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Development and Validation for Concurrent Identification of Docetaxel and Fosamprenavir in Pharmaceutical Dosage Forms by using RP-HPLC

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Abstract
This research work details the advancement & validation of an HPLC technique for the simultaneous estimation of Docetaxel & Fosamprenavir. Utilizing a Thermo Scientific Hypersil C18 column, the technique employs a MP of 50% Na3PO4 buffer (pH 5) & 50% MeOH, with a flow of 1.0 mL per min and detection at 245 nanometer. System suitability tests showed a tailing factor below 2.0 and theoretical plates above 2000, confirming the method's reliability. Validation parameters included assay, scale of linearity, precision, ID precision, accuracy, LOD, LOQ, & robustness. The assay demonstrated high precision with %RSD within acceptable limits. Linearity was confirmed over a concentration range of 20 to 100 ppm for both drugs, with a strong correlation coefficient. Accuracy was validated through recovery studies at 50%, 100%, and 150% concentrations, while robustness assessments confirmed the method's reliability under variations in flow &MP ratio. This optimized HPLC technique is suitable for regular analysis of Docetaxel & Fosamprenavir, offering a reliable, efficient, and reproducible approach for pharmaceutical quality control.
Keywords: Docetaxel, Fosamprenavir, Hypersil C18 column, validation, reliability, RP-HPLC, ICH guidelines.

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1. Introduction

The rapid advancement in pharmaceutical sciences has led to the development of numerous drugs aimed at treating complex diseases and conditions. Among these, Docetaxel and Fosamprenavir stand out due to their significant roles in cancer treatment and HIV management, respectively. Docetaxel, a widely used chemotherapeutic agent, is employed primarily in the treatment of various cancers, including breast, lung, prostate, and gastric cancers. Fosamprenavir, on the other hand, is a prodrug of amprenavir, which is utilized in the management of HIV infection. The concurrent identification and quantification of these drugs in pharmaceutical dosage forms are crucial for ensuring their efficacy, safety, and quality. Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) has emerged as a preferred analytical technique in pharmaceutical analysis due to its high resolution, sensitivity, and reproducibility. The development and validation of an RP-HPLC method for the concurrent identification of Docetaxel and Fosamprenavir involve several critical steps. This process ensures that the method is robust, reliable, and capable of accurately quantifying both drugs in a single run. The application of RP-HPLC in

this context is particularly advantageous as it allows for the separation and analysis of compounds with varying polarities, making it suitable for the concurrent estimation of Docetaxel and Fosamprenavir.

The development of an RP-HPLC method for these drugs begins with the optimization of chromatographic conditions. This involves selecting the appropriate mobile phase, column type, flow rate, and detection wavelength. The mobile phase must be carefully chosen to achieve optimal separation and resolution of the drug compounds. Commonly used mobile phases in RP-HPLC include combinations of water, methanol, and acetonitrile, often with the addition of buffer solutions to maintain pH stability. The choice of column, typically a C18 column, is also critical as it directly impacts the separation efficiency and resolution.

Drug Profile

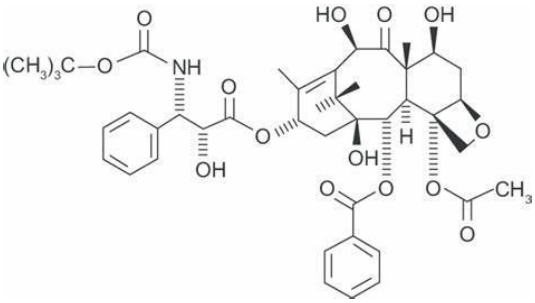

Fig.1.Docetaxel Structure

IUPAC Name: (2R,3S)-N-carboxy-3-phenylisoserine, N-tert-butyl ester, 13-ester with 5β-20-epoxy-1,2α,4,7β,10β,13α-hexahydroxytax-11-en-9-one 4-acetate 2-benzoate
Molecular Formula: $C_{43}H_{53}NO_{14}$
Molecular Weight: 807.88 g/mol
Melting Point: 232 to 234 ℃
pKa: Approximately 7.61
Category: Chemotherapy agent (Taxane)
Solubility: Poorly soluble in water

Pharmacokinetics

Absorption: Administered intravenously.
Distribution: Widely distributed in body tissues.
Metabolism: Metabolized in the liver by the cytochrome P450 enzyme system.
Route of Elimination: Primarily excreted in the feces.
Protein Binding: Approximately 94%.
Half-Life: Around 11 hours.

