.4
Photo degradation (Closed stress)	As per ICH Q1B	99.5	0.56	100.1
Acid hydrolysis (0.1N HCl, 27˚C)	16 hours	93.4	7.4	100.8
Base hydrolysis (0.001N NaOH, 27˚C)	Immediately	92.5	6.6	99.1
Oxidation (30% H_2O_2)	2 days	94.9	5.1	100.0
Water hydrolysis (90˚C)	3 days	98.8	2.3	101.1

Purity1 Angle, Threshold, and Flag Values

Stressed Condition	Purity1 Angle	Purity1 Threshold	Purity1 Flag
Normal	0.344	0.611	No
Impurities spiked sample	0.398	0.572	No
Thermal treatment (105˚C)	0.289	0.357	No
Photo degradation (Open stress)	0.256	0.339	No
Photo degradation (Closed stress)	0.297	0.376	No
Acid hydrolysis	0.570	0.650	No
Base hydrolysis	0.702	0.736	No
Oxidation	0.310	1.010	No
Water hydrolysis	0.409	0.460	No

In summary, the degradation studies confirm that the mass balance for Docetaxel is consistent under various stressed conditions. The Purity1 values indicate that no significant co-elution or impurities were detected during these studies.

System Suitability Test (SST)

- A mixture of Docetaxel and its impurities was injected into the UPLC system.
- **Resolution** between Docetaxel and Impurity-A: 3.0.
- **Tailing factor**: 1.0.
- **% RSD** for replicate injections: 0.7.

Precision

- **Assay Precision**: Six independent assays of Docetaxel showed a %RSD of 0.4, indicating high repeatability (see Figure 6).
- **Related Substances Precision**: %RSD for various impurities ranged from 0.4% to 3.7%, demonstrating reliable repeatability (see Figure 7).

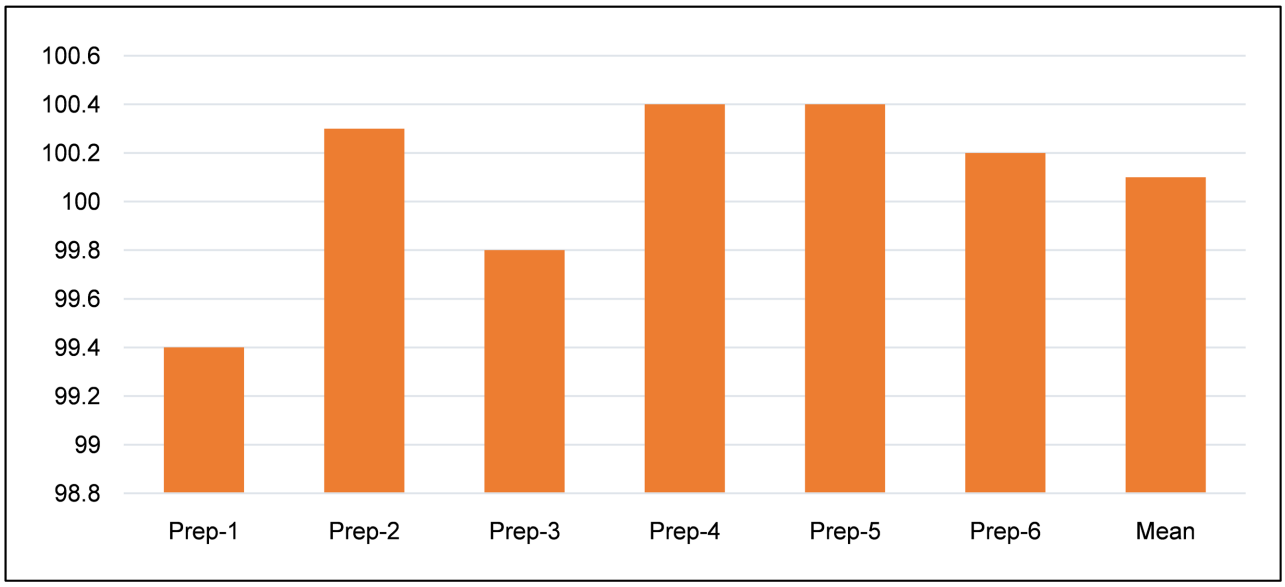

Figure 6. Graphical representation of assay method precision study.

