doi.org/10.36721/PJPS.2022.35.6.SP.1713-1718.1

A novel approach for the quantification of paclitaxel loaded in ethyl cellulose/kollicoat and ethylcellulose/eudragit colloidal particles by UPLC-PDA

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Abstract: An optimized rapid reversed phase ultra-performance liquid chromatography (UPLC-PDA) method has been developed and validated for precise and accurate quantification of paclitaxel in drug delivery systems. The chromatographic separation was attained on L1 (USP) column (2.1 ×50 mm, 1.7µm) with an isocratic mobile phase comprised of acetonitrile and water (1:1; flow rate 0.6 mL/min) and detection was executed at 227 nm by PDA detector. The proposed UPLC-PDA method is found to be rapid with retention time of 1.37 min, selective with homogenous peaks and sensitive with Limit of Detection (LOD) of 0.08µg/mL and Limit of Quantification (LOQ) of 2.6µg/mL. The method showed excellent linearity (R^2>0.998) over the range of 0.1 to 0.4mg/mL and applied for the paclitaxel quantification in different formulations with no inference of excipients. Thus, the proposed approach has potential for rapid estimation of drug purity, assay and release profile from pharmaceutical preparations.

Keywords: UPLC-PDA, rapid estimation, paclitaxel, validated approach, application.

INTRODUCTION

Paclitaxel, a taxane diterpene amide (as shown in fig. 1) was first obtained from the bark of the plant, *Taxus bravifolia*, therefore commonly called as taxol (Andersen *et al*., 2006). It is an effective antineoplastic agent used for the treatment of various solid tumors in humans, for instance ovarian cancer, breast cancer, head and neck tumors, kaposi's sarcoma and lung carcinomas (Bardelmeijer *et al*., 2004). It is a cytoskeletal drug that inhibits proliferation of cells by interrupting networks of microtubules during mitosis (Foa *et al*., 1994; Gao *et al*., 2003). Intravenous administration of paclitaxel is linked with multiple side effects, while oral administration is limited for low oral bioavailability (up to 4.6%) due to water insolubility (ICH, 2005; Jain *et al.,* 2014). This problem can be resolved by preparing more soluble derivatives and/or by formulating paclitaxel bound to vehicles (Wenk *et al*., 1996). Recently developed micellar based and liposomal formulations have shown reduced untoward effects (Kesharwani *et al.*, 2011). Taxol®, commercially available paclitaxel formulation is a micellar dispersion of paclitaxel in dehydrated ethanol and Cremophor EL® in 1:1 v/v (Montazeri *et al*., 2018).

Literature review indicated several analytical methods for analysis of paclitaxel in drug delivery systems and biological samples including bio-analytical method UV-Spectrophotometer, HPLC, simultaneous estimation of paclitaxel and topotecan by HPLC, Ultra-fast chromatography, stress degradation studies by HPLC, liquid chromatography-mass spectrometry (LC-MS) (Mowafy *et al*., 2012; Musteata & Pawliszyn, 2006; Prashanth *et al.,* 2011). These reported analytical techniques have multiple drawbacks like complicated sample preparation method, complex mobile phase mixture, high retention time, rigorous monitoring of analytical parameters such as flow rate of mobile phase, column temperature, pH maintenance etc. In order to overcome these limitations, the current study aimed to develop a simple, precise, rapid, sensitive and efficient ultra-pressure liquid chromatography procedure for quantification of paclitaxel in bulk and in developed colloidal drug delivery systems.

MATERIALS AND METHODS

Solvents and chemicals

Paclitaxel was a generous gift from Novartis Pharmaceuticals, Karachi, Pakistan. HPLC grade acetonitrile was purchased from Sigma Aldrich (Germany). All chemicals used were of analytical grade.

Instrumentation and chromatographic conditions

The Ultra-High Performance Liquid Chromatography (UPLC) of Waters (Acquity UPLC-PDA Class H) equipped with a Pump (isocratic solvent manager B17QDI686M-QDI), Auto sampler (sample manager-FTN, C17SOI685G Acquity H-Class Sm-FTN), Photodiode Array detector (M16UPL758A-UPL, QDA Detector KAD3142 Aquity QDA) and Column heater

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(A17CHA512G Acquity CHA) was used for chromatographic separation. Data collection and analysis were performed using LC solution, Empower 3 Software. Chromatographic system was calibrated according to USP <621> and GMP/FDA, Requirements on HPLC Systems in Lab. Quantification of paclitaxel was achieved using optimized chromatographic conditions as given in table 2.