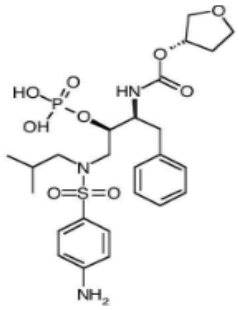

Fig.2.Fosamprenavir structure

2. Materials and Methods

List of Proposed Materials:

For the estimation of drugs, various chemicals and reagents are employed. Phosphate buffer of HPLC grade is sourced from Qualigens and is used for the estimation of Docetaxol and Fosamprenavir. Similarly, acetic acid, also of HPLC grade from Qualigens, is used for the same drugs. Water, of HPLC grade from Qualigens, is utilized for all drugs. Acetonitrile, another HPLC grade chemical from Qualigens, is also used for all drugs. Lastly, methanol, of HPLC grade from Rankem, is employed across all drugs.

Equipment's and instruments used in the study:

The equipment used includes an electronic balance, model SAB2032, manufactured by Scaletech. An ultra-sonicator, model SE60US from Labman Scientific India, is employed. Additionally, a thermal oven, model i-THERM A17782, produced by Dwaraka Scientific, is utilized. The pH levels are measured using a pH meter, model ORION STAR A111, from Thermo Scientific. For filtration purposes, filter paper with a pore size of 0.45 microns from Millipore is used. Lastly, the HPLC system employed is the Waters 2690 Separation Module, manufactured by Waters.

Method Development

Choosing λmax:

UV spectrum of 10µg per ml Docetaxel & Fosamprenavir in diluents (MP ratio) was noted by examining in the scale of 200 to 400nm and the isobestic λmax of both the drugs obtained at 245 nm.

Chromatographic Optimized Conditions

Instrument : RP-HPLC having Auto Sampler & PDA or UV detector
Temperature : Ambient
Column : Thermo Scientific Hypersil C18,(150×4.6mm, 3mm)
Buffer : Na2HPO4Phosphate buffer (pH-5)
MP : 50%PNa2HPO4 buffer PH-5: 50% Methanol
Flow : 1ml per min
λmax : 245 nanometers
volume Injected : 10µl
Run time : 10 min.
Buffer & mobile phase making:

Phosphate buffer PH-5 Preparation :

By adding 13.7 g of Na2HPO4&6.6 g of NaH2PO4 in 1LHPLC grade water. Adjust the solution to PH 5 with base or acid.

Composition of MP (mobile Phase): Mix a 500ml Na2HPO4 buffer (50%), 500ml Methanol (50%) and remove gases in ultra-sonication water bath for 5 min. Filter using 0.45µ filter by vacuum filtration instrument.

Diluent: MP as Diluents.

System Suitability: Tailing factor for Metformin & Voglibose in Std solution shouldn 't >2.0.

For Standard solution Theoretical plates for the Metformin & Voglibose peaks shouldn 't <2000.

Calculation: (For Docetaxel and fosamprenavir)

$$\%\ Assay = \frac{AT}{AS} * \frac{WS}{DS} * \frac{DT}{WT} * \frac{Average\ weight}{Label\ Claim} * \frac{P}{100} * 100$$

Acceptance criteria of System Suitability: Tailing factor should be < 2. Theoretical Plates should be > 2000.

Assay:

Procedure: After Injecting every conc. into the HPLC & get the data of peaks. Construct a CC graph (Calibration curve) area of peak on X-axis vs conc. on Y-axis & find out R^2.

