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RP-HPLC ANALYTICAL METHOD DEVELOPMENT, FORMULATION AND EVALUATION OF ENTERIC COATED TABLETS OF PACLITAXEL USED AS BIO-ENHANCER

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ABSTRACT

An easy, fast and validated RP-HPLC method was invented to quantify paclitaxel in drug solution and orally disintegrating tablet. The separation was carried out using reversed phase C-18 column (Agilent Eclipse Plus C-18) with UV detection at 268 nm. Method optimization was tested using various compositions of organic solvent. The mobile phase comprised of phosphate buffer (0.01M), methanol and acetonitrile (50:30:20, v/v) adjusted to pH 2.7 with phosphoric acid (80%) was found as the optimum mobile phase. The method showed intraday precision and accuracy in the range of 0.24% to -1.83% and -1.83% to 1.99% respectively. The standard calibration curve was linear from 0.125µg/mL to 16µg/mL. In formulation development, five different tablets were prepared as shown in table with constant amount of quercetin but varying concentration of mannitol, calcium phosphate etc. Disintegration studies, observed that F1 as per table shows the best disintegration time of 1.5±0.20 min. This formulation was further chosen for the enteric coating. Formulation evaluations were found to be bulk density; 0.45±0.02 g/cc and tapped density 0.53±0.01 g/cc. The angle of repose was found to be 29.16±0.82 θ and Carr's Index was found to be 18.26±0.25. Hardness was found to be 5.1±0.41 kg/cm2 and friability was found to be 0.19 ± 0.01%. To confirm the equal distribution of the weight the weight variation was tested which was found to be 199.16±2.36. This parameter confirms the granules are good in physical properties and can be used further for compression. The drug solution was stable under room temperature at least for 6 hours.

Keywords: Paclitaxel, RP-HPLC, Disintegration, Bulk density.

1. INTRODUCTION

"Bio-enhancers are substances which stimulate and enhance the bioavailability of the drugs, which are mixed with drugs and do not exhibit synergistic effect with the drug [1]." Lipophilicity and size of molecule both are the most important preventive factors for molecules to pass the biological membrane and to be immersed systematically following administration through oral or topical route [1-4]. Several plant extracts and phytoconstituents, despite having excellent bioactivity *in vitro* demonstrate less or no *in vivo* actions due to their poor lipid solubility or inappropriate size of molecule or both, resulting reduced absorption and poor bio-availability. It is often found that, when individual contents are extracted from the plant extract there is loss of specific bioactivity [5-8].

Sometimes some constituent of the many constituents plant extract are damaged in gastric environment when taken orally. They reduce the dose, reduce the duration of treatment and therefore reduce problems of drug resistance. Due to dose economy, they make treatment cost- effect. Herbal bio-enhancer is phyto-molecules that at low doses promote and augment the bioavailability or biological activity of drug. Bio-enhancers are such agents, which by themselves are not therapeutic entities but when combined with an active constituent proceed to the potentiating of the pharmacological effect of the drug [9-10]. Such formulations have been found to increase the bioavailability or bio-efficacy of a number of drugs even when reduced doses of drugs are present in such formulations. Many synthetic and herbal drugs suffer from the problem of low bioavailability. Bioavailability is the extent and rate to which a substance enters total systematic circulation and becomes available at the required site of action. For the purpose of this paper, the term bioenhancer is reserved for molecules of natural origin that are capable of increasing the rate and/or extent at which co-administered drug molecules reach

the systemic circulation unchanged (i.e., increased bioavailability). The main mechanisms that have been identified through which bioenhancers can improve the bioavailability of drug molecules include alteration of the plasma membrane fluidity to increase passive transcellular drug permeation; modulation of tight junctions to allow for increased paracellular diffusion; and active efflux transporter modulation, [11].

2. MATERIAL AND METHOD

2.1. Pre-formulation study

2.1.1. Physical appearance

Paclitaxel powder was examined for its organoleptic properties like colour, taste and odor.

2.1.1.1. Determination of Wavelength Maxima (λ max)

Accurately weighed 10 mg of drug was dissolved in 10 ml of methanol in a 10 ml volumetric flask. 0.1ml of this stock solution was pipetted into a 10 ml volumetric flask and volume made up to the mark with methanol. The resulting solution was scanned between 200-400 nm using UV/Vis double beam spectrophotometer.

2.1.2. Preparation of calibration curve of Paclitaxel

2.1.2.1. Calibration curve in methanol

A 10 mg of drug was weighed accurately and dissolved in 5 ml of methanol in a 10 ml of volumetric flask and volume was made up to 10 ml with methanol. This resulted 1000µg/ml solution and from this solution 1ml was pipetted out and transferred into 10 ml volumetric flask and volume was made up with methanol. Suitable dilution was prepared to make a concentration range of 5-25µg/ml of Paclitaxel. The spectrum of this solution was run in 200-400 nm range in U.V. spectrophotometer (Labindia-3000+). The absorbances of these solutions were measured against a blank methanol. The calibration curve was obtained by plotting the absorbance versus the concentration. The straight line of best fit was obtained byusing linear regression analysis program.

2.2. FTIR Spectra of Paclitaxel

FTIR spectra of drugs were recorded by KBr method using Fourier Transform Infrared Spectrophotometer. A base line correction was made using dried potassium bromide pellet [1]. The potassium bromide-drug pelletof approximately l mm diameter was prepared by grinding 3-5 mg of drug with 100- 150 mg of potassium bromide in pressure compression machine. The sample pellet was mounted in IR compartment and scanned at wavelengths 4000 cm^{-1} to 400 cm^{-1}.

2.3. Melting point determination by DSC

It is one of the parameters for the purity of drugs. In case of pure chemicals, melting points are very sharp and constant. Since the drug contains the mixed chemicals, they are described with certain range of melting point. Melting point was determined by Differential scanning calorimetry $(DSC)^2$. The sample (2-4 mg) was heated in a non-hermetically crimped aluminum pan at a rate of 5°C/min, over the range of 50 to 200°C. DSC thermogram obtained and evaluated for melting point of Paclitaxel.

2.4. Drug excipient compatibility study by DSC

The thermograms were monitored using a differential scanning calorimeter. Weighted samples (2-4mg) were placed in metal pans with flat bottoms and hermetically sealed. These samples were heated at a continuous rate of 10°C per minute in an environment of nitrogen (200ml/min) over a temperature range of 50- 400°C, with alumina as the reference standard.

2.5. Solubility study

Solubility studies were performed in distilled water; 0.1 N hydrochloric acid, 0.1 N NaOH, ethanol, methanol and chloroform at room temperature (25±2°C) [3]. An excess amount of drug was added to 5ml of solvent in screw-capped glass vials; these were mechanically shaken for 48 hours at 25°C until equilibrium was achieved. Aliquots were withdrawn, filtered through a membrane filter (0.45µ) and analyzed for solubility.