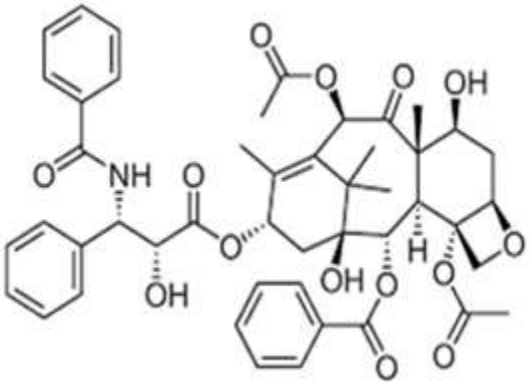

Fig. 1: Structural formula of Paclitaxel

Preparation of diluent

200uL of glacial acetic acid was added to 1L volumetric flask containing about 500mL of methanol; mixed and diluted with methanol to volume.

Preparation of mobile phase and standard solution of paclitaxel

Water and Acetonitrile (50:50, v/v) were mixed, filtered and degassed. Standard solution of paclitaxel (200µg/mL) was prepared by mixing 20mg of paclitaxel in 30mL of diluent, the mixture was ultrasonicated for 5 minutes and final volume was made up to 100mL with diluent.

Validation parameters of developed RP-UPLC-PDA method

The RP-UPLC-PDA procedure developed in the current study was validated in accordance with guidelines of International Conference on Harmonization (ICH) guidelines (ICH, 2005).

Linearity and range

Paclitaxel stock solution (400g/mL) was appropriately diluted with mobile phase to obtain concentrations in the linearity range of 0.1 to 0.4mg/mL. For each solution 0.4µL was injected onto the column (in triplicate) and chromatograms were recorded. Concentrations (mg/mL) versus average peak areas were plotted to get the calibration curve of paclitaxel and slope intercept and correlation coefficient were calculated by applying linear regression.

Accuracy and precision

For the estimation of method accuracy, the standard addition technique was applied. A known quantity of standard (50%, 100% and 150%) was added to a pre-analyzed sample. The samples were analyzed by the developed procedure and percent recoveries were estimated.

Table 1: Chromatographic parameters

Parameters	Conditions
Stationary Phase	Acquity C_{18} coloumn/ L1 (USP)(2.1×50mm, 1.7µm) (PN: 186004661, SN:0293 3710815602) (base deactivated or equivalent)
Mobile phase	Acetonitrile: Water (50:50)
Flow Rate	0.6 mL / min
Mode of Operation	Isocratic
Run time	3 min
Volume of injection loop (µL)	0.4
Detector	PDA
Detection wavelength (nm)	227 nm
Sample Temperature	30℃
Tailing Factor	NMT 2

Table 2: System suitability

	Peak area	Tailing factor	Theoretical plates
Mean (n=6)	50770.65	1.78	
SD	65.03		5535.84
% RSD	0.128		

The precision of the method was estimated in terms of intra-day (repeatability) and inter-day (intermediate precision) studies. For this purpose, peak area and retention time of three different paclitaxel concentrations (0.4, 0.25 and 0.1mg/mL) were measured. Intra-day precision was evaluated by analyzing injections of 0.1, 0.25 and 0.4mg/mL under the same experimental conditions on the same day, while inter-day precision was estimated by repeating injections of 3 different concentrations on three different days.

Sensitivity

Limit of detection (LOD) and limit of quantitation (LOQ) are crucial parameters for the determination of sensitivity of analytical methods and were estimated from the calibration curve using equation 1 and 2 respectively.

$$\mathrm{LOD} = \frac{3.3\,\mathrm{S.D}}{\mathrm{s}} \quad (1)$$

$$\mathrm{LOD} = \frac{10\,\mathrm{S.D}}{\mathrm{s}} \quad (2)$$

Where, *S. D`* is the standard deviation of the peak area (n=5) of sample and *s* is slope of the corresponding calibration curve.