3. Results and Discussion

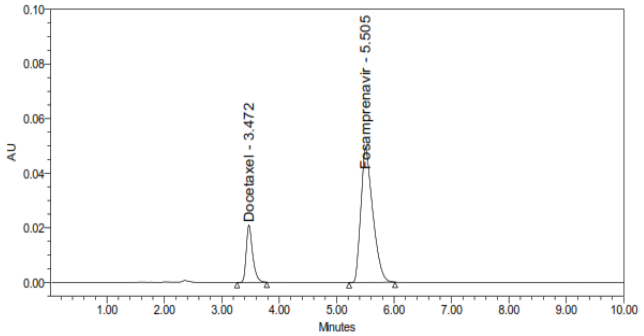

Figure 3: Chromatograph for system suitability of Standard solution

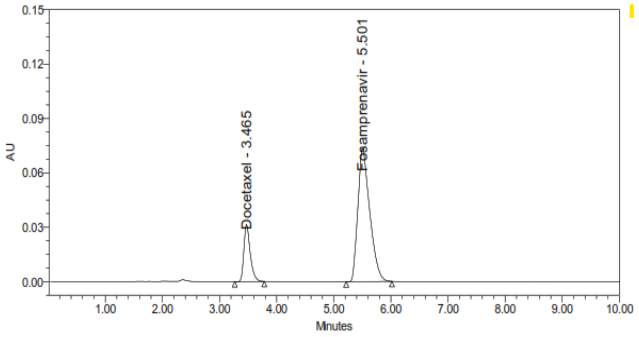

Figure 4: Chromatograph for system suitability of Sample solution

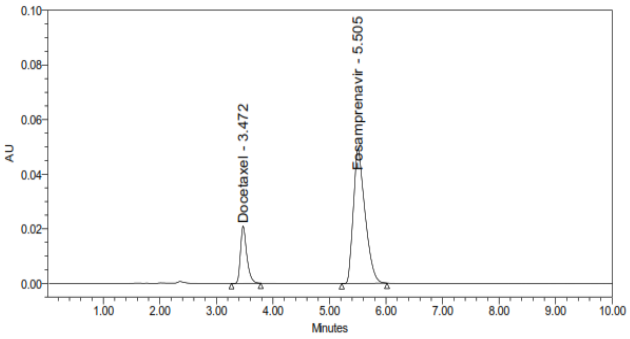

Figure 5: Standard Chromatogram

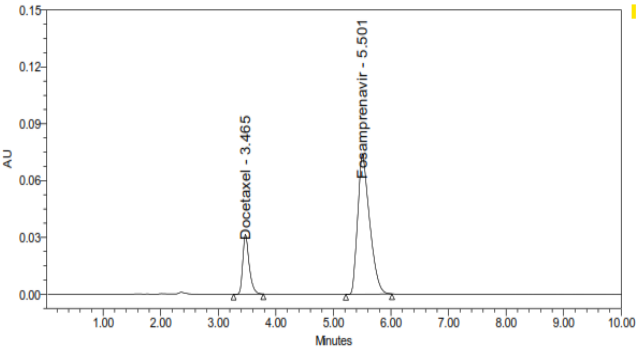

Figure 6: Sample Chromatogram

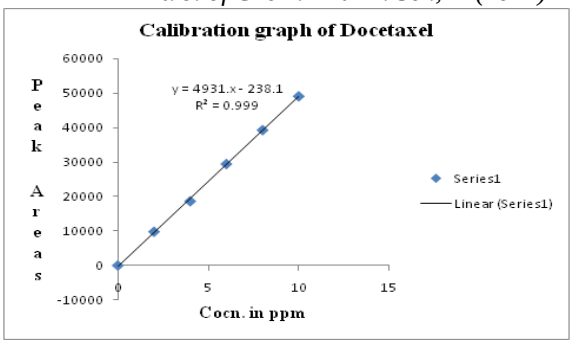

Figure 7: Calibration graph for Docetaxel

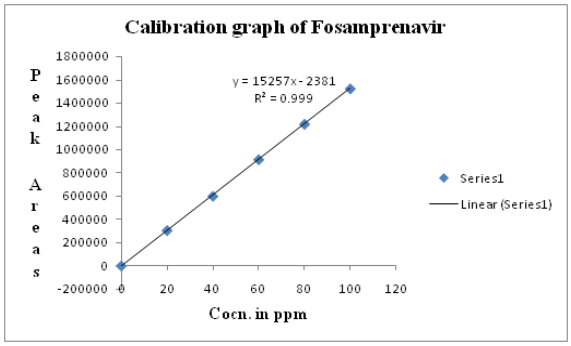

Figure 8: Calibration graph for fosamprenavir

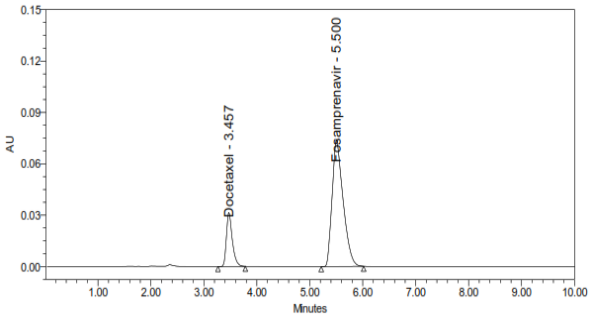

Figure 9: Precision Chromatogram

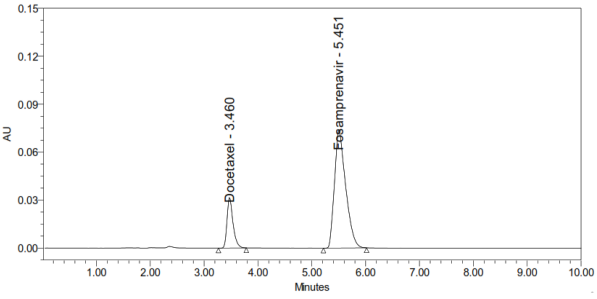

Figure 10: ID Precision Chromatogram

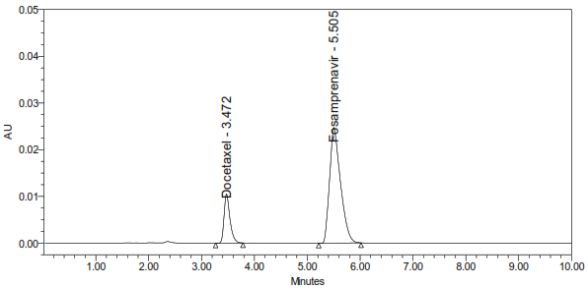

Figure 11: Accuracy 50% Chromatogram

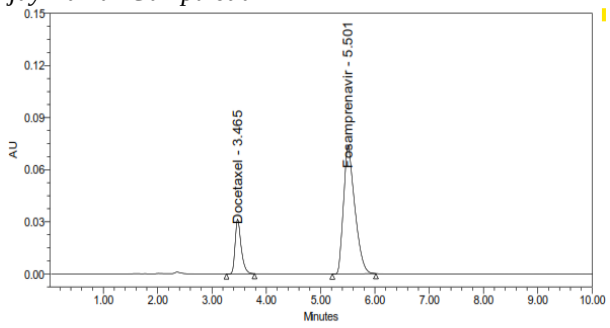

Figure 12: Accuracy 100% Chromatogram

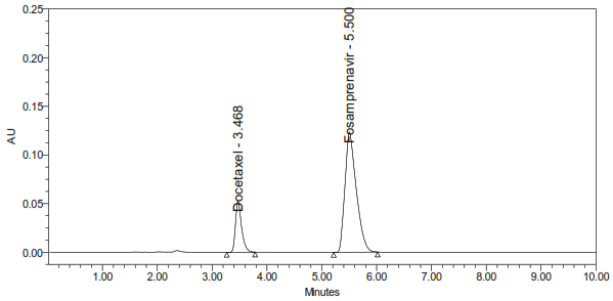

Figure 13: Accuracy 150% Chromatogram

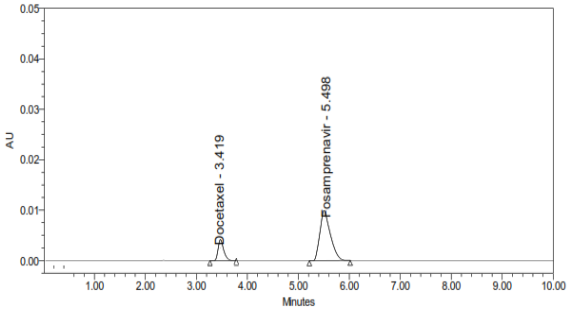

Figure 14: Docetaxel and fosamprenavir dipicting LOD Chromatograph

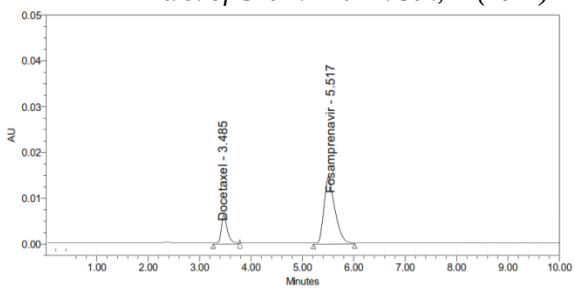

Figure 15: Docetaxel and fosamprenavir dipicting LOQ Chromatograph

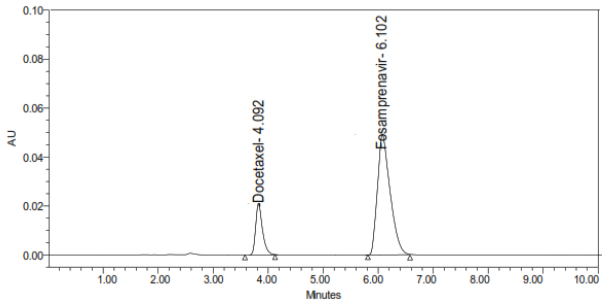

Figure 16: Less flow depicting Chromatograph

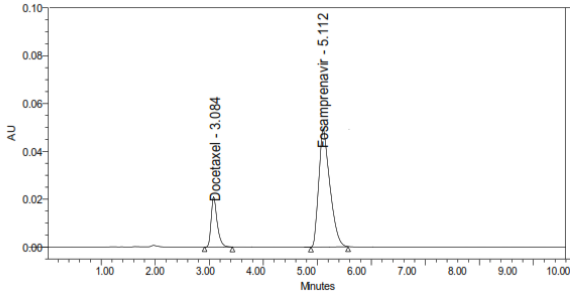

Figure 17: More flow depicting Chromatograph

Table 1: Areas of various conc. of Docetaxel