2.6. Analytical Method Development - RP-HPLC Method

Optimization and evaluation of various stages of sample preparation, chromatographic separation, detection and quantification are the key factors for ideal method development. Optimization of various parameters was performed in order to develop a selective and sensitive method for analysis of PT on reverse phase high performance liquid chromatography (RP-HPLC) [12].

2.7. Material

Methanol, Acetonitrile, water and triethylamine (TEA) and 85 % *ortho*-phosphoric acid (H_3PO_4) were purchased from Rankem, New Delhi, India. All these chemicals and solvents were of HPLC grade and were used without any further purification.

All the solutions were prepared in HPLC grade water. Market formulation was procured from the local drug store. Unless otherwise specified, all solutions were

filtered through a 0.2 µm Ultipor® N66® Nylon 6, 6 membrane filter (Pall Life Sciences, USA) prior to use. The analysis was performed on HPLC system of WATERS (Milford, USA) composed of 515 solvent delivery system equipped with Rheodyne injection valve with a 20 µl loop, PDA detector was set at wavelength range 190-800 nm. All the chromatograms were processed using Empower software and sigma plot software.

2.8. Optimization of chromatographic conditions

The effects of different chromatographic conditions on the instrument response create a situation where one has to compromise between different experimental variables in order to achieve the best chromatographic separation. Chromatographic separations are significantly affected by the mobile phase conditions, such as the type and composition of the organic modifiers and therefore before selecting the conditions for the optimization, a number of preliminary trials were conducted with different combinations of different organic solvents and buffers at various pH, compositions, and flow rate to check the retention time, shape, resolution, and other chromatographic parameters. From those experiments the mobile phase combination of ACN and TEA in the acidic pH range was found to be most suitable [13].

2.9. Validation of the proposed method

After chromatographic method development and optimization, it was validated. The validation of an analytical method verifiesthat the characteristics of the method satisfy the requirements of the application domain. The proposed method was validated according to ICH guidelines for linearity, precision, accuracy sensitivity, and recovery.

2.9.1. Linearity

For linearity studies, working standard solutions equivalent to 0.1 to 1.0 µg/ml of PT were prepared with the mobile phase. Calibration graph was prepared by plotting the mean peak area versus concentration of PT.

2.9.2. Precision and Accuracy

Intra-day precision and inter-day precision for the developed method was measured in terms of % R.S.D. The experiments were repeated three times a day for intra-day precision and on three different days for inter-day precision. The concentration values for both intra-day precision and inter- day precision were calculated six times separately and percent relative standard deviation were calculated. Finally, the mean of % R.S.D. (% R.S.D. = [S/X] 100, where S is standard deviation and X is mean of the sample analyzed) were taken for conclusion.

2.9.3. Detection and quantification limits (LOD & LOQ)

The limits of detection and quantification were calculated by the method based on standard deviation (σ) and slope (S) of the calibration plot using the formula LOD = $3.3\sigma/S$ and LOQ = $10\sigma/S$.

Percentage Purity: Twenty tablets were weighed and powdered equivalent to 10 mg of PT was accurately weighed and diluted with mobile phase. Working dilution was prepared using mobile phase and used for analysis.

2.10. Physical Mixture of Paclitaxel and bioenhancers

The different weight ratio of the PT and QU were prepared using the physical blending method. PT and QU binary system was prepared at 5 different points or weight ratio levels, 3:0.5, 3:1, 3:1.5, 3:2.0, 3:2.5 w/w of the PT and QU. In this method both the drug and bioenhancers weight individually and sealed in the bag and blended for 60 min. all the blends were tested for confirmatory of uniformity using HPLC.

2.11. Compatibility Studies of Paclitaxel and bioenhancers

To conduct the compatibility studies, the samples prepared and used, in this study the FTIR, DSC and melting point were used. The individual of each PT and QU FTIR, DSC spectrum were recorded and compared with the binary mixture. Similarly, the melting point for the mixture as well as individual were recorded and noted for any physical interaction among the drug and bioenhancer [14].

2.12. Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectrum of PT, QU, PT: QU were obtained using Avatar 360 E.S.P FTIR spectrometer, Thermo Nicolet Corp, Madison, WI, USA. Kbr was used to keep the sample dry in 1:100 and pellet was prepared by compressing the pellet machine. All the samples were scanned for 16 times in the range of 500-4000 cm^{-1}. The Peaks correspond to the major functional groups of the drug has been noted and compared with the physical mixture of PT and QU.

2.13. Differential Scanning Calorimetry (DSC)

PT, QU and their physical mixture DSC thermograms were recorded using the Mettler-Toledo, Schwerzenbach, Switzerland. The thermograms were recorded in the range of 0-350°C. The ratio of the temperature increment was kept at 10°C min^{-1}. The empty aluminum pan was used as the reference thermogram to check the any influence of the blank.

2.14. Franz Diffusion Studies Ex-Vivo

The *ex-vivo* permeation studies were conducted in the Franz diffusion cell having an area of 3.20 cm^2 with the facility of donor and acceptor compartment. To conduct these studies the intestinal tissues of the goat was collected from local slaughter house and was stored in normal saline under deep fridge conditions. Before the start of the experimentation, the gut tissue was cleaned by the slow infusion of the normal saline and air. The tissue was cleaned to make sure there was no content in it. The prepared binary systems using the physical mixture method was assessed for the permeation using the intestinal tissue of the goat as it is morphological close to human intestine tissue [15-17].

2.15. *In vivo* pharmacokinetic study in rabbits

2.15.1. Animal preparation for In-vivo studies

In vivo pharmacokinetic study performed in New Zealand white rabbits (2-3 Kg) provided by the animal house at Pharmacy Department. The rabbits were given free access to food and water. The rabbits were fasted for 12 h prior to the experiments with free access to water.

2.15.2. Dosing

In pharmacokinetics study rabbits were divided in to 3 group having 5 animals in each group. The PT (oral), PT (IV), PT: QU were orally administered as suspension, at a dose of 50 mg/kg. The rabbits had free access to water during the entire experiment. Blood samples of 400 µL were collected at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24 and 48 hr after dosing via the orbital venous plexus using isoflurane as anesthesia.

Table 1: Pharmacokinetics Study Design for the PT

S. No	Groups	Dose	Route	Total no of animals
1	Control	50 mg/kg	Oral	5
2	PT : QU	5:1.5	Oral	5
3	IV	5 mg/Kg	I.V	5

The whole blood was collected in heparinized tubes, and the plasma from the sample was separated by centrifugationat 9000 rpm for 10 min and stored at-20°C prior to analysis by LC-MS.

