DEVELOPMENT AND VALIDATION OF RAPID AND SIMPLE BIOANALYTICAL METHOD FOR DOCETAXEL WITH ONE STEP PROTEIN PRECIPITATION

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ABSTRACT

A rapid and simple bio-analytical method with one step protein precipitation and extraction using acetonitrile as extraction solvent was developed for docetaxel. The extraction efficiency was 87.81% with satisfactory separation of docetaxel and IS peaks by isocratic elution with C_{18} column (25 cm X 4.5 mm, 0.5µm), acetonitrile and water (53:47 % V/V) as a mobile phase at ambient temperature and flow rate of 1mL/min. Paclitaxel solution in acetonitrile (10 mcg/ mL) was used as internal standard. The calibration curve was linear over the concentration range 50 – 5000 ng/mL, regression coefficient R^2= 0.99936 and slope 0.00034. The limit of quantification and limit of detection were found to be 33 ng/ mL and 100 ng/mL, respectively. Coefficient of variation for within day and between the days was in the range of 10.9 to 14.9 and 12.5 to 15.05, respectively. Accuracy of the method indicated % recovery of 97.92 – 104.24%. Thus, a precise, accurate and robust method was developed and validated as per FDA guidelines.

Keywords: Docetaxel, Paclitaxel, Pharmacokinetic, bio-analytical, validation, plasma.

INTRODUCTION

Docetaxel is one of well-known semisynthetic anti-mitotic anticancer agents from taxane class and derived from the needles of *Taxus baccata*[1,2]. It is very effectively used for treating various types of cancers such as breast, ovarian and non-small cell lung cancer[1,2,3]. Being a chemotherapeutic agent, it shows various toxicities[1]. In order to minimise these toxicities, achieve site specific delivery and prolongation of presence in blood; attempts were made to develop targeted novel formulations like liposomes, solid lipid nanoparticles, NLCs and polymeric nanoparticles with and without ligand[4-16].

The development of novel formulations was aimed at reduction of toxicity associated with solubilizer present in conventional formulation and achievement of cancer cell specific targeting. In addition, modification of surface characteristics for these novel carrier-based systems were done to increase residence time of the drug in circulation. Hence, it becomes very important to assess the residence time by pharmacokinetics studies for such drug delivery systems.

Since results of these studies depend on the method that is used to determine concentration of the drug in the circulation, the method should be sensitive. In addition, it should be simple, precise and accurate.

For pharmacokinetic studies of docetaxel containing formulations, various bio-analytical methods have been developed. All these methods are chromatographic methods with different types of detector as well as extraction techniques and are utilized for evaluation of pharmacokinetics of docetaxel from various carrier systems. Various detectors that have been used include UV detector[17,18], PDA detector[19], radioactivity detector[20] and mass spectrophotometry[21]. Similarly, column specifications include use of C_{18} column[2], C_{18} radial compression column[3], solid-phase cartridges[23] and C_8 column[26]. Researchers have extracted docetaxel from biological fluid samples using methods such as solid phase extraction[3, 21, 23-25] and liquid-liquid extraction using extraction solvents such as diethyl ether[2], methanol[7], methanol: acetonitrile (1:1 V/V)[22], or tert-butyl methyl ether[27]. Mobile phase compositions reported by researchers in the literature include: acetonitrile–35 mM ammonium acetate buffer (pH 5)–tetrahydrofuran (45:50:5 V/V)[2], 0.1% formic acid in methanol–water (70:30 V/V/V)[21], acetonitrile–0.02 *m* ammonium acetate buffer, pH 5 (43:57V/V)[3], 0.2% formic acid and acetonitrile[22],

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0.1% formic acid and acetonitrile, gradient elution[23], 0.2% triethylamine (pH 6.4 with orthophosphoric acid) and acetonitrile (ratio of 45:55 V/V)[26], acetonitrile and 0.1% formic acid aqueous solution (60:40 V/V)[27].

Thus, it is evident that many HPLC methods have been published. But limitations of these methods are multi step protein precipitation and solid phase extraction technique that makes them inconvenient to use in the laboratory setup. Also, other chromatographic conditions such as column dimensions, packing and temperature and method of elution may further lengthen the process of analysis of plasma samples[3, 28-35].