System suitability tests and robustness

The system suitability was estimated by analyzing 6 successive injections of a standard solution of paclitaxel (0.15mg/mL).

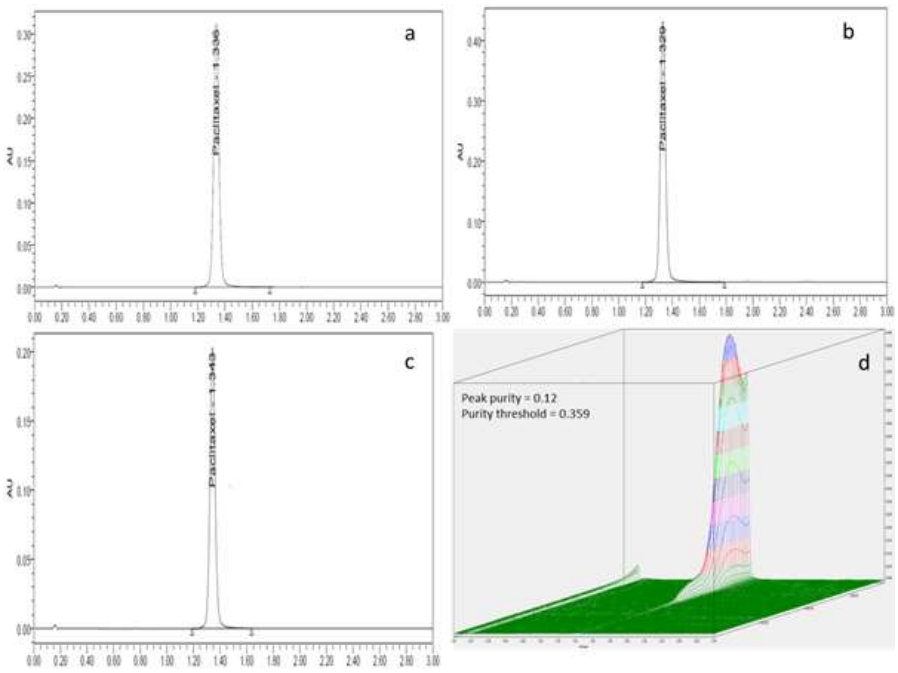

Fig. 2: UPLC Chromatogram of (a) Paclitaxel standard Solution (b) Paclitaxel Injection (c) Colloidal Particles (EK) and (d) 3-D plot showing peak purity

Table 3: Recovery data of paclitaxel

	Formulation concentration (mg/mL)	Spiked concentration (mg/mL)	Total concentration (mg/mL)	Concentration obtained (mg/mL ± SD)	% Recovery
50%	0.1	0.05	0.15	0.147± 0.005	98%
100%	0.1	0.1	0.2	0.203±0.009	101.7%
150%	0.1	0.15	0.25	0.247±0.004	98.8%

Table 4: Precision study

Conc. (mg/mL)	Intraday Precision				Interday precision			
	Retention time		Con. Found		Retention time		Conc. found	
	Mean	%RSD	Mean	%RSD	Mean	%RSD	Mean	%RSD
0.4	1.339	0.24	0.394	1.68	1.34	0.23	0.392	1.493
0.25	1.336	0.21	0.247	1.61	1.35	0.19	0.246	0.619
0.1	1.335	0.198	0.097	1.56	1.36	0.21	0.098	1.17

Table 5: Robustness in terms of change in mobile phase composition and flow rate

Conc. (mg/mL)	Change in mobile phase Composition (Acetonitrile: Water)				
	(55:45)			(45:55)	
	Conc. found (mg/mL) Mean ± SD	RSD (%)		Conc. found (mg/mL) Mean ± SD	RSD (%)
0.4	0.398 ± 0.007	1.85		0.393 ± 0.005	1.42
0.25	0.243 ± 0.006	2.78		0.24± 0.006	2.83
0.1	0.097 ± 0.002	2.71		0.098 ±0.001	1.02
	Change in flow rate				
	1.2 ml/min			0.8 ml/min	
0.4	0.39 ± 0.007	2.03		0.395 ± 0.006	1.54
0.25	0.251 ± 0.004	1.96		0.249 ± 0.007	3.03
0.1	0.096 ± 0 .002	2.16		0.098 ± 0.002	2.55