2.16. Development of Enteric Coated tablets

Hydroxy propyl methyl cellulose phthalate (HPMC phthalate), cellulose acetate phthalate and Eudragit L-30 D-55 was procured for Torrent Pharmaceutical Ltd., India. The acryl-EZE® and opadry® was procured as gift sample from the Colorcon, Goa, India and other than these additives required were procured commercially. All the other reagents and solvents has been used were of analytical grade. In vitro analysis of the prepared tablets was carried out as per official pharmacopoeia [18].

2.17. Drug excipient interaction study

The PT: QU blend was tested for computability with the other excipients by blending in 1:1 ratio. These blends were filled in the sampling vials and kept under controlled conditions of 35°C±2°C / 60%±5% RH for a period of 4 weeks. Then these samples were analysed by IR.

2.18. Preparation of uncoated tablets

The Granulation for the tablet was prepared using the wet granulation method. The polymer and other excipients were passed through sieve no 60 before blending, the turbula mixer was used for the blending. Coating of the blended powder was done using PVP K-30 solution in the isopropyl alcohol and the prepared granules were dried. These dry granules were used for the compression on the cadmach 10 station machine using the biconvex round shape die and punches of 7 mm.

2.19. Granules evaluation

The prepared granules were evaluated for the bulk density, tapped density and carr's index using standard methods. In this method, 100 gm of granules were filled in the clean and dry cylinder and the final volume of the granules were recorded. The cylinder was then tapped for 25 times using tapping instrument and the volume after tapping was recorded. Using the respective mass and volumes of the granules, the bulk and tapped density was calculated for the granules. Carr's index (I) was calculated using the equation 4.10, where Dt is the tapped density of the powder and Db is the bulk density of the powder.

$I = Dt - Db / Dt \times 100$

Table 2: Formulations for the uncoated tablets

Ingredient (mg)	F1	F2	F3	F4	F5
Paclitaxel	50	50	50	50	50
Quercetin	15	15	15	15	15
Mannitol	15.0	----	30.0	35.0	40.0
Dibasic Calcium Phosphate	----	55.0	25.0	20.0	15.0
Magnesium Stearate	4.8	4.8	4.8	4.8	4.8
Talc	6.5	6.5	6.5	6.5	6.5
PVP-K 30	10.0	10.0	10.0	10.0	10.0
Croscarmellose Sodium	5.0	5.0	5.0	5.0	5.0
Isopropyl alcohol	QS	QS	QS	QS	QS

Table 3: Composition of enteric coating material and different trials

Ingredient	F1	F2	F3	F4
Eudragit L-30 D-55	6.8	---	---	---
Talc	0.90	0.90	0.90	0.90
Triethyl Citrate	1.02	1.02	1.02	1.02
CAP	--	---	---	6.8
HPMC-P	--	6.8	--	--
Isopropyl Alcohol	--	QS	--	QS
Purified Water	QS	--	QS	--
Di Chloro Methane	--	QS	--	QS
Acryl-EZE	--	--	6.8	

2.20. Angle of repose

Angle of Repose was measured using the fixed funnel method, in this the granules were poured carefully until the apex of the conical pile just touches the tip of the stem of the funnel. The angle of repose was calculated using theformula , where H is the height of the pile and R is the radius of the base of the conical pile.

Tan $\alpha = H/R$.

2.21. Evaluation of Tablets

2.21.1. Physical parameters

2.21.1.1. Weight Variation

Twenty tablets were randomly selected from the batch and their weight was recorded separately on the analytical balance. Average weight was calculated with the standard deviation.

2.21.1.2. Friability Test

Another important parameter for physical strength of the tablet was conducted using Roche friabilator (Electrolab, Bangalore, India). In this study, 20 tablets were weighed and instrument was set at 100 revolutions for 4 min. After 4 min, the tablets were collected and dedusted by air and weight was recorded, from the difference of pre weight and current weight the percentage weight loss was calculated.

2.21.1.3. Hardness

Strength of the tablet was analysed using the Monsanto tablet hardness tester, in which the tablet was crushed and weight force was recorded to crush the tablet. Ten tablets were crushed and average force was recorded.

2.21.1.4. Disintegration Testing

The Disintegration for the tablets was studied using the two different solutions, 0.1 N HCl and Phosphate buffer of pH 6.8. Starting with 0.1 N HCl, the tablets were tested for disintegration using USP apparatus for 2hrs. The tablets which show no disintegration in this media were then further tested in phosphate buffer of pH 6.8. In the experimentation the temperature was maintained at 37°C ± 2.

2.21.1.5. Chemical ParametersDrug content studies

In this, twenty tablets were drawn from the batch randomly and crushed to the powder in mortar & pestle. Then the quantity equivalent to 50 mg of paclitaxel was weighed and analysed on HPLC using the method developed earlier.

2.21.1.6. In Vitro Drug Release Profile

The Drug release profile was evaluated using the USP XIII Type II (Paddle) method using a dissolution test

apparatus (Electro Lab, TDT-08L, Mumbai, India). The Dissolution studies were conducted as per the pharmacopoeial method in which two dissolution media were used. Firstly, tablets were kept for 2 h in 0.1N HCl and then media was changed into phosphate buff er pH 6.8. The Sample from the basket was withdrawn at regular intervals and analysed as per the analytical method developed [19].

3. RESULTS AND DISCUSSION

3.1. Pre-formulation Studies

3.1.1. Organoleptic evaluation

The above observations of colour, odour, taste and appearance are as per I.P.

Table 4: Organoleptic property of Paclitaxel

S. No.	Organoleptic properties	Paclitaxel
1	Color	White to off-white
2	Odor	Odorless
3	Taste	Bitter
4	Appearance	crystalline powder

3.1.2. Determination of wavelength maxima in methanol

The wavelength maxima for Paclitaxel was found 238 nm.

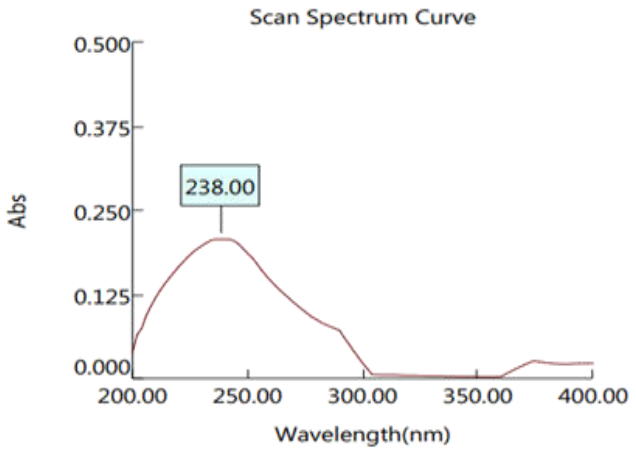

Fig. 1: Wavelength maxima of Paclitaxel in methanol

3.1.3. Calibration curve of Paclitaxel in methanol

The calibration curve was plotted between concentration and absorbance. Paclitaxel showed a linear relationship with correlation coefficient of 0.999 in the concentration range of 5-25µg/ml in methanol.