Hence the current work was aimed at development of easy, precise, accurate analytical method with one step protein precipitation and extraction estimation of for docetaxel from plasma samples.

MATERIALS AND METHODS

Solvents and Chemicals

Docetaxel and internal standard (IS) paclitaxel were obtained as generous gift samples from Naprod Life Sciences, Thane, India. HPLC grade acetonitrile and water were purchased from S.D. Fine Chemicals Ltd., Mumbai, India. All other chemicals and reagents were of analytical grade.

Instrumentation and chromatographic conditions

The chromatographic system used for the method development was of Agilent Technologies, Model: 1260 with quaternary Pump G1311C, UV detector and manual sample injection port. The control of system components and data acquisition was done using Ezchrom Elite software during development of the method.

Method was developed using Qualisil BDS C_{18}, (25 cm X 4.5 mm, 0.5µm) at ambient temperature. The optimized mobile phase for the analysis with proper resolution of peaks for docetaxel and internal standard (IS)(paclitaxel) was acetonitrile: water (53:47% V/V) of HPLC grade. For the analysis, mobile phase was pumped isocratically at the flow rate of 1 mL/ min for the run time of 15 min. The sample volume injected was 20µL and the peaks for docetaxel and internal standard (IS)were resolved at λ_{max} of 230 nm.

Preparation of stock solutions

Docetaxel stock and working solutions

The stock solution for docetaxel1000ppm (1000 mcg per mL) was prepared by dissolving docetaxel10mg accurately using Metter Toledo high precision balance in10mL acetonitrile. This solution was further diluted using acetonitrile to get working standard of 10 ppm (10 mcg per mL). This working standard was further diluted with acetonitrile to obtain solutions with concentration 50, 100, 250, 500, 1000, 2500 and 5000 ng per mL for preparation of calibration curve in plasma.

Internal standard stock and working (Paclitaxel IS) solution[10, 18, 23, 27, 36]

The literature search on the existing methods for determination of docetaxel in plasma revealed paclitaxel being used as internal standard. Accurately 10 mg of paclitaxel was weighed on Metller Toledo high precision balance and dissolved in acetonitrile completely to get concentration of 1000ppm (1000 mcg per mL). Further dilution of this solution with acetonitrile produced working standard of 10 ppm (10 mcg per mL) that was used as internal standard.

Collection of plasma

Fresh blood plasma was obtained by centrifugation of fresh blood collected from healthy rats. The blood from healthy rats was collected through retro-orbital plexus using heparinised capillaries in EDTA tubes. Tubes were immediately centrifuged at 2000rpm for 20 min; supernatant (plasma) was separated and stored at -20°C until further study.

METHOD DEVELOPMENT

Optimization of mobile phase[37- 40]

Docetaxel and paclitaxel both are official in IP[37,38] and USP[39, 40]. Analytical method for both these agents as reported in the respective pharmacopoeias indicated use of acetonitrile and water as mobile phase with linear gradient elution. Hence, in the current method, mixtures of acetonitrile and water in varying ratios were tried to find appropriate composition. Various compositions of mobile phase containing acetonitrile and water (both HPLC grade) were prepared by mixing and then degassing. The ratios of both solvents selected were ACN: water 65:35, 60:40, 55:45 and 50: 50%V/V. The judgement was made on the basis of peak separation for both the compounds in a mixture.

Mixture containing docetaxel 1 ppm (1 mcg per mL) and IS 10 ppm (mcg per mL) in acetonitrile was prepared and injected into the column to find peak resolution. Since both the compounds are practically water insoluble and have good solubility in acetonitrile the latter it was selected as solvent for preparation of mixture.

Optimization of mobile phase flow rate

The flow rate of mobile phase was varied from 0.8 mL/ min to 1.2 mL/ min and retention time for both compounds was found. The appropriate flow rate selected was the one which gave minimum retention time i.e. for docetaxel approx 9.8 ± 0.5 min and for IS about 10.7 ± 0.5 min leading to minimize action of overall run time for the method. This in turn will result in the reduction of total analysis time during the study.

