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Quantitative Estimation, Validation and Stability Indicating Assay Method for Determination of Related Substances in Paclitaxel.

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Abstract: This research will contribute to the development of a method that will reduce manufacturing costs for drugs by being more accurate, precise, and affordable than previously established approaches. The method can be used on an industrial scale. The estimation of impurity profile, which aims to detect, identify, and quantitatively determine organic and inorganic impurities as well as residual solvents in bulk pharmaceuticals and pharmaceutical formulations, is also included in this study.

Keywords: Paclitaxel, Anticancer, UV spectroscopy, HPLC, Validation, Stability studies.

Introduction: Anticancer drugs were quantified using a variety of analytical techniques, either alone or in conjunction, including radioimmunoassay, UV, reverse HPLC, HPTLC, HPLC, LC-MS, and MS. Anticancer medication manufacturers are requiring routine analysis, therefore efforts are being made to create straightforward and precise instrumental procedures for the quantitative estimate of the drugs' determination in formulation. Therefore, for the purpose of effectively estimating anticancer drugs, newer, simpler, more sensitive, accurate, and cost-effective analytical methodologies must be developed. To guarantee a pharmaceutical product's safety and effectiveness for its whole shelf life, analytical monitoring of the product or a particular constituent is required. [1]

Materials and method

Selection of solvents

Methanol (AR grade) was selected as the solvent after considering the solubility and stability factor of both the drugs as well as the interference due to the excipients matrix present in the tablet formulation.

Preparation of stock solutions

To prepare stock solution of Paclitexel, (100 µg/ml) 100mg of Paclitexel was placed in 100 ml volumetric flask and dissolved in 75 ml of methanol and the volume was made up to the mark with methanol, to obtain the solution of 1000 µg/ml . 10 ml of the solution was diluted up to 100ml with methanol to produce final stock solution of 100 µg/ml of Paclitexel. [2]

Preparation of Standard for the test of linearity

From the stock solution of 100µg/ml appropriate dilution with methanol was made to prepare the solution with concentration. The absorbance was measured and the calibration curves were plotted from the mean values of observation.

Intermediate Precision (Inter-day and Intra-day precision)

The intra and inter-day precision was calculated by assay of the sample solution ones the same day and on different days at different time intervals respectively.

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

100 mg each of the reference standards of Paclitexel, were weight and transferred to separate 100 ml volumetric flasks. All three drugs were dissolved in methanol and the volume were made up to the mark with, the same solvent to get solutions of concentration 1000 μg/ml. 1 ml of each of these was diluted to 10 ml with methanol in two separate volumetric flasks to get solutions of concentration 100 μg/ml. For each drug appropriate aliquots were pipetted from the final solutions into a series of 10 ml volumetric flasks. The volumes were made with methanol to get a set up solutions of each drug in various concentration ranges (2, 4, 6 … 20 μg/ml for PCT). The absorbance was measured at 254 nm. This was repeated ten times and the standard deviation of the analyte was calculated.[3]

Stability studies of Paclitexel

Design

A minimum of four samples should be generated for every stress condition,

1. The blank solution is stored under normal conditions.
2. The blank subjected to stress in the same manner as the drug solution.
3. Zero-time sample containing the drug which is stored under normal conditions.
4. Drug solution subjected to stress treatment.

Chromatographic conditions used for the developed and validated HPTLC method for Paclitaxel

The following densitometric conditions were used for HPTLC studies:

The following densitometric conditions were used for HPTLC studies:

Stationary phase	:Precoated plates of Silica Gel 60 GF254 (Merck)
Mobile phase	: Chloroform: methanol: formic acid (8.2:1.5:1)
Saturation time	: 15 min
Development time	: 15 min
Wavelength	: 254 nm
Lamp	: Deuterium
Band width	: 7 mm
Length of chromatogram	: 8 cm

Forced Degradation Studies of Paclitaxel [4]

Acid degradation

The hydrochloric acid (HCl) was prepared by diluting 8.5 ml of concentrated HCl to 100 ml of distilled water. 1mg/ml solution was prepared of Paclitaxel.1 ml of Paclitaxel solution and 4 ml of 1N HCl were mixed and the mixture was refluxed ina water bath for 3 hours at 60°C. The refluxed solution of Paclitaxel and HCl was allowed to attend ambient temperature and then the refluxed solution was neutralized by 1 N NaOH to pH 7 and the volume was made up to 10 ml with methanol. Then the final solution was applied to the TLC plates.

Total degradation was found when the Paclitaxel was refluxed 1 N HCl for 3 hr., therefore the exposure time was reduced to 1 hour with the same concentration of HCl. Then the stressed sample was analyzed. The chromatogram of the 1 hr. refluxed sample showed the same pattern of degradation as that of the 3-hr. refluxed sample.