Where y is the response, x is the concentration, m is the slope and c is the intercept of a best fit line to the data.

Table 5: Calibration curve points of the proposed method for the estimation ofPaclitaxel

Concentration (µg/ml)	Mean Absorbance
5	0.156±0.002
10	0.324±0.002
15	0.481±0.003
20	0.659±0.002
25	0.821±0.004

**Average of three readings*

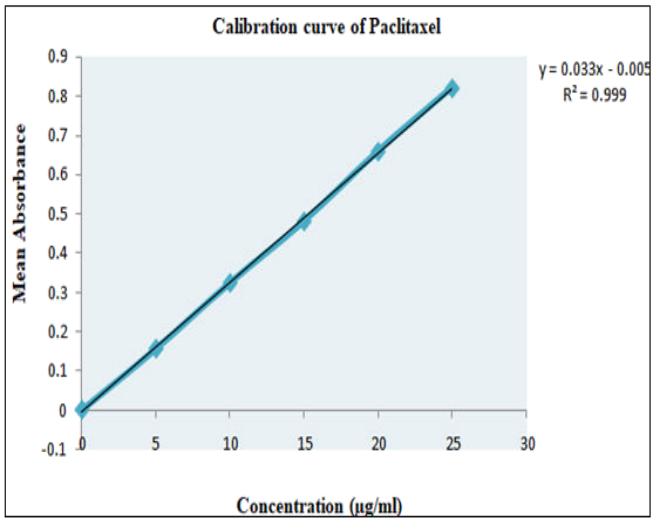

Fig. 2: Calibration curve of Paclitaxel in methanol

Table 6: Statistical parameters related to standard curve of Paclitaxel

Parameters	Values
Wavelengt maxima	238nm
Beer's Law Range	5-25 µg/ml
Regression Coefficient	$R^2 = 0.999$
Regressed line equation (y = mx + c)	y = 0.033x - 0.005

3.1.4. Fourier-Transform Infra Red Spectroscopy (FTIR)

The spectrum of drug was authenticated by FTIR spectroscopy. The presences of characteristic peaks associated with specific structural characteristics of the drug molecule were noted.

3.1.5. Melting point determination

Thermograms were recorded using a differential scanning calorimeter.

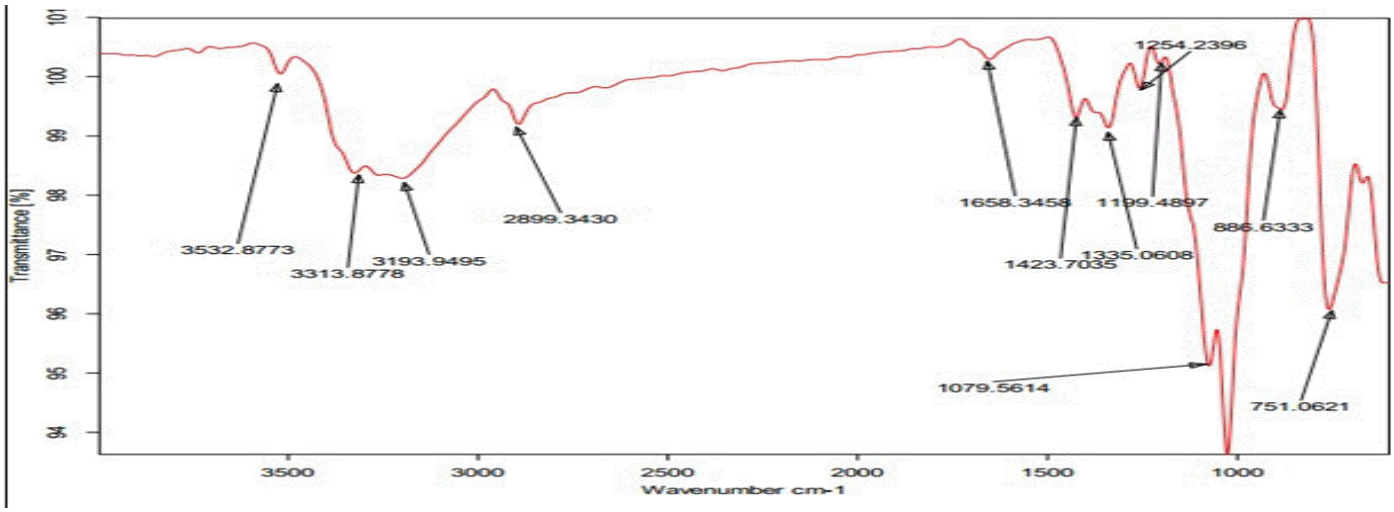

Fig. 3: FT-IR Spectrum of Pure Drug (Paclitaxel)