Table 6: Results of assay of Drug

Pharmaceutical Preparation	Amount found (mg)	Measurement uncertainty	Assay
Paclitaxel injection (Paclitaxel USP) 30 mg/5mL	29.92	±1.02	99.73
EK colloidal particles (100mg)	97.13	±1.05	97.13
EE colloidal particles (100mg)	95.25	±1.18	95.25

Peak area, theoretical plates (N) and tailing factor (T) were recorded (ICH, 2005). Robustness of the method was analyzed by altering the flow rate of the mobile phase as well as changing the mobile phase ratio deliberately.

Assay procedure for paclitaxel injection (commercial preparation)

1mL of the intravenous injection (30mg/5ml USP) containing 6 mg was transferred into a clean volumetric flask (25mL), sufficient amount of diluent was added to it, mixed well and diluted up to volume with diluent. Solution was filtered (0.25µm) to remove any particulate matter and analyzed for paclitaxel.

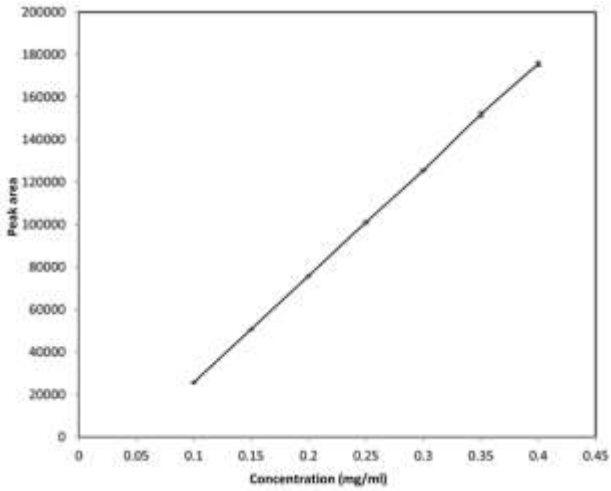

Fig. 3: Calibration Curve of Paclitaxel

Analysis of Paclitaxel loaded in Ethyl Cellulose/Kollicoat (EK) and Ethylcellilose/ Eudragit L100 D 55 (EE) colloidal particles

The amount of paclitaxel loaded in EK and EE colloidal particles designed for controlled drug delivery, was analyzed by the UPLC-PDA method described above. Paclitaxel loaded colloidal particles (equivalent to 100mg drug) were powdered and incubated (20-25C) in diluent (15mL). The suspension was sonicated, filtered by a 0.25µm filter to remove particulate matter. The amount of paclitaxel present in the sample solution was determined by the regression equation of the calibration curve. Each determination was performed on six replicate injections.

STATISTICAL ANALYSIS

SPSS (IBM, version 20) was used to express mean ± SD and one way ANOVA for the collected data. *p*-value less than 5% ($p<0.05$) was considered statistically significant.

RESULTS

Chromatographic separation

In order to optimize the separation and to obtain highest analytical sensitivity for paclitaxel, different mobile phase compositions were examined and the optimum parameters with which the best result obtained (table 1) were used for the analysis. The representative UPLC chromatograms of standard paclitaxel solution, paclitaxel Injection and colloidal formulation are depicted in fig. 2 showing that paclitaxel was well separated at retention time of 1.37 min without any inference and the calculated purity angles were less than the purity threshold.

Analytical method validation

Linearity, accuracy, precision, LOD, LOQ and robustness were assessed to validate the method as per ICH requirements.

System suitability

System suitability was established by measuring % RSD of peak areas, theoretical plates and tailing factor of six replicates of standard solution. Results are presented in table 3.

Sensitivity, Linearity and range

The linearity of the assay procedure was validated over the concentration range of 0.1mg/mL to 0.4mg/mL and established by least squares linear regression analysis of calibration curve (fig. 3). The LOD and LOQ were estimated at 0.8µg/mL and 2.6µg/mL, respectively.