Selection of extraction solvent based on extraction efficiency

In the analytical method development, it is very important to recover analyte from the test sample by the process of extraction. The amount of analyte recovered is represented as percentage of original amount of analyte and it is known as extraction efficiency for any particular solvent. Various organic solvents like methanol, acetonitrile, ethyl acetate, dichloromethane, petroleum ether are known to be used for extraction of pharmaceutically active compounds from the plasma. Since docetaxel and paclitaxel both are known to have very good solubility in acetonitrile and also pharmacopoeia reveals use of acetonitrile as a component of mobile phase; acetonitrile was tried as solvent for extraction [37-40].

Spiked sample of plasma was prepared in triplicate at single concentration of 250 ng/mL of docetaxel and 10 mcg/ mL of IS and injected into the chromatographic column. Similarly the standard non- biological sample solution containing docetaxel (250 ng/ mL) and IS (10 mcg/ mL) was prepared and injected into the column to find peak area of docetaxel and IS. Comparison between peak area ratios for biological and non- biological samples yielded extraction efficiency. The method can be employed for assay determination of the docetaxel from the formulation i.e. from the non-biological samples. The method of extraction was liquid- liquid extraction process which makes it simple, and convenient for performing in the existing laboratory facilities.

Preparation of analytical samples

Based on the results for extraction efficiency of the docetaxel from plasma samples as well as solubility study for docetaxel, acetonitrile was used as an extraction solvent and analytical samples for the linearity and other method validation parameters were prepared. A known amount of plasma (200µL) was taken in stoppered test tube and this was spiked with 300 µL docetaxel standard solution in acetonitrile (conc. range 50 ng- 5000 ng/ mL). The solution was vortex mixed for 5 min by keeping the tube closed and then 200 µl of internal standard solution 10 mcg/ mL and 300 µL of acetonitrile were added into it. The solution was again vortex mixed for another 5 min and then subjected to centrifugation at 2000 rpm for 5 min. The supernatant was separated and 20 µL was injected into the column for analysis.

METHOD VALIDATION[41,42]

For any newly developed method or modification of existing method; validation plays a key role. This confirms that the method developed is reliable, reproducible and robust for intended use. The developed method was validated for precision, accuracy, recovery, intraday and inter-day variation, LOD and LLOQ, development of calibration curve to check linearity in the observations with respect to concentration and freeze thaw stability for the analyte in biological sample.

Calibration curve (Linearity and range)

Calibration curve is developed to assess ability of analytical method to obtain test results that are directly proportional to the concentration of analyte in the sample. The calibration curve was prepared in the concentation range from 50 to 5000 ng/ mL using solutions prepared by spiking the plasma sample with working standard of docetaxel and IS.These solutions were injected (20 µL) into the column and chromatogram was obtained at 230 nm wavelength, mobile phase acetonitrile: water 53:47% V/V. The peak area for docetaxel and I in each analytical sample was determined and the graph was constructed by plotting ratio of peak area for docetaxel to peak area for internal standard on Y- axis against concentration of docetaxel spiked in each solution on X- axis.

Extraction efficiency

Selected extraction solvent (acetonitrile) was evaluated to determine extraction efficiency. In order to determine extraction efficiency two different analytical samples were prepared. One was a mixture of docetaxel (250 ng/ mL) and IS (10 mcg/ mL) in acetonitrile. The other samples was prepared by extraction from plasma spiked with docetaxel (250 ng/ mL) and IS (10 mcg/ mL). Both the samples were injected (volume 20 µL) into the column.

The peak area of docetaxel and IS for both samples was determined. The extraction efficiency was calculated by comparing ratio of peak areas for both samples.

PRECISION

Closeness of results obtained for each analyte solution is precision of analytical method. It should be measured

for five determinations per concentration and minimum of three concentrations in the range. The precision of the proposed HPLC method was determined as inter and intra- day variations in the peak areas of the drug in plasma. The ratio of peak area of drug to peak area of IS was calculated. The concentrations selected for precision determination were LLOQ, 250, 500 and 1000 ng per mLof docetaxel spiked plasma samples and precision was found in terms of %RSD[42].