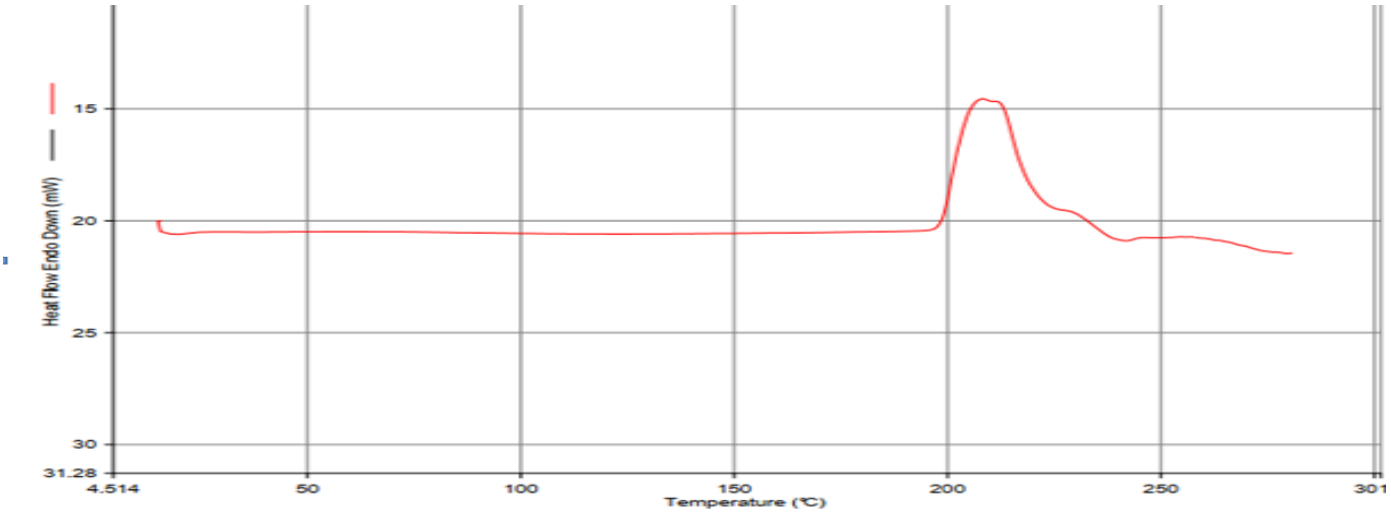

Fig. 4: DSC thermogram of pure Paclitaxel

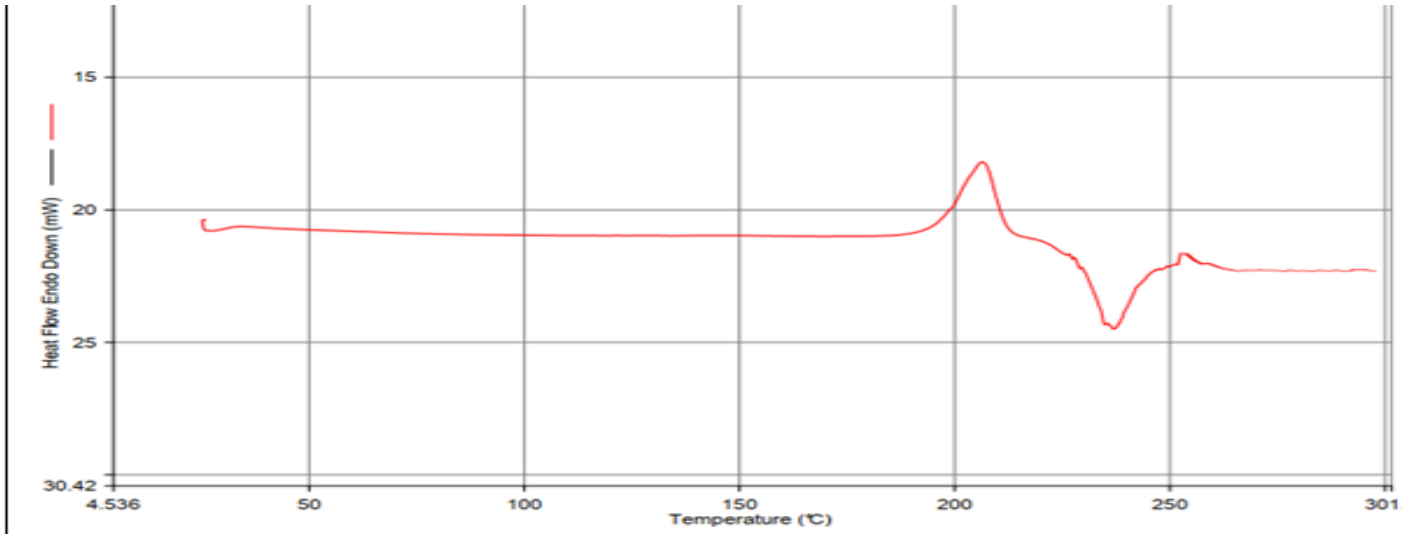

Fig. 5: DSC thermogram of pure drug (Paclitaxel) + Excipients

From the DSC data of drug and the physical mixture (drug and excipients) it is clear that functionalities of drug have remained unchanged including melting point of drug. This suggests that during the process drug and excipients has not reacted with the drug to give rise to reactant products. So there is no interaction between them which is in favor to proceed for formulation of blend microspheres. The DSC study shows that the drug and excipient are compatible with each other.

3.1.6. Solubility studies

Table 7: Solubility studies of Paclitaxel in different solvent

S. No.	Solvent used	Solubility of Paclitaxel
1.	Water	Slightly soluble
2.	0.1 N HCl	Sparingly soluble
3.	Ethanol	Soluble
4.	Methanol	Soluble

3.1.7. Uniformity of physical mixture

The physical mixture prepared for the studies has been checked out for the content of uniformity and results were found to be in the range of 98-102 %.

3.2. Fourier Transform Infrared Spectroscopy (FTIR)

In our pursue to check the compatibility among the drug and bioenhancer, the FTIR spectrums of PT, QU, PT: QU (Physical Mixture) were recorded. These recorded spectrums were compared to check any major or drastic change in the peak of PT. The Characteristic Peaks of PT shows a C-O stretch at 1045-1090 cm^{-1}. The amine (N-H) group in the PT shows a Peak at 3340 cm^{-1}. The Carbonyl ground C=O shows a major peak at 1720-1725 cm^{-1}. These were the major peaks which are identified for the characterization of the PT in the powder. Now these major peaks were compared with the Physical mixture spectrum, in which it has been observed that there was no major peak shift in the physical mixture although the intensity of the peaks has been reduced to some extent which may be due to the variation sample concentration. So, from FTIR it has been clearly elucidated that there are no structural changes in the drug due to the presence of the QU.

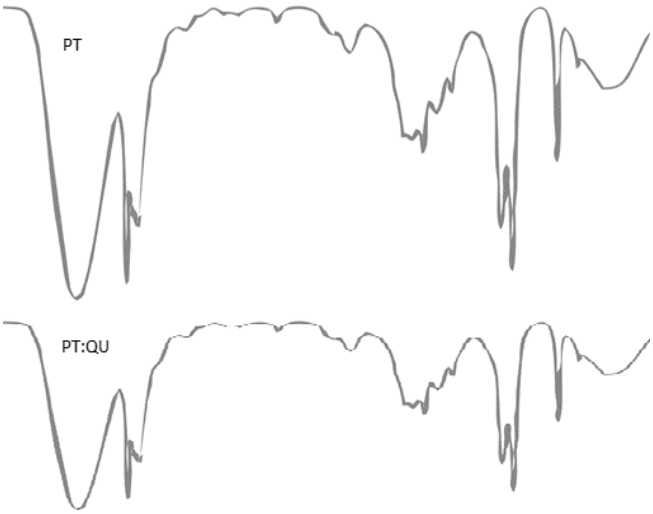

Fig. 6: FTIR Spectrum Comparison of PT and PT: QU

Table 8: Physical Mixture Content of Uniformity

Physical Mixture Ratio	3:0.5	3:1.0	3:1.5	3:2.0	3:2.5
PT: QU	98.56±0.35	99.36±0.45	98.76±0.75	99.26±0.65	101.2±0.35

3.3. Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry (DSC) thermograms of the PT, QU, PT: QU has been recorded as per the details shared in the method section. The thermograms obtained from the experiment were compared for any variability or interaction. The Thermogram clearly elucidate that there is no interaction among the drug and bio-enhancer, as there was no drastic change in the endothermic peak of the physical mixture of PT and QU. Thermogram of the PT shows a sharp endothermic peak at 215°C. This sharp peak is also in confirmation with the melting point that was observed during the pre-formulation studies of the drug. The Melting point of QU is 322°C and thermogram shows an exact sharp endothermic peak range at the same point which confirms the QU. The Physical mixture of PT and QU in 1:1 thermogram shows a sharp endothermic peak at 212°C. This peak is very near to the actual peak of the PTdrug which again proves the no compatibility issues among the drug and bio-enhancer. These DSC thermograms futures encourage the research to extrapolate to the permeation studies and dosage form design and act as lying foundation in the current research work.