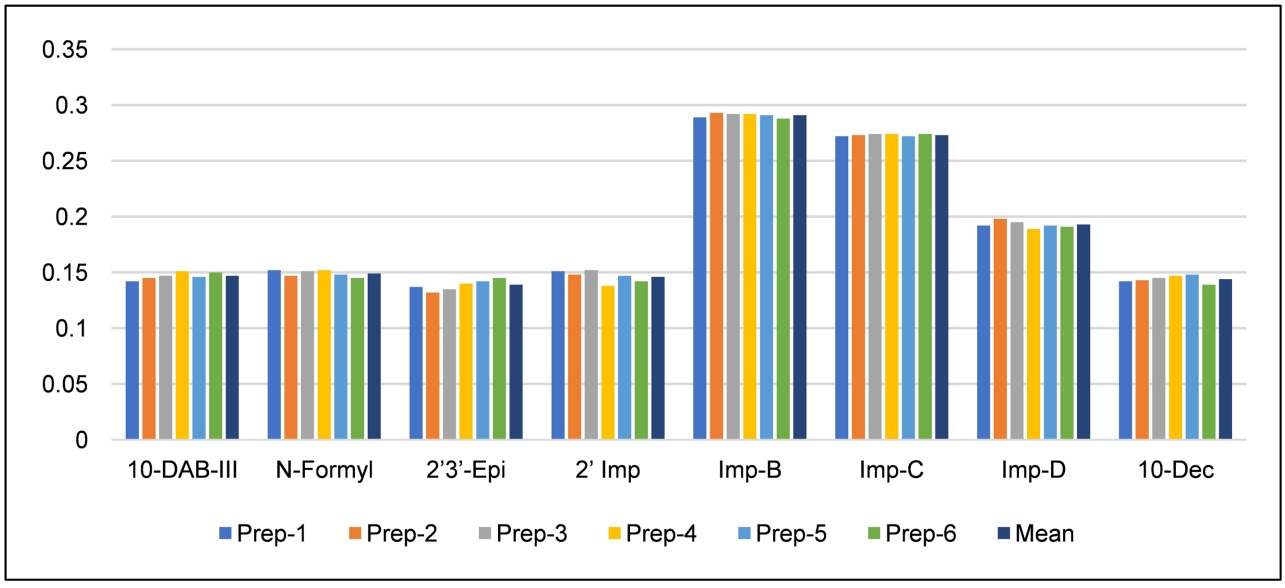

Figure 7. Graphical representation of impurities precision study results.

Limit of Quantification (LOQ) and Limit of Detection (LOD)

- **LOD**: Lowest concentration detected for impurities ranged from 0.004% to 0.006%.
- **LOQ**: Lowest concentration quantifiable with precision ranged from 0.015% to 0.023%.
- **Precision and Accuracy at LOQ**: %RSD for impurities at LOQ was less than 2.3%, ensuring good precision and Accuracy at LOQ ranged from 98.4% to 100.6% (see Figure 8).