S.NO	**Concentration(µg per ml)**	**Areas of Docetaxel**
1	2	9834
2	4	18668
3	6	29502
4	8	39336
5	10	49170

Table 2: Areas of various conc. of fosamprenavir

S.NO	**Concentration(µg per ml)**	**Areas of Fosamprenavir**
1	**20**	304854
2	**40**	599708
3	**60**	914562
4	**80**	1219416
5	**100**	1524270

Table 3: Regression equation parameters of Docetaxel & fosamprenavir

Parameters	**Docetaxel**	**Fosamprenavir**
Slope(m)	4931.3	15257
Intercept(c)	238.1	2381
Coefficient of Correlation (R^2)	0.9995	0.9999

Table 4: Recovery(Accuracy) data for Docetaxel

%Conc. (at specified Level)	Docetaxel Area*	Amount Added(mg)	Amount Found(mg)	% Recovery	Avg Recovery
50%	14751	2.5	2.48	99.2	98.1
100%	29502	5	4.9	98.0	
150%	44253	7.5	7.3	97.3	

Table 5: Recovery(Accuracy) data for fosamprenavir

%Conc. (at specified Level)	Fosamprenavir Area*	Amount Added (mg)	Amount Found(mg)	% Recovery	Avg Recovery
50%		25	24.5	98.0	98.8
100%	914562	50	49.6	99.2	
150%		75	74.5	99.3	

Table 6: LOD Results

Drug's names	Baseline noise (µV)	Signal Attained (µV)	Signal/Noise ratio	Conc. In ppm
Docetaxel	92	270	2.93	0.1µg/ml
Fosamprenavir	92	274	2.97	0.04µg/ml

Table 7: LOQ Results

Drug's name's	Baseline noise (µV)	Signal attained (µV)	S/N ratio	Conc. In ppm
Docetaxel	92	910	9.89	0.4 µg/ml
Fosamprenavir	92	914	9.93	0.1 µg/ml

Table 8: Outcomes of difference in flow speeds for Docetaxel

S.No	Flow (ml per min)	SST outcomes of Docetaxel	