3.4. Franz Diffusion Studies Ex-Vivo

In the intestine tissue, permeation of the physical mixture of PT with QU shows a significant

improvement in the when compared to the alone PT. The different weightratios of the PT and QU were used in this study, all the combinations were evaluatedfor the Permeation coefficient (Peff), comparison of the different mixture has been elucidated in the Table.

Table 9: Permeation coefficient for PT and PT: QU

Weight Ratio	Permeation Coefficient (Peff) × 10^{-6}cm/s
PT	0.659 ± 0.06
PT: QU (5:0.5)	0.719 ± 0.03
PT: QU (5:1.0)	0.742 ± 0.04
PT: QU (5:1.5)	0.963 ± 0.04
PT: QU (5:2.0)	0.926 ± 0.05
PT: QU (5:2.5)	0.904 ± 0.02
PT: QU (5:3.0)	0.886 ± 0.06

The PT and QU physical mixture in the ratio of 5:15 shows permeation coefficient around 0.963 × 10^{-6}cm/s which is significantly high than the PT alone which shows Peff, 0.659 × 10^{-6}cm/s. Although, at the lower weight ratios it also shows increment in the coefficient but as weight ratios moves from 1.5 to 3.0 the Peff, starts decreasing which clearly indicated the 5:1.5 that this ratio is the best for the PT and can be used further for future studies, also it validated the hypothesis of bioavailability enhancement of PT using the bioenhancers.

Table 10: Enhancement Ratio for PT and PT: QU

Weight Ratio	Enhancement Ratio
PT: QU (5:0.5)	1.09
PT: QU (5:1.0)	1.12
PT: QU (5:1.5)	1.46
PT: QU (5:2.0)	1.40
PT: QU (5:2.5)	1.37
PT: QU (5:3.0)	1.34

The PT and QU physical mixture in the ratio of 5:15 shows the maximum enhancement ration nearly equal to 1.46 times. As due to high variability in these kinds of model so this needs to be further validated using the cell lines studies. Also, to have an idea of the increment in the drug concentration, the drug release profile in the franz diffusion was also studied which shows an increment in the release with the time from the intestinal tissue.

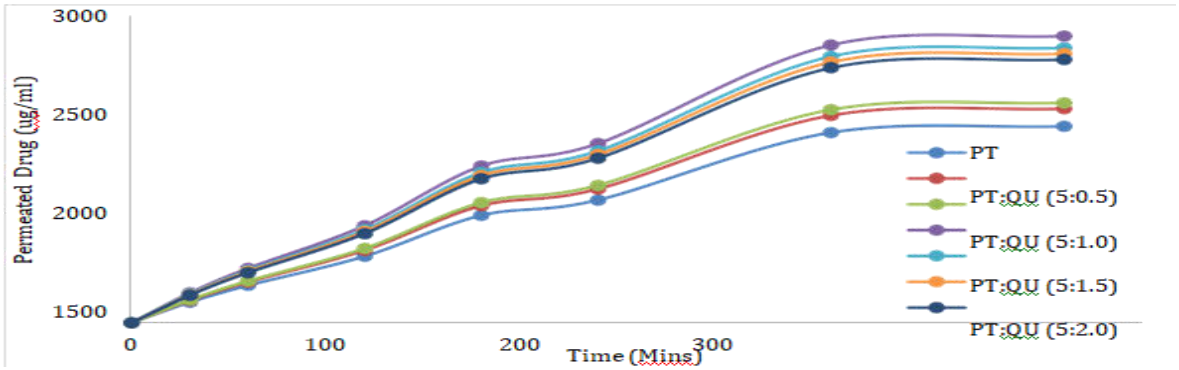

Fig. 7: Release Profile of PT and Mixture in the Presence of different WeightRatios of QU

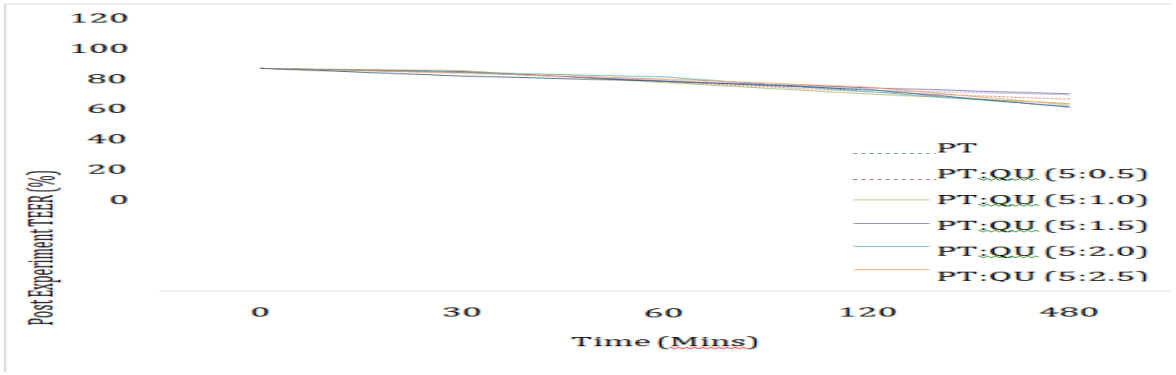

Fig. 8: TEER Values at Different Concentration of QU in the presence of PT

To further, confirm the permeation enhancement is genuine through actual transport mechanism, we conducted the cell viability of cell lines which are treated with the study group and it was observed that there was no permeation due to the cell deaths as cell viability percentage was found to be above 90%. In figure the release profile of the PT in the presence of different concentration has been illustrated, which shows there is increase the amount of PT in the BL side with the time. The Comparative data of different concentration of QU shows a good increment in the 5:1.5 for PT and QU. Papp values for the PT and combination of the PT and different concentration of the QU also illustrated in the figure. It has been observed that the QU and PT in the ratio of 1.5 to 5 has shown a dramatically increase in the release profile as well as in the Papp value. Enhancement ratio chart in figure also shows that at the ratio of 5:1.5 of PT and QU is good, this was again confirmed with the in-vivo pharmacokinetic studies.