There were six peaks that were degradants as none of the peaks showed similar Rf as that of standard. Among all degradants peak at Rf 0.29 was in the highest percent (64.99%) as compared to other degradation compounds.

Thus, the exposure time of the Paclitaxel to HCl was kept for 1 hr and the concentration of HCl was decreased to 0.1N. Further on analysis the stressed sample showed almost no change compared to the previous conditions. Hence it was concluded that the Paclitaxel was not stable under any stressed acidic conditions tested.[5]

Base degradation

1M of NaOH was prepared by dissolving 4 g of sodium hydroxide pellets in 100 ml of distilled water.

1 ml of Paclitaxel solution (1 mg/ml) and 4 ml of 1N NaOH were mixed and refluxed ina water bath for 3 hours at 60°C. The solution was allowed to attend ambient temperature and then the solution was neutralized by 1 N HCl to pH 7 and the volume was made up to 10 ml with methanol. Then the final solution was applied to the TLC plates.

Total degradation was found when the Paclitaxel was refluxed 1 N NaOH for3 hr., therefore the exposure time was reduced to 1 hr. with the same concentrationof NaOH. Then the stressed sample was analyzed. The chromatogram of the 1 hr. refluxed sample showed the same pattern of degradation as that of the 3-hr. refluxed sample. Thus, the exposure time of thePaclitaxel to NaOH was kept for 1 hr. and the concentration of NaOH was Decreased to 0.1N. Further on analysis, the stressed sample when analyzed showed degradants peak at Rf 0.22, 0.41, 0.59, 0.78. The peak at Rf 0.22 was in higher concentration with 84.47%. Hence it was concluded that the Paclitaxel was not stable under any stressed alkaline conditions tested.

Oxidative degradation

1 ml of Paclitaxel solution (1 mg/ml) and 9 ml 3 % H202 solution were mixed and the mixture was refluxed ina water bath for 3 hr. at 60°C.The solution was allowed to attend to ambient temperature and applied to the TLC plates. There was no oxidative degradation Paclitaxel found when studied using 3% of H2O2 for 3 hr. The exposure time to oxidative condition was increased gradually up to 8 hr. When the stressed sample was analyzed, there were no additional peaks. There was no difference in the peak area of the stressed sample and the untreated sample of Paclitaxel. Thus, it indicates that there was no degradation due to oxidative stress (Fig.11.12). Hence it was concluded that Paclitaxel was stable under the conditions tested.[7]

Wet degradation

10 ml of aqueous Paclitaxel solution (1 mg/ml) were refluxed ina water bath for 3 hr at 60°C. The solution was allowed to attend to ambient temperature and then applied to the TLC plates.There were three peaks of degradants beside the Paclitaxel peak with the Rf values 0.34, 0.56, 0.59, and 0.76 (Fig. 11.13). The Rf of Paclitaxel was shifted from 0.76 to 0.92. At Rf 0.76 there was a degradant peak was observed with a higher percentage of peak area (51.81%). Paclitaxel peak at Rf 0.92 was confirmed by comparing the UV spectrum with the untreated standard Paclitaxel (Fig. 11.14). The λmax of the tested Paclitaxel and of the standard Paclitaxel λ max were 254 nm and 385 nm. Thus, it was confirmed that 86.15% of Paclitaxel was degraded. [8]

Dry heat

5mg of Paclitaxel was placed in an oven for 3 hr. at 100°C and then the hated sample of Paclitaxel was dissolved in 5ml of methanol. 5mg of Paclitaxel was placed in an oven for 24 hr at 60°C and then the heated sample was dissolved in 5ml of methanol. Both the solutions were allowed to attend ambient temperature and applied onto the TLC plates.

When the stressed sample of 3 hr. at 100°C was analyzed, no degradation was found (Fig.11.15). But the sample of 24 hr. at 60°C showed a total of three peaks out of which one with Rf 0.77 was of Paclitaxel which was confirmed by the UV spectrum (Fig. 11.16). The other two were (with Rf 0.88, 0.96) degradants and the total percent of degradation of Paclitaxel was found to be 5.22 %.[9]

Photo stability study

5mg of Paclitaxel was exposed to UV short (254 nm) light for 24 hr to study the UV degradation. Then the exposed sample of Paclitaxel was dissolved in 5ml of methanol and applied to the TLC plates.When the stressed sample was analyzed, there was no degradation observed in the Paclitaxel sample. The sample was again exposed for 48 hr. Further chromatographic studies showed no degradation. Hence, it was concluded that the Paclitaxel sample was stable under tested conditions.[10]

