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Research Article

ANALYTICAL METHOD DEVELOPMENT VALIDATION FOR SIMULTANEOUS ESTIMATION OF PACLITAXEL, GEMCITABINE IN PH DOSAGE FORM BY HPLC

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ABSTRACT:

Gemcitabine and Paclitaxel method validation was estimated by using Agilent column of dimension 150 x 4.6 mm, OPA: MeCN was selected as Mp in 55:45 v/v ratio. This combination was optimized at a spectrum of 256 nm. The Retention of this drugs were eluted at 2.205 and 3.080 for Gemcitabine and Paclitaxel, its RSD was 0.3 and 0.5 with in limit. And the regression was obtained at is y = 89619x + 2823.2, and y = 88164x + 5630.1 respectively. Mean recovery was achieved at 99.20% and 99.50% for Gemcitabine and Paclitaxel respectively and this method was specific with any interference of other drug peak.

Keywords: Paclitaxel, Gemcitabine, Rp HPLC, Validation, Method Development.

INTRODUCTION

Gemcitabine and paclitaxel are widely used chemotherapeutic agents, often combined to treat a variety of malignancies, including breast, ovarian, and lung cancers. Gemcitabine, a nucleoside analog, acts as an antimetabolite that interferes with DNA synthesis. It is incorporated into the DNA strand during replication, leading to chain termination and apoptosis of rapidly dividing tumor cells[1].

Paclitaxel, a microtubule-stabilizing agent, inhibits the normal breakdown of microtubules during mitosis. This leads to mitotic arrest and subsequent cell death, making it effective against fast-proliferating cancer cells[2].

The combination of gemcitabine and paclitaxel has shown synergistic effects in various clinical settings. The regimen exploits the different mechanisms of action, with gemcitabine enhancing the cytotoxic effects of paclitaxel by synchronizing cells in the G1/S phase of the cell cycle, where paclitaxel exhibits maximum efficacy[3]. This synergy has been particularly beneficial in treating triple-negative breast cancer and advanced non-small cell lung cancer (NSCLC), offering improved response rates and progression-free survival compared to single-agent therapy[4].

Despite its effectiveness, the combination is associated with notable toxicities, including myelosuppression, neuropathy, and fatigue. However, these side effects are often manageable with supportive care and dose modifications[5]. Ongoing research continues to optimize dosing schedules and explore biomarkers to identify patients most likely to benefit from this combination therapy[6]. Gemcitabine is a Chemotherapy drugs are used alone to treat pancreatic cancer, in combination with other drugs, to treat advanced stages of breast and ovarian cancer, and to treat a particular kind of lung cancer. Chemically known as 4-amino-1-[(2R,4R,5R)-3,3-difluoro-4-hydroxy-5-(hydroxymethyl)oxolan-2-yl]-1,2-dihydropyrimidin-2-one.[7] Paclitaxel is an antitumor drug used to treat breast, ovarian, and lung cancer. Known as (1S,2S,3R,4S,7R,9S,10S,12R,15S)-4,12-bis(acetyloxy)-1,9-dihydroxy-15-{[(2R,3S)-2-hydroxy-3-phenyl-3-(phenylformamido)propanoyl]oxy}-10,14,17,17-tetramethyl-11-oxo-6-oxatetracyclo[11.3.1.0^{3,10}.0^{4,7}]heptadec-13-en-2-yl benzoate.[8]

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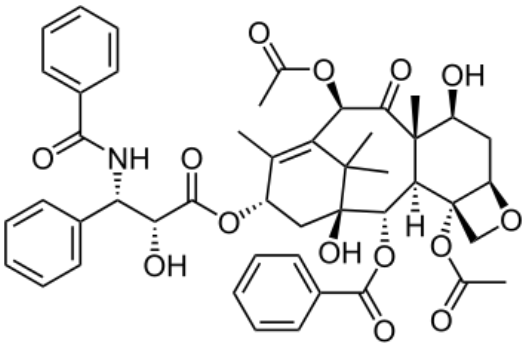
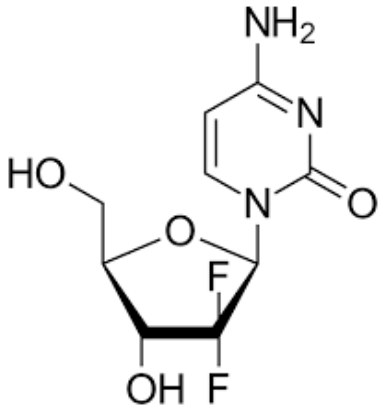


[A]



[B]

Figure 1 and 2: structure of [A] Paclitaxel and [B]Gemcitabine

Extensive literature research has unearthed a multitude of recorded analytical procedures, including the discovery of more economically efficient ways. Hence, a reliable and cost-effective approach is suggested for assessing the stability of Gemcitabine, Paclitaxel, and their medicinal dose form using RP-HPLC.[9-14] must be validated and developed as per ICH guidelines.