		Plate Count USP	Tailing USP
1	0.8	3253	0.98
2	1	3262	1.02
3	1.2	3269	1.10

Table 9: Outcomes of difference in flow speeds for fosamprenavir

S.No	Flow (ml per min)	SST outcomes of Fosamprenavir	
		Plate Count USP	Tailing USP
1	0.8	8207	1.02
2	1	8212	1.05
3	1.2	8230	1.14

4. Conclusion

This research focuses on the advancement & verifications of a RP-HPLC technique for the concurrent identification of Docetaxel &Fosamprenavir in dosage forms. Docetaxel is an anticancer agent, while Fosamprenavir is an antiretroviral drug used in HIV treatment. The goals of the proposed work is to establish an efficient, accurate, & reproducible technique for quantifying both compounds in combination dosage forms, ensuring quality control and compliance with pharmaceutical standards. The RP-HPLC technique was setup using an optimized MP, selected to achieve appropriate retention times for both drugs. Key chromatographic parameters like flow rate, detection wavelength, and column temperature were fine-tuned. Validation of the method followed International Council for Harmonisation ICH rules, assessing parameters like specificity, linearity, accuracy, precision, LOD, LOQ, and robustness. This validated method can be employed in both research and industrial applications for the effective quality assurance of combination drug formulations containing these two active ingredients.

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