ACCURACY

Recovery studies for ensuring reliability and accuracy of method can be performed by standard addition method. As per this method, known amount of pure analyte solution is added to pre-analysed samples i.e. the sample with known amount of analyte in it (specific concentration selected from the calibration curve) and reanalysed by the proposed method. During accuracy study the prepared plasma samples containing 500 ng/mL of docetaxel and 10 mcg/mL IS was spiked at 50, 100 and 150% levels of docetaxel were prepared in triplicate. These prepared samples were then injected into the column and analyzed using selected mobile phase. From resultant chromatographs, the concentration of each solution was determined using equation and % recovery was calculated.

LOD, LOQ and LLOQ

Lowest limit of quantification (LLOQ) is the lowest amount of an analyte in a sample that can be quantitatively determined with acceptable precision and accuracy. Selectivity of the developed method was determined at LLOQ. The plasma extract was obtained from blank as well as analyte spiked plasma and injected into the column. The absence of interference peak at the retention time of analyte and coefficient of variance less than 20% of the nominal value of LLOQ indicates the method to be selective for the analyte. The limit of detection (LOD) and limit of quantification (LOQ) was determined based on calibration curve using standard deviation and slope of regression line. The LOD and LOQ were calculated using the formulae given below:

$$LOD = 3.3\sigma/S$$

$$LOQ = 10\sigma/S$$

Where, "σ" is the standard deviation of the response and "S" is the slope of the calibration curve.

Stability

As per the guidelines[41, 42], it is essential to determine the stability of the analyte in the biological sample as time elapses between the collection of biological sample and analysis can vary depending upon the facilities as well as number of samples to be analyzed. Factors affecting stability of analyte in the biological sample are storage conditions, physicochemical characteristics of analyte, biological matrix and container system. The biological samples obtained at each time point during pharmacokinetic studies are known to contain analyte in specific concentration. The time duration between collection, extraction and estimation may range from few hours to few months. Hence it becomes an important parameter to evaluate stability of the analyte in biological samples. The stability of analyte in biological matrix is evaluated on the basis of expected time lapse between collection of sample and analysis of sample. Evaluation of stability is usually done for long term storage in frozen conditions, short term at room temperature and freeze thaw cycling

In this research work, expected time lapse between collection and analysis of sample is not more than 48 hrs. Hence, stability of the samples was determined by freeze thaw cycling, at room temperature and for one week in freezing condition.

Three sets of analytical samples at two concentration levels viz. LQC (250 ng/ mL) and HQC (1000 ng/ mL) were prepared according to the method described in extraction efficiency above and subjected to stability testing. One set of samples was subjected to freeze thaw cycle where samples were kept in freezer at -20^{0}C for 24 h and then at room temperature for another 24 h. Another set was maintained at room temperature for 24 h and the third set was maintained at -20^{0}C.for one week. After each time-point, the samples were withdrawn from storage and analyzed for docetaxel content using above mentioned chromatographic conditions. The samples were considered stable if deviation from the mean calculated concentration of freshly prepared quality control samples was within ±15%.

RESULTS AND DISCUSSION

Docetaxel, the drug under study and paclitaxel, used as internal standard (IS), belong to the taxane class of anticancer drugs. Hence, both these compounds have structural similarity. In addition, there is similarity in their physico-chemical properties such as solubility, lipophilicity and partition coefficient. In IP and USP;

Table I: Mobile phase optimization

Sr. No.	Mobile Phase composition (Acetonitrile: water)	Flow rate (mL/ min)	Wavelength (nm)	RT for Docetaxel (min)	RT for IS (min)	Peak shape
1	65:35	1 mL/ min	230	5.33	5.55	Merged peak
		0.8 mL/ min	230	6.71	6.99	Merged peak
2	60:40	1 mL/ min	230	6.52	6.94	Merged peak
		0.8 mL/ min	230	8.25	8.81	Merged peak
3	55:45	1 mL/ min	230	8.66	9.48	Two distinct peaks
		0.8 mL/ min	230	10.84	11.87	Two distinct peaks
4	50:50	1 mL/ min	230	12.40	14.01	Two distinct peaks
		0.8 mL/ min	230	15.64	17.71	Two distinct peaks
5	51:49	1 mL/ min	230	11.50	12.91	Two distinct peaks
6	52:48	1 mL/ min	230	10.66	11.89	Two distinct peaks
7	53:47	1 mL/ min	230	9.91	10.92	Two distinct peaks