Materials and Methods: Gemcitabine and Paclitaxel pure pharmaceuticals (API) are available as gift samples from Spectrum Pharma Research Solution. The chemicals and buffers used in this estimation were supplied by Rankem, an Indian source.

Instrumentation: The development and method validation were conducted using a WATERS HPLC, specifically the model 2695 SYSTEM, equipped with a Photo diode array detector. The system also included an automated sample injector and the Empower 2 software.

Objective: In order to ful fill ICH standards, we need to design and test an HPLC technique that can detect Paclitaxel and Gemcitabine in pharmaceutical formulations at the same time.

Table 1 : Chromatographic Conditions

Mobile phase	**Acetonitrile: OPA (45:55 v/v)**
Flow rate	1.0 ml/min
Column	Agilent C18 Column, 5 µm, 4.6 x 150 mm
Detector wave length	256 nm
Column temperature	30°C
Injection volume	10µL
Run time	10.0 min

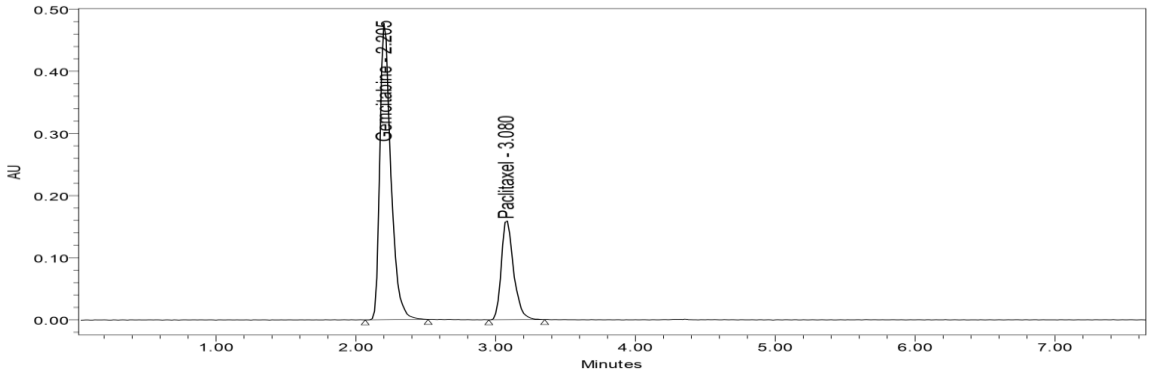


Figure 3: Optimized Chromatogram

Preparation of Standard stock solutions: In each 50 millilitre of vf, 62.5 milligrammes of gemcitabine and 25 milligrammes of paclitaxel were injected. Prior to adding further diluent, the mixture was sonicated for 10 minutes after being filled to a fourth of its capacity with diluent. (1250µg/ml of Gemcitabine and 500µg/ml of Paclitaxel). Then, one millilitre of the stock solution was added to a ten millilitre vial of diluent, and the volume was adjusted to fit the needs. Gemcitabine has a dose of 125 µg/ml, while paclitaxel is 50 µg/ml.

Preparation of Sample stock solutions: One vial of the drug's commercial formulation was injected into one hundred millilitres of volumetric flask along with twenty-five millilitres of diluent; the mixture was then subjected to sonication. Subsequently, it was compounded using diluent (Gemcitabine - 2500µg/ml, Paclitaxel - 1000µg/ml). Two millilitres of the stock solution were spiked and added to a ten millilitre volumetric flask, which was diluted to the mark using a diluent. The concentrations of Gemcitabine and Paclitaxel are 125 µg/ml and 50 µg/ml, respectively.

System suitability parameters: The system appropriateness characteristics, including peak tailing, resolution, and USP plate count, were assessed after six injections of standard solutions of gemcitabine (125 ppm) and paclitaxel (50 ppm). An RSD of no more than 2% should be present in the region of six standard injection results.

Specificity: Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So, this method was said to be specific.