Table 1 Linearity studies of Paclitaxel at 276 nm

S. No.	Conc. (µg/ml)	Absorbance of Paclitaxel						SD(±)
		Replica 1	Replica 2	Replica 3				
1	5	0.172	0.162	0.158				0.00709
2	10	0.309	0.298	0.284				0.0118
3	15	0.443	0.449	0.433				0.00623
4	20	0.592	0.608	0.603				0.00756
5	25	0.788	0.786	0.782				0.0054
6	30	0.904	0.898	0.892	0.904	0.886	0.895	0.00782
7	35	1.036	1.046	1.041	1.032	1.034	1.038	0.00567
8	40	1.180	1.186	1.183	1.170	1.178	1.179	0.00607
9	45	1.336	1.332	1.328	1.339	1.338	1.335	0.00456
10	50	1.462	1.469	1.457	1.460	1.458	1.461	0.00476

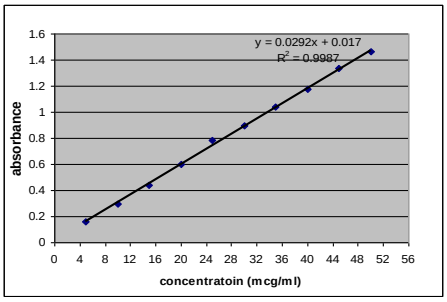

Fig 1 a) Linearity studies of Paclitaxel at 276 nm b)LOD and LOQ of Paclitaxel

Table 2: Intra and inter day precision study of Paclitaxel.

Replicate No.	Intraday			Interday		
	Percentage obtained			Percentage obtained		
	1st hr	2nd hr	3rd hr	Day 1	Day 2	Day 3
Replicate-1	99.71	99.61	98.67	99.97	99.87	98.10
Replicate-2	99.67	98.56	98.98	99.65	99.90	99.13
Replicate-3	99.87	99.12	98.03	100.12	99.45	99.01
Replicate-4	99.34	99.12	100.12	99.89	99.01	98.12
Replicate-5	99.98	100.12	98.56	100.01	99.39	99.12
Mean	99.71	99.30	98.87	99.92	99.52	98.69
S.D.	0.243	0.588	0.777	0.176	0.370	0.537
%CV	0.24	0.59	0.79	0.18	0.37	0.54

Table 3: LOD and LOQ of Paclitaxel

S. No.	Conc. (µg/ml)	Absorbance of Paclitaxel							SD(±)
		Replica 1	Replica 2	Replica 3	Replica 4	Replica 5	Replica 6	Mean	
1	5	0.172	0.162	0.158	0.154	0.164	0.174	0.162	0.00709
2	10	0.309	0.298	0.284	0.309	0.287	0.308	0.296	0.0118
3	15	0.443	0.449	0.433	0.447	0.441	0.443	0.443	0.00623
4	20	0.592	0.608	0.603	0.596	0.590	0.592	0.598	0.00756
5	25	0.788	0.786	0.782	0.796	0.784	0.782	0.787	0.0054
6	30	0.904	0.898	0.892	0.904	0.886	0.892	0.895	0.00782
7	35	1.036	1.046	1.041	1.032	1.034	1.041	1.038	0.00567
8	40	1.180	1.186	1.183	1.170	1.178	1.183	1.179	0.00607
9	45	1.336	1.332	1.328	1.339	1.338	1.328	1.335	0.00456
10	50	1.462	1.469	1.457	1.460	1.458	1.457	1.461	0.00476

Mean standard deviation	0.0067
Slope	0.0292
LOD	3.3 x 0.0067/0.0292= 0.76 µg/ml
LOQ	10 x 0.0067/0.0292= 2.31 µg/ml