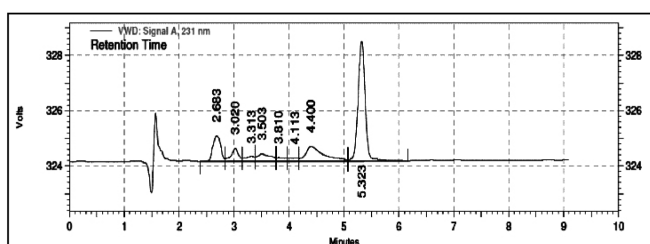


Fig. 1:Docetaxel 1 mcg/mL

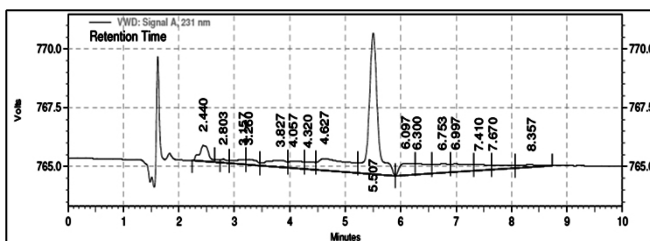


Fig. 2: Paclitaxel 1 mcg/mL

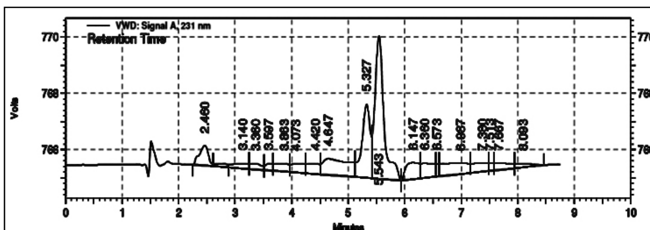


Fig. 3: Docetaxel 1 mcg/ mL and Paclitaxel 1 mcg/ mL

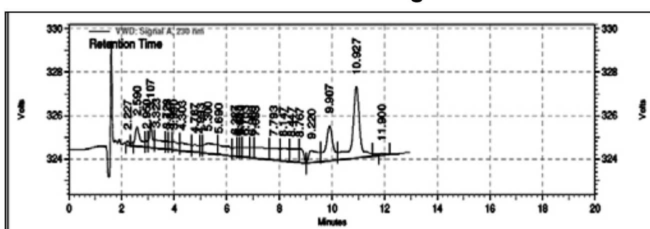


Fig. 4: Docetaxel 1 mcg/ mL and Paclitaxel 1 mcg/ mL with optimized mobile phase

assay method to assess purity of these compounds has been described.

In the current research work, method to analyze and quantify amount of docetaxel in biological samples has developed.

The most convenient and rugged chromatographic method is reverse phase chromatography resulting in satisfactory separation. Also, highly hydrophobic compounds are strongly retained on the column with this method due to less polarity of the column than mobile phase containing mixture of water and organic solvent.

Optimization of mobile phase and flow rate

Docetaxel as well as IS being highly lipophilic in nature C_{18} column with mixture of ACN and water as mobile phase were considered as most appropriate chromatographic conditions. Composition of mobile phase governs the retention time; where increase in polarity increases retention time and decrease in polarity decreases retention time for the hydrophobic substances. This was observed in the current work and was found true when mobile phase (ration of ACN: water) was optimized.

Analytical method developed in our laboratory conditions for assay of docetaxel showed retention time of 5.33 ± 0.5 min using C_{18} column as stationary phase and ACN: water (65:35% V/ V) as mobile phase (shown in Fig. 1). Use of same chromatographic conditions for IS revealed retention time of 5.55 ± 0.5 min as shown in Fig. 2.