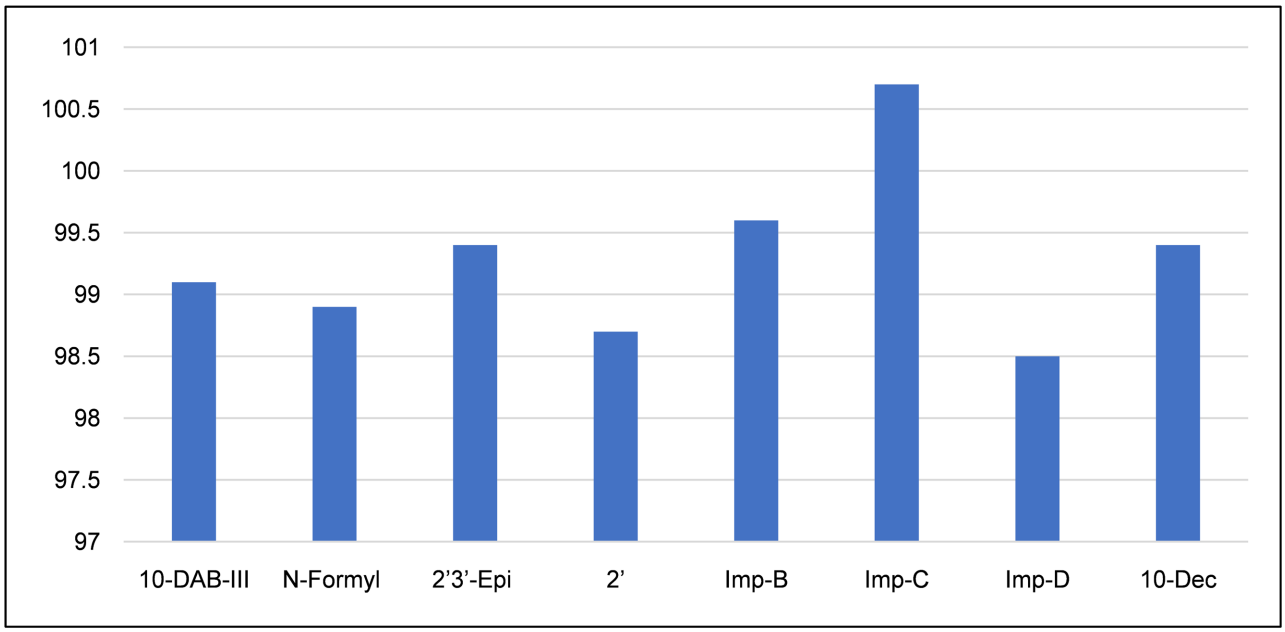

Figure 8. Graphical representation of impurities accuracy at LOQ.

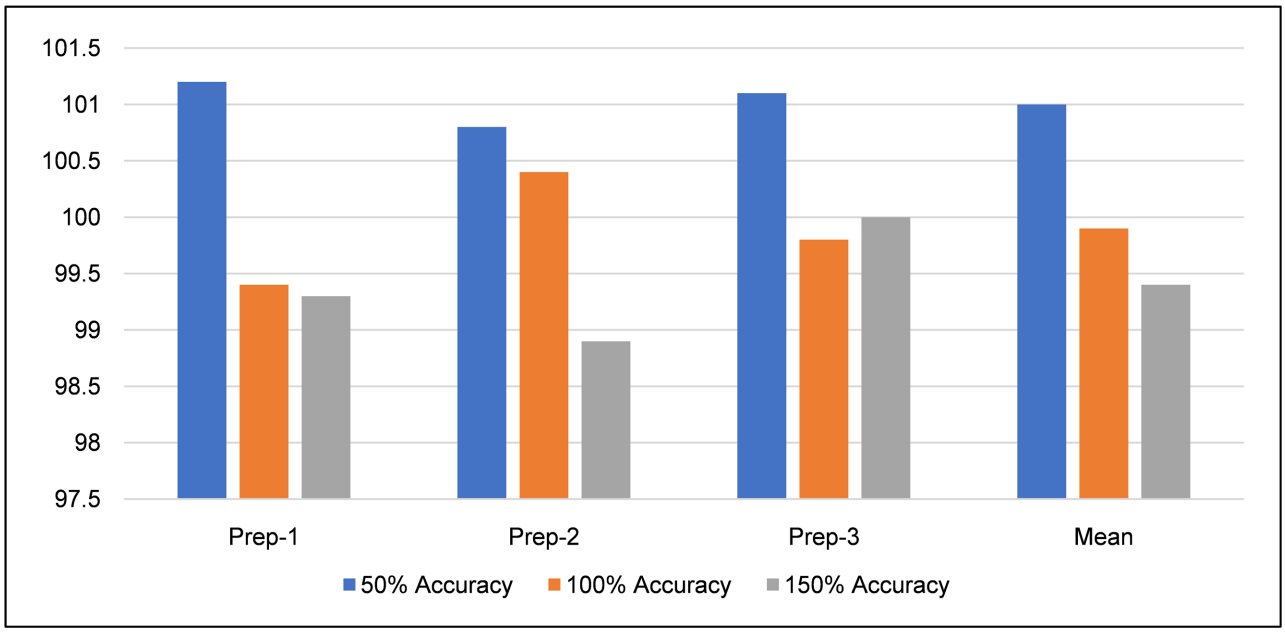

Figure 9. Graphical representation of assay accuracy.