Table 2: System suitability results

S.no	Gemcitabine			Paclitaxel			
Inj	RT (min)	USP Plate Count	Tailing	RT (min)	USP Plate Count	Tailing	Resolution
1	2.197	3442	1.33	3.065	5666	1.34	5.5
2	2.198	3910	1.43	3.069	6576	1.28	5.7
3	2.200	3293	1.42	3.069	5650	1.36	5.5
4	2.201	3405	1.37	3.070	6115	1.32	5.5
5	2.202	3981	1.37	3.071	5969	1.26	5.7
6	2.203	4184	1.34	3.073	5372	1.29	5.7

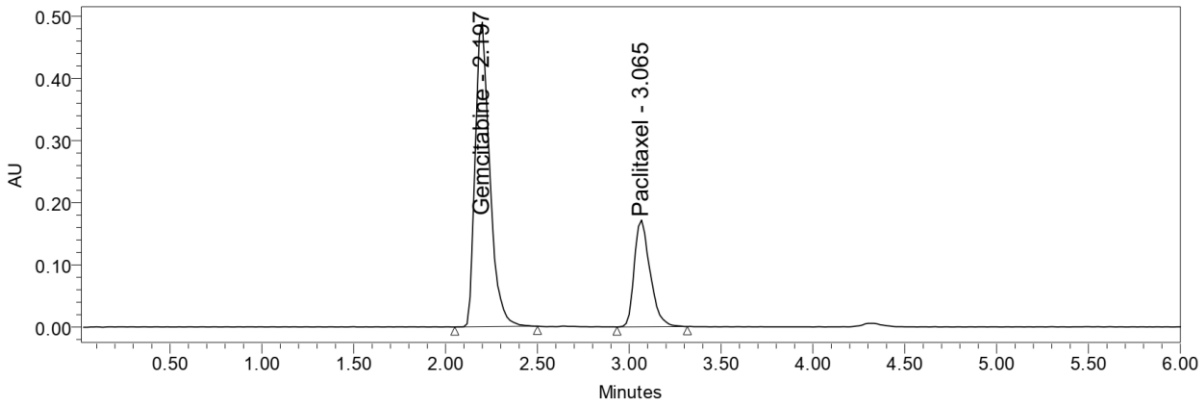


Figure 4: system suitability Chromatogram

Table 3: Specificity data

Sample name	Retention time(mins)	Area
Gemcitabine	2.205	479867
Paclitaxel	3.080	160136

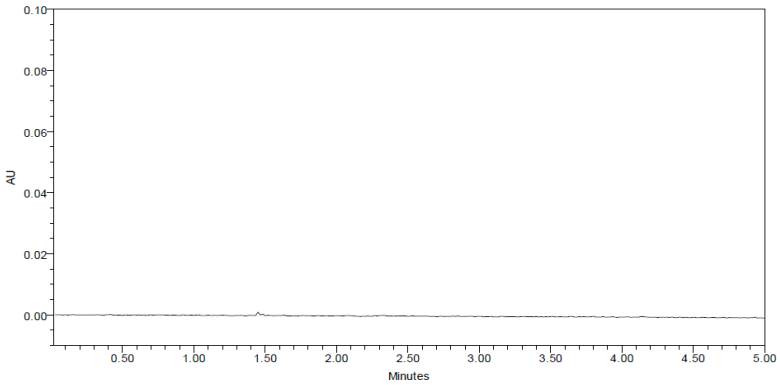


Figure 5: Blank

Linearity:

Calibration data and regression data in table 4 and calibration curve in figure 6, 7.

Table 4: Calibration data of Gemcitabine and Paclitaxel

	Gemcitabine		**Paclitaxel**	
S. no	**Conc (µg/mL)**	**Peak area**	**Conc(µg/mL)**	**Peak area**
1	0	0	0	0
2	7.5	672955	3	265667
3	15	1367402	6	541071
4	22.5	1960846	9	789355
5	30	2736331	12	1081999
6	37.5	3394717	15	1350741
7	45	4002518	18	1564892
Concentration range	**7.5-45**		**3-18**	
Regression Equation	**y = 89619x + 2823.2**		**y = 88164x + 5630.1**	
Co-relation	**0.9994**		**0.9991**	
LOD	**0.15**		**0.45**	
LOQ	**0.11**		**0.33**	