This in turn leads to peak merging when mixture of these compounds in concentration of 1 mcg/ mL was

analyzed with the same chromatographic conditions as depicted in Fig. 3. This can be attributed to similar lipophilicity of both compounds belonging to same chemical class and structural similarity.

Thus, to resolve both compounds as distinct peaks mobile phase was modified. Various compositions tried for the resolution of docetaxel and IS peaks were ACN: water; 65:35, 60: 40, 55:45, 50: 50, 51: 49, 52: 48 and 53:47% V/V.

In addition, flow rate of mobile phase affects the elution time. This was observed when flow rate was changed from 1 mL/ min to 0.8 mL/ min. This led to increase in retention time for both compounds.

Mobile phase containing ACN: water (53: 47 % V/V) at flow rate of 1 mL/ min resolved the mixture of components into two distinct peaks viz. docetaxel at about 9.91± 0.5 min and IS at 10.82 ± 0.5 min when separation was carried out using C_{18} column.

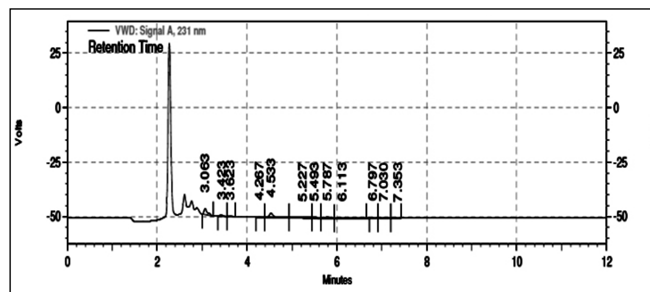
Thus, it was considered as optimum with which chromatographic separation was within 11 min. and the overall run length lasted for 15 min. Table I depicts effect of composition and flow rate on the peak resolution and retention time. Final chromatograph with desired peak resolution is shown in Fig. 4.

Selection of extraction solvent based on extraction efficiency

For determination of extraction efficiency, analytical samples containing docetaxel (250 ng/ mL) and IS (10 mcg/ mL) were prepared (as described in Extraction efficiency) and analyzed. Also, blank plasma extract was prepared by extracting only plasma with acetonitrile. This extract was injected into the column and analyzed.

It was observed that the peak at the RT of docetaxel and IS was absent in the blank plasma extract prepared using acetonitrile as extracting solvent. The results reveal no interference due to plasma matrix components in the analysis of docetaxel and IS. The results are shown in Fig. 5 (A and B).

Ratio of peak areas (peak area for docetaxel to peak area for IS) in biological sample extracted with acetonitrile was found to be 0.1042 ± 0.0079 and in non- biological sample (only mixture of docetaxel and IS) was found to be 0.1187 ± 0.0096. Thus, the extraction efficiency was 87.81% when acetonitrile was used as extracting solvent. Extraction and analysis revealed symmetrical peak shape and uniform baseline.



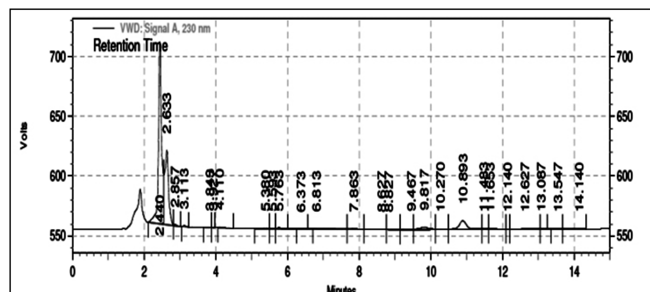


Fig. 5A: Chromatogram for blank plasma extract with ACN chromatograph

Fig. 5B: Chromatogram for Docetaxel spiked plasma sample after extraction

Physicochemical properties of ACN are favourable for separation and detection and hence it is the most preferred solvent. Also, it is acceptable in terms of UV absorptivity and viscosity. In addition, it is miscible with water and found to be selective for extraction of taxanes. Thus, the selected extracting solvent is found to be most appropriate.