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Accuracy and precision

Accuracy of the procedure was calculated as percent recovery (table 4). The percent recovery was found between 98% and 101.7%. The results of intraday and interday precision are presented in table 5. The % RSD of retention time and peak area were in range of 0.198% to 0.24% and 1.56% to 1.68% respectively for intraday precision, while the % RSD of retention time and peak area for interday precision were in range of 0.19% to 0.23% and 0.619% to 1.49%, respectively.

Robustness

Robustness was accessed by small intentional changes in flow rate of mobile phase and composition of the mobile phase and results obtained are presented in table 6.

Application

The developed UPLC method was applied for the quantification of paclitaxel in a commercially available injection and novel colloidal preparations synthesized by authors for controlled delivery of drug. The results of paclitaxel Injection assay (99.7%±1.02) and percent paclitaxel loaded (97%±1.05 in EK and 95%±1.18 in EE) in colloidal particles are presented in table 7.

Table 7: Results of assay and percent drug loading

Pharmaceutical Preparation	Amount found (mg)	Measurement uncertainty	Assay/Percent drug loading (%)
Paclitaxel injection (Paclitaxel USP) 30 mg/5mL	29.92	±1.02	99.73
EK colloidal particles (100mg)	97.13	±1.05	97.13
EE colloidal particles (100mg)	95.25	±1.18	95.25

DISCUSSION

The chromatographic analytical procedure should be able to separate the desired analyte with good resolution, sensitivity, accuracy, precision and reproducibility. In current study a simple mobile phase comprised of Acetonitrile: Water (50:50 v/v) has successfully eluted the analyte in 1.37 min, thus allowing rapid estimation with short run time, which would permit reduced mobile phase consumption. The peaks were homogenous and showed good resolution. The purity angles of the peaks were less than the purity thresholds indicating that the peaks were homogenous, thus establishing the selectivity of assay (Srinivasa *et al*., 2017).

The developed procedure was validated in accordance to ICH guidelines (ICH, 2005) for establishing documented evidence providing assurance that the analytical procedure is capable of consistently meeting its predetermined specifications and quality attributes. System suitability, an integral of analytical methods was accomplished before the initiation of validation. All parameters (table 3) were satisfactory as per ICH requirements % RSD of peak area ≤2%, theoretical plates ≥ 1000 and tailing factor ≤2.0 confirming the acceptable performance of the system (Wang *et al*., 2017; Zhai *et al.*, 2018).

The method indicated excellent linearity over the range of 0.1mg/mL to 0.4mg/mL with R^2 more than 0.999. The sensitivity of method was good enough to estimate paclitaxel in bulk and various drug delivery systems. The results confirmed the accuracy (≥98% recovery) of the analytical procedure, further the assay method was found precise with %RSD of retention time and peak area <1% and <2%, respectively for both intra-day and inter-day assay precision. Robustness, an important validation parameter, measures method's scope to remain unaltered by small intentional changes in analysis conditions. The proposed UPLC-PDA method was found robust for changes in mobile phase concentration and flow rate, thus is reliable under normal usage (Pyla *et al*., 2013; Sathyamoorthy *et al*., 2014; Siddiqui *et al*., 2012).

The validated method was applied for the quantification of paclitaxel in paclitaxel injection (available in market) and controlled release colloidal formulations. The chromatograms showed separation of drug without any inference with the excipients suggesting that the method might allow a rapid, economical, accurate and precise approach for the quantification of paclitaxel (Srinivasa *et al*., 2017).

CONCLUSION

In conclusion an accurate, precise, selective and reproducible UPLC-PDA method for the quantification of paclitaxel in pure and in pharmaceutical formulations has been developed and validated. Moreover, it is an economical, simple and rapid approach with potential for routine application in research and drug development for the determination of paclitaxel's purity, assay and dissolution profile.

ACKNOWLEDGEMENT

The authors are grateful to deanship of Scientific Research at King Khalid University for provision of funding through large research group project under grant number RGP2/3/43. Authors are also grateful to Bahauddin Zakariya University Multan for providing the facilities for this research project.

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