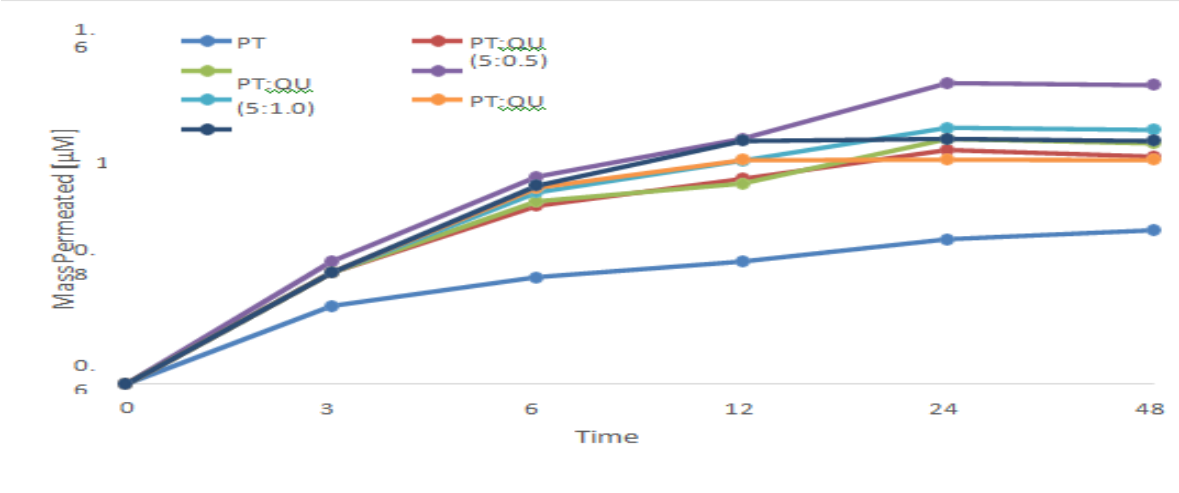

Fig. 8: Release Profile of PT alone and in presence of QU at differentconcentrations

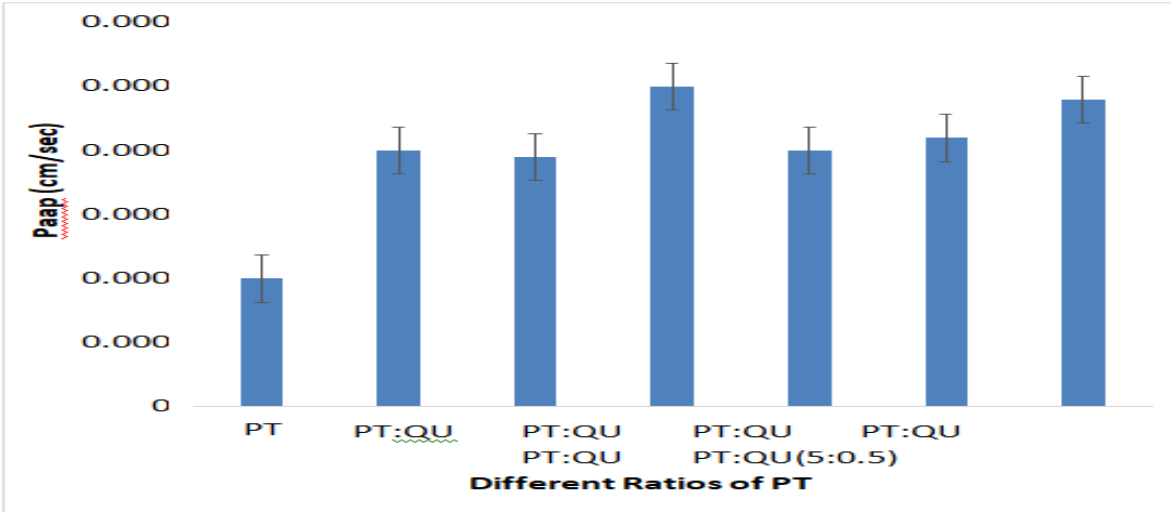

Fig. 9: Papp value comparison for PT alone and PT in combination of QU

3.5. Development and evaluation of tablets

In formulation development, five different tablets were prepared as shown in table with constant amount of quercetin but varying concentration of mannitol, calcium phosphate etc. These prepared tablets were then studied for the disintegration studies, it was observed that F1 as per table shows the best disintegration time of 1.5±0.20 min. The disintegration was carried out in phosphate buffer of pH 6.8. This formulation was further chosen

for the enteric coating. These formulation granules were also tested for other parameters such as bulk density, tapped density which were found to be bulk density; 0.45±0.02 g/cc and tapped density 0.53±0.01 g/cc. The angle of repose was found to be 29.16±0.82 θ and Carr's Index was found tobe 18.26±0.25. This parameter confirms the granules are good in physical properties and can be used further for compression.

Hardness was found to be 5.1 ± 0.41 kg/cm2 and friability was found to be 0.19±0.01%. To confirm the equal distribution of the weight the weight variation was tested which was found to be 199.16±2.36. Then these developed tablets of formulation F-1 were used for the coating, in this research work we have used the four different types of coating materials as discussed in table. These all are anionic polymers which are insoluble in acidic medium and solubilize near the neutral pH.

It was observed that Eudragit L30 required plasticizer and detackifier during dispersions as suggested by literature and vendor. So, we have used triethyl citrate and talc for the same reason with the polymer. The Four different type coating was conducted on the F1, in 4 different parts such as F1, F2 etc. The Drug content and other parameters were studied before the dissolution and have been shown in table.

It has been observed that F1 shows no-disintegration of the tablets in the acid media (0.1 N HCl) during the study period (2 h). As, these formulation also tested for the disintegration in phosphate buffer ph 6.8 and shows some good difference among time. Figure shows the dissolution profile of F1-F4 in 0.1 N HCl and Phosphate buffer medium.

Table 11: Parameters of Enteric Coating Tablets

Parameters	F1	F2	F3	F4
Weight Variation	199.16±2.36	191.11±1.85	196.36±3.26	198.25±3.12
Drug Content	98.22	92.86	96.58	97.56
Disintegration (0.1 N HCl)	-	115.67	114.29	113.69
Disintegration (6.8 pH)	12.35	09.63	10.63	11.05