Linearity and range

The calibration curve of docetaxel in rat plasma was found to be linear in the concentration ranges from 50ng/ mL– 5000 ng/mL. The ratio of peak area for docetaxel to peak area for IS was calculated. These ratios were then plotted on Y- axis against spiked concentration of docetaxel on X- axis to determine coefficient of variation (R^2) and linearity. The results are shown in Fig. 6 and Table II.

The relevant regression equation describing calibration curve in plasma was Y = 0.00034X, where, Y is the ratio of peak areas of docetaxel to internal standard and X is the concentration of docetaxel (ng/mL) spiked in the plasma sample. Slope was found to be 0.00034 with regression coefficient R^2 = 0.99936.

Precision

Precision for the developed method was determined for concentrations LLOQ (50 ng/mL), 250, 500 and 1000 ng/ mL as per ICH guidelines and results are

Table II: Calibration curve

Sr. No.	Conc. of Docetaxel Spiked (ng/ mL)	Ratio of Docetaxel to IS	
		Mean ± SD (n = 6)	%RSD
1	50	0.0214 ± 0.0034	15.93
2	100	0.0376 ± 0.0013	3.46
3	250	0.1042 ± 0.0079	7.63
4	500	0.1791 ± 0.0165	9.23
5	750	0.2710 ± 0.0251	9.26
6	1000	0.3183 ± 0.0054	1.69
7	2500	0.8456 ± 0.0209	2.48
8	5000	1.7290 ± 0.0160	0.93

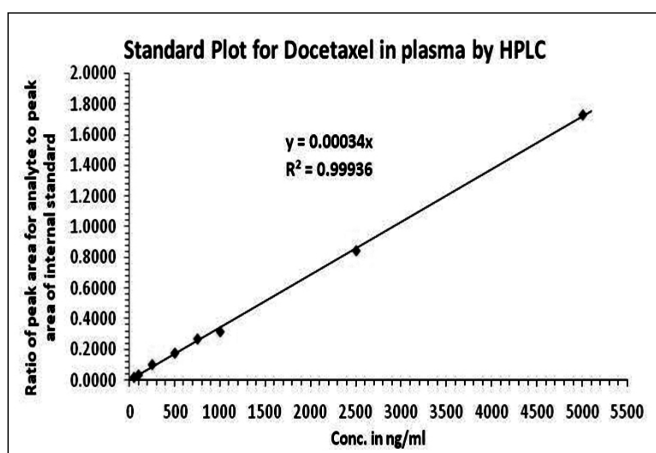


Fig. 6:Calibration curve for Docetaxel in plasma by HPLC

presented in table no. III and IV. The experimental concentrations were determined using calibration curve equation Y = 0.00034X. The values for ratio were substituted as ‘Y’ and concentration of docetaxel spiked to prepare experimental solution was determined. This concentration was expressed as % recovery from nominal value.

Table III: Inter- day validation

Sr. No.	Nominal value conc. (ng/ mL)	Recovered conc. (ng/ mL) (n= 9)	SD (ng/mL)	% Recovery
1	LLOQ	48.797	±15.047	97.24
2	250	266.736	±13.339	106.69
3	500	497.211	±13.053	99.44
4	1000	999.192	±12.540	99.91

Table IV: Intra- day validation

Sr. No.	Nominal value conc. (ng/ mL)	Recovered conc. (ng/ mL) (n= 9)	SD (ng/ mL)	% Recovery
1	LLOQ	49.565	±14.951	99.12
2	250	269.106	±10.971	106.69
3	500	504.504	±13.575	99.44
4	1000	996.017	±14.666	99.91

Inter-day validation revealed % recovery from 97.24 to 106.69 % whereas for intra- day variation % recovery was found to be between 99.12- 106.69 % with SD within limits[42]. As per guidelines, the recovered concentration for all selected concentrations should have standard deviation of NMT 15% whereas this should be below 20% for LLOQ. Thus, the results obtained indicate that the method is precise where the standard deviation for LLOQ does not exceed 20%[41] and for other concentrations less than 15%, as given in the guidelines[41].