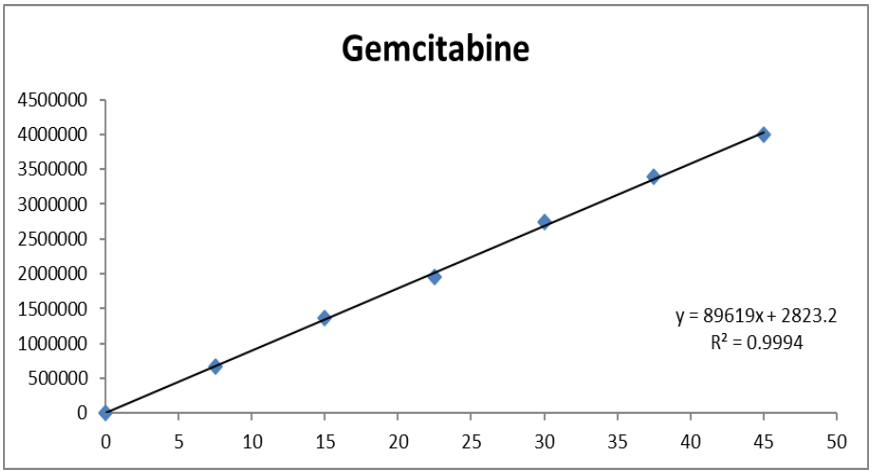


Figure 6: Calibration curve of Gemcitabine

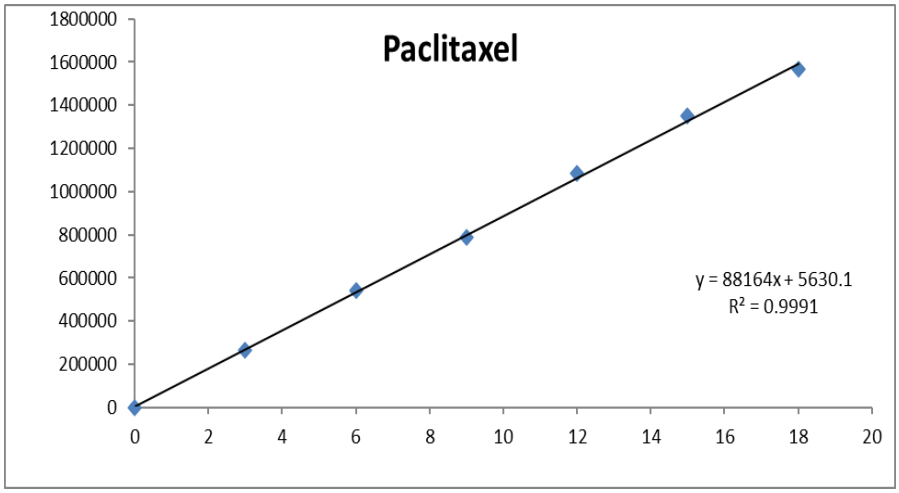


Figure 7: Calibration curve of Paclitaxel

Accuracy:
Recovery data shown in table 5

Table 5: recovery data of Gemcitabine and Paclitaxel

	Gemcitabine			Paclitaxel		
% Level	**Amount Spiked (µg/mL)**	**Amount recovered (µg/mL)**	**% Recovery**	**Amount Spiked (µg/mL)**	**Amount recovered (µg/mL)**	**% Recovery**
50%	15	15.00	99.99	6	5.92	98.73
	15	14.82	98.79	6	6.00	99.98
	15	14.71	98.08	6	5.92	98.66
100%	30	29.57	98.55	12	11.94	99.52
	30	29.82	99.39	12	12.00	100.03
	30	29.93	99.78	12	11.94	99.49
150%	45	44.85	99.66	18	17.94	99.64
	45	44.53	98.95	18	18.16	100.89
	45	44.81	99.57	18	17.74	98.55
% recovery	**99.20**			**99.50**		

System precision was performed and the data was shown in table 6

Table 6: System precision of Gemcitabine and Paclitaxel

S. No	Area of Gemcitabine	Area of Paclitaxel
1.	2624141	1050947
2.	2636171	1064651
3.	2621425	1055562
4.	2634743	1059850
5.	2632248	1054778
6.	2639258	1059278
Mean	**2631331**	**1057511**
S.D	**7050.8**	**4773.4**
%RSD	**0.3**	**0.5**

The % RSD for the peak areas of Gemcitabine and Paclitaxel obtained from six replicate injections of standard solution was within the limit.

Method Precision: The precision of the method was determined by analyzing a sample of Gemcitabine and Paclitaxel and shown in table 7.