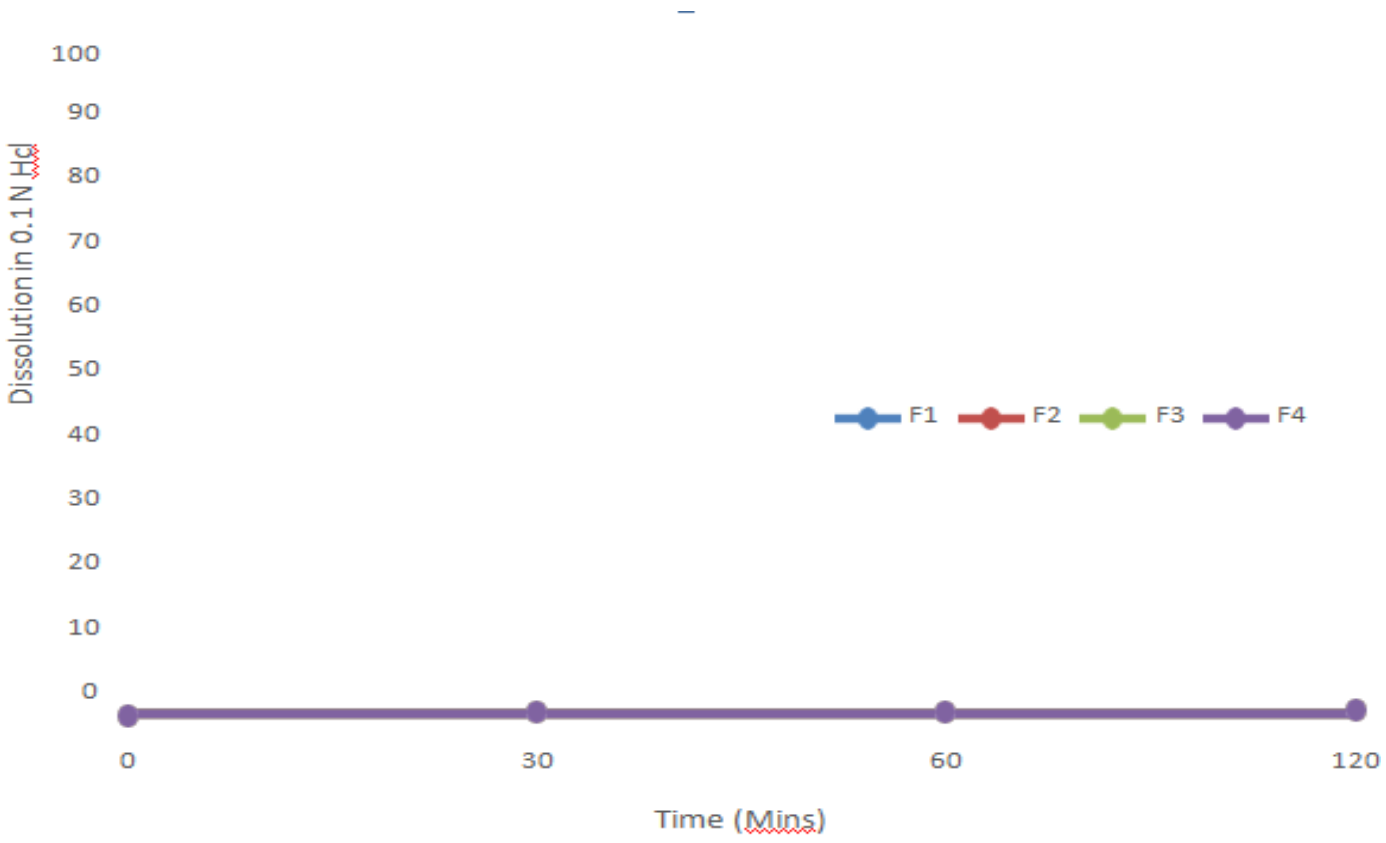

Fig. 10: Dissolution profile in 0.1N Hcl for F1, F2, F3 and F4

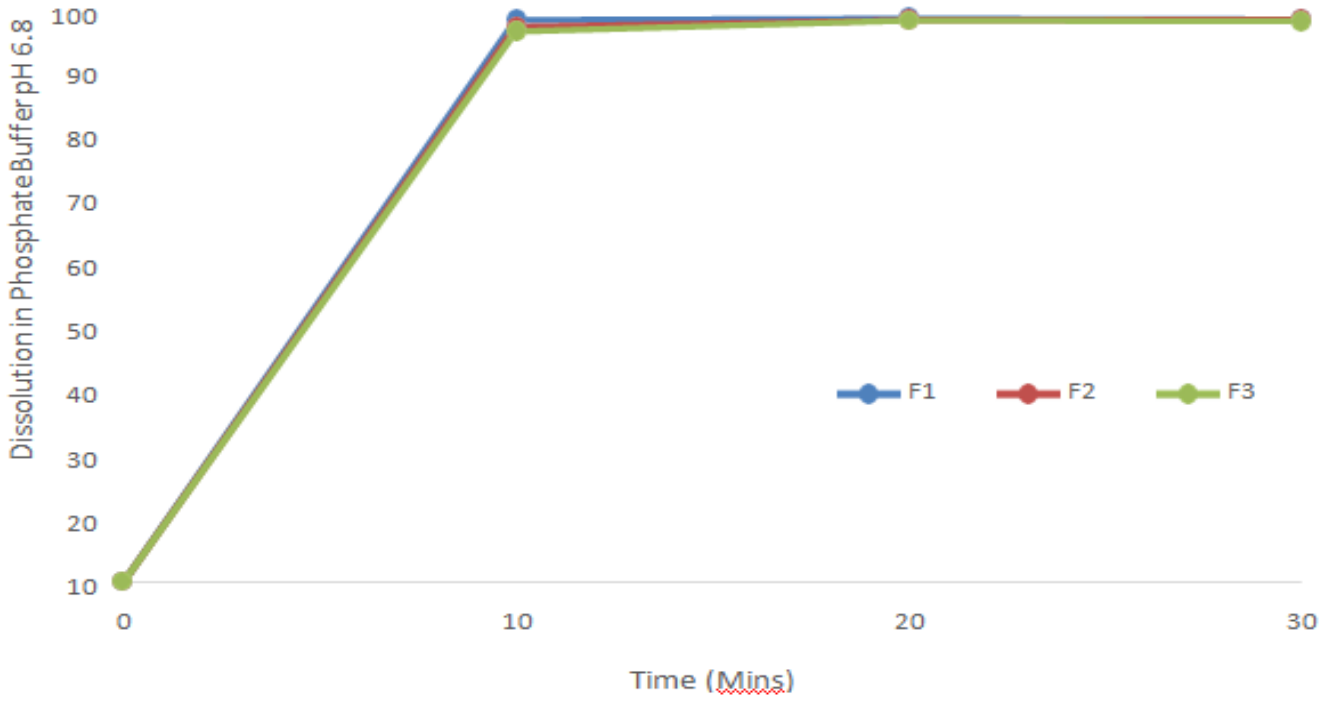

Fig. 11: Dissolution profile in phosphate buffer 6.8 pH for F1, F2, F3 and F4.

4. CONCLUSION

A simple RP-HPLC method of paclitaxel was developed and optimized. Optimization of the method for the combination of drugs by the conventional trial and error method would have been a tedious job. The optimized RP-HPLC method developed for the analysis of paclitaxel was found to be simple, precise, and reproducible. The validation of the RP-HPLC method was carried out according to ICH guidelines. The absence of significant interfering peaks and lower % RSD values

Conflict of interest

None declared

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