Accuracy

Recovery study was carried out as per ICH guidelines at 50, 100 and 150% levels. The percentage recovery for the docetaxel from the prepared samples was found by determining difference between measured concentration and added concentration of the docetaxel. It was found that percent recovery is between 97.92 and 104.24% and

Table V: Accuracy results for Docetaxel in plasma

Accuracy Level	Amount recovered (ng)	% Recovery	Avg. % recovery	Standard deviation	% RSD
50%	758.3122	101.1083	99.8478	±1.6945	1.6970
	734.4118	97.9216			
	753.8511	100.5135			
100%	1026.4438	102.6444	101.7830	±0.7803	0.7667
	1011.2334	101.1233			
	1015.8124	101.5812			
150%	1277.6634	102.2131	102.2506	±1.9738	1.9304
	1253.6975	100.2958			
	1303.0360	104.2429			

Table VI: Stability of docetaxel in plasma

Stability	Nominal concentration of docetaxel (ng/ mL)	Initial concentration (ng/ mL) [mean ± SD]	Final concentration (ng/ mL) [mean ± SD]	Deviation (%)
Freeze- Thaw stability	250.00	247.466	245.672	0.725
	1000.00	985.888	984.457	0.145
24 hr stability at room temp	250.00	247.784	236.500	4.554
	1000.00	991.969	973.881	1.823
One-week stability at -20^{0}C	250.00	249.530	247.140	0.958
	1000.00	988.420	986.006	0.244

reproducibility of the results were found satisfactory. The results are shown in Table V.

From the results it was observed that the SD as well as % RSD is less than 2%. This in turn confirms that the method is accurate.

LLOQ, LOD and LOQ

LLOQ is the lowest concentration in the calibration curve and was found to be 50 ng/ mL. The specificity determination of the docetaxel at LLOQ concentration level revealed absence of peak at the retention time of drug in blank plasma samples extracted with acetonitrile. Hence, the method is selective for detection of docetaxel in plasma. There is no interference from the plasma components. Thus, the method is specific.

Considering calibration curve, LOD was found to be 33 ng/ mL where as LOQ was found to be 100ng/ mL which were calculated using formulae,

LOD= 3.3σ/S

LOQ= 10σ/S

where standard deviation 'σ' was taken for 500 ng/ mL and slope of linearity plot 'S' was 0.00034.

STABILITY

The results for stability validation for the plasma extracts of docetaxel are summarized in Table VI. The concentration of docetaxel in each analytical sample subjected to freeze thaw stability, stored at room temperature for 24 h and 1 week at -20^{0}C was similar with initial concentration. The deviation of final concentration from initial concentration was found to be less than 15 % in all the storage conditions. This indicates that the sample is stable at room temperature, during freeze thaw stability as well for one month when maintained at -20^{0}C. Hence it can be inferred that docetaxel is stable in plasma.

CONCLUSION

The aim of the study was to develop a precise, accurate, reproducible and simple analytical method with one step plasma protein precipitation and liquid liquid extraction technique in order to analyse the docetaxel in rat plasma. The method developed is RP- HPLC method with UV detection and liquid- liquid extraction technique. Selection of suitable mobile phase revealed mixture of solvents acetonitrile and water (53:47 %V/V) as most appropriate mobile phase. In addition, extraction efficiency of 88.87% confirms use of acetonitrile as solvent for extraction[7,8].

The method was found to be precise, accurate and selective with LLOQ of 50 ng/ mL, LOD of 33 ng/ mL and LOQ of 100 ng/ mL. In addition, method was linear in the concentration range of 50- 5000 ng/mL with regression coefficient R^2= 0.99936 and slope of line 0.00034. Docetaxel, the analyte under study, was found to be stable in plasma samples as indicated by the results of stability validation of method.

Thus, method was developed successfully and validated indicating that it can be applied for pharmacokinetics of docetaxel in rat plasma.

Since there are some differences with reference to composition and enzymatic activity between rat and human plasma, the method can be extrapolated to human plasma samples with partial validation of the method for analyte samples prepared in the same manner for human plasma samples.

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