Table 7: method Precision

S. No	Area of Gemcitabine	Area of Paclitaxel
1.	2647498	1062561
2.	2654655	1065414
3.	2649206	1058795
4.	2666802	1063070
5.	2658287	1059199
6.	2651537	1068304
Mean	**2654664**	**1062891**
S.D	**7090.5**	**3639.1**
%RSD	**0.3**	**0.3**

From the above results, the % RSD of method precision study was within the limit for Gemcitabine and Paclitaxel.

Robustness: Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus (40B:60A), mobile phase plus (50B:50A), temperature minus (27°C) and temperature plus(33°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

Table 8: Robustness data for Gemcitabine and Paclitaxel.

Condition	%RSD of Gemcitabine	%RSD of Paclitaxel
Flow rate (-) 0.9ml/min	0.5	0.4
Flow rate (+) 1.1ml/min	1.2	1.3
Mobile phase (-) 40A:60B	1.0	1.1
Mobile phase (+) 50A:50B	0.5	0.4
Temperature (-) 27°C	1.1	1.1
Temperature (+) 33°C	0.3	0.3

Force Degradation Studies: table 9 shows degradation conditions and the obtained degraded data in table 10 and purity plot chromatogram in figure 8,9

Table 9: degradation conditions

Stress condition	Solvent	Temp(^{0}C)	Exposed time
Acid	2N HCL	60^0c	30 mins
Base	2N NAOH	60^0c	30 mins
Oxidation	20% H_2O_2	60^0c	30 mins
Thermal	Diluent	105^0c	6 hours
Photolytic	Diluent	-	-
Hydrolytic	Water	60^0c	

Table 10: degradation data

Type of degradation	Gemcitabine			Paclitaxel		
	area	%recovered	%degraded	area	%recovered	% degraded
Acid	2498962	94.78	5.22	1048313	98.93	1.07
Base	2548861	96.68	3.32	981088	92.59	7.41
Peroxide	2519281	95.55	4.45	1027513	96.97	3.03
Thermal	2470316	93.70	6.30	1018212	96.09	3.91
UV	2493869	94.59	5.41	1036133	97.78	2.22
Water	2610660	99.02	0.98	998721	94.25	5.75

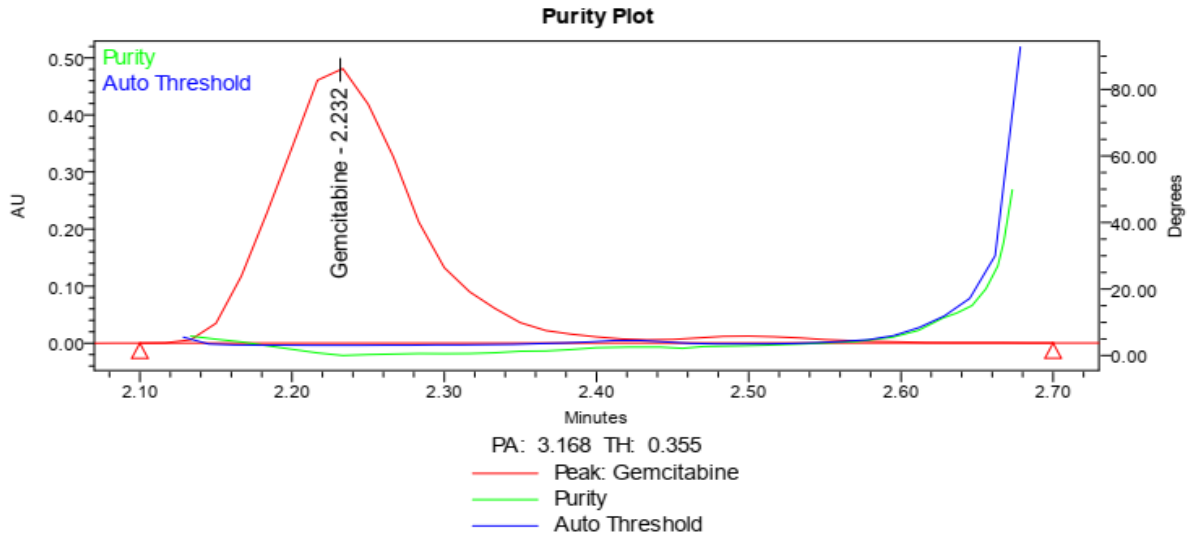


Figure 8: Purity plots for Acid Condition for Gemcitabine