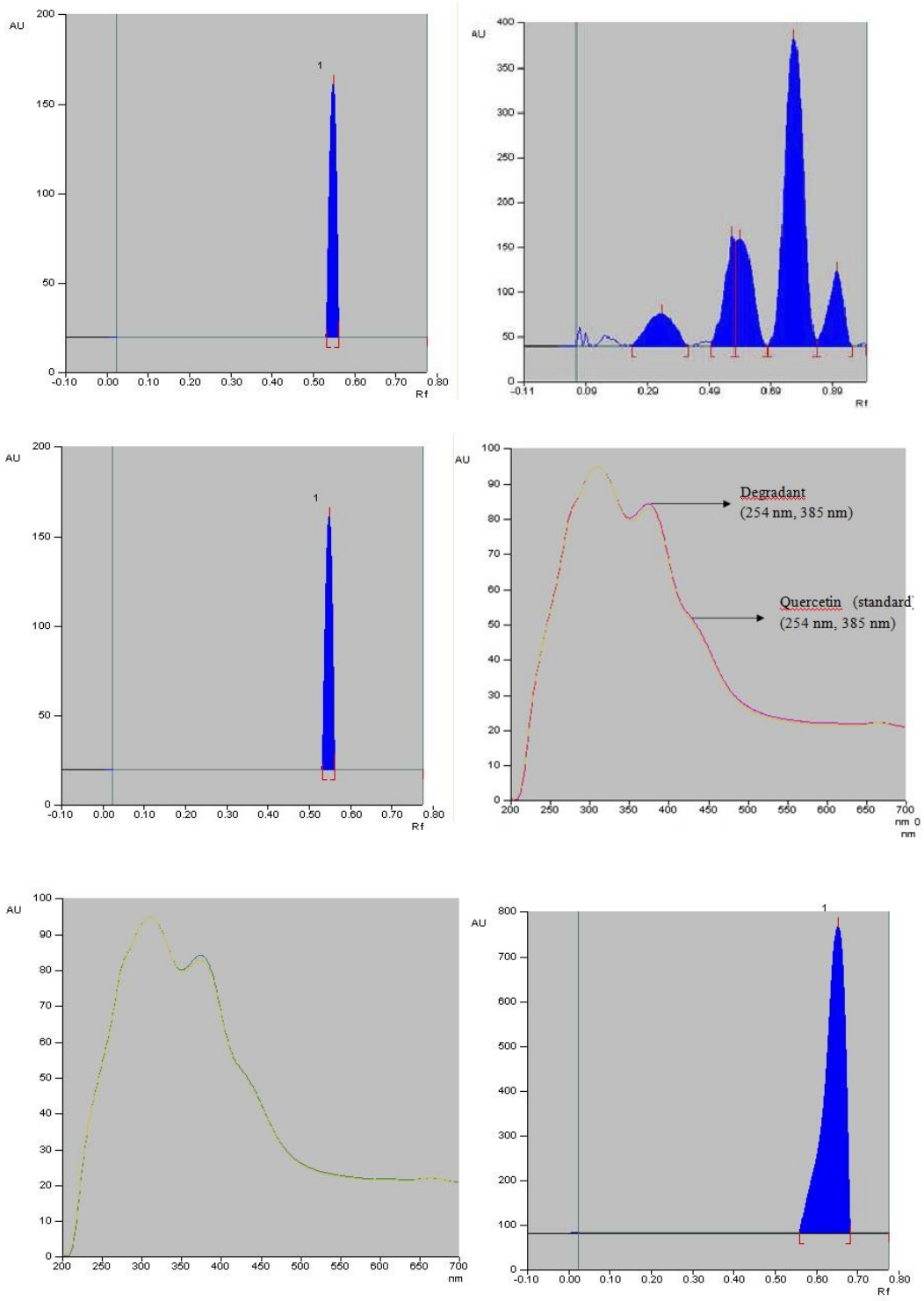

Fig. 2: HPTLC Chromatogram of Paclitaxel a) after acid degradation b) after base degradation c)after oxidative stress d) after wet degradation e) after dry degradation f) after UV exposure

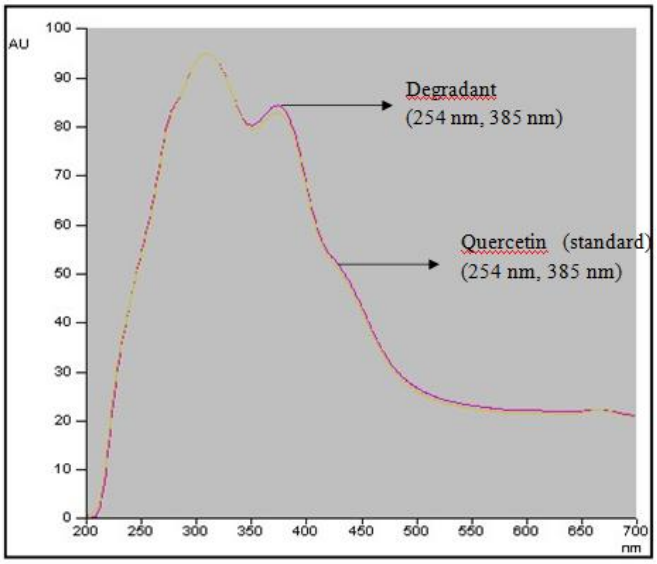

Fig. 3: UV spectrum of Paclitaxel (standard) and degradant (UV exposed condition)

CONCLUSION

The application of developed and validated HPTLC methods for Paclitaxel as stability indicating methods was successfully employed. It was observed that Paclitaxel was stable only under dry conditions but the other conditions had altered the concentration of Paclitaxel. Paclitaxel is a biomarker with very good anti-oxidants and many other therapeutic activities. It is even present in many medicinal plants. Thus, the stability indicating method can be very well adapted for the evaluations of many different formulations containing these two biomarkers.

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