Linearity

- **Assay Method**: Linear response with a correlation coefficient of 0.9999 for Docetaxel concentrations ranging from 50% to 150% of the target.
- **Related Substances Method**: Linear responses with correlation coefficients ranging from 0.999 to 0.9999 for impurities across their concentration ranges.

Accuracy

- **Assay Method**: Mean recoveries for Docetaxel were 99.4% to 101.0% (see Figure 9) across concentrations (50%, 100%, and 150%).
- **Related Substances Method**: Mean recoveries of impurities ranged from 93.6% to 101.0% (see Figure 10) at different levels (50%, 100%, and 150%).

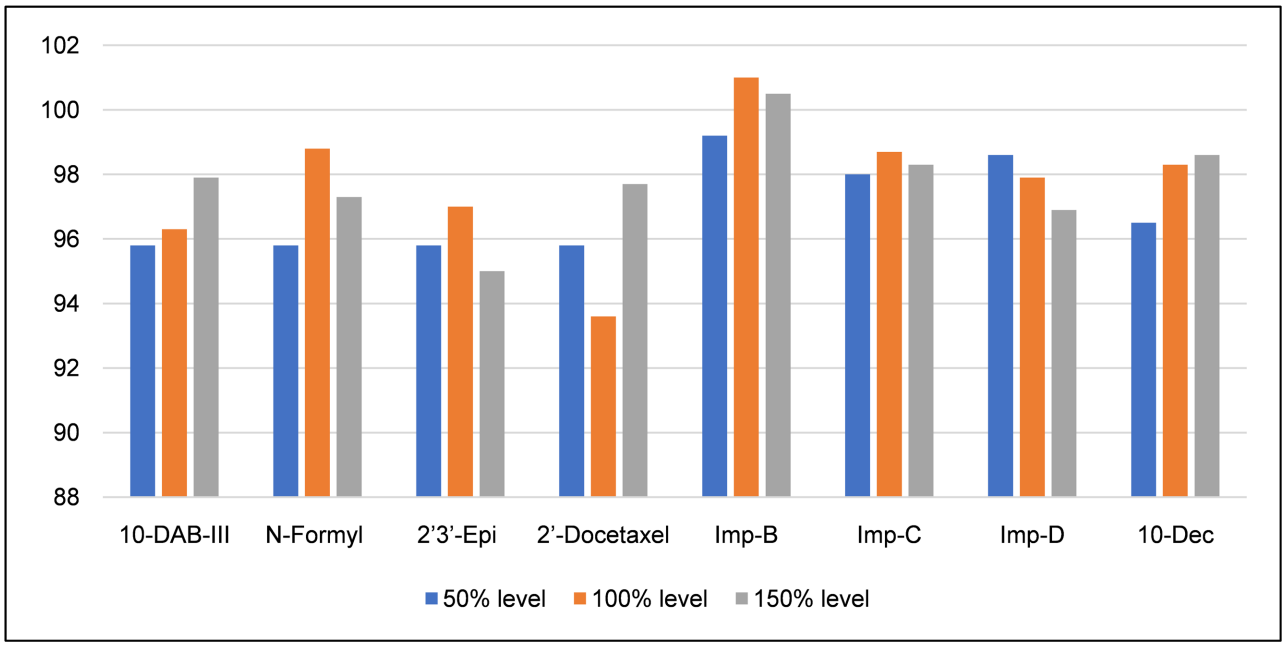

Figure 10. Graphical representation of accuracy of docetaxel impurities.

Solution and Mobile Phase Stability

- **Impurities**: Solutions were stable for up to 2 days with less than 7% variation.
- **Docetaxel Assay**: Stability was confirmed with less than 0.3% variation over 2 days.

6. Conclusion

The UPLC stability-indicating method was developed to determine the stability of Docetaxel under different conditions such as acidic, basic, oxidative, thermal, and photolytic degradation. All the method development and validation studies were carried out according to ICH guidelines. The developed method is selective, fast, cost-effective, precise, accurate and selective, much superior to the pharmacopeial methods. In conclusion, the developed method will be suitable for quantification and identification of Docetaxel and its impurities in any pharmaceutical formulations.

Human and Animal Rights

The authors declare that the work described has not involved experimentation on humans or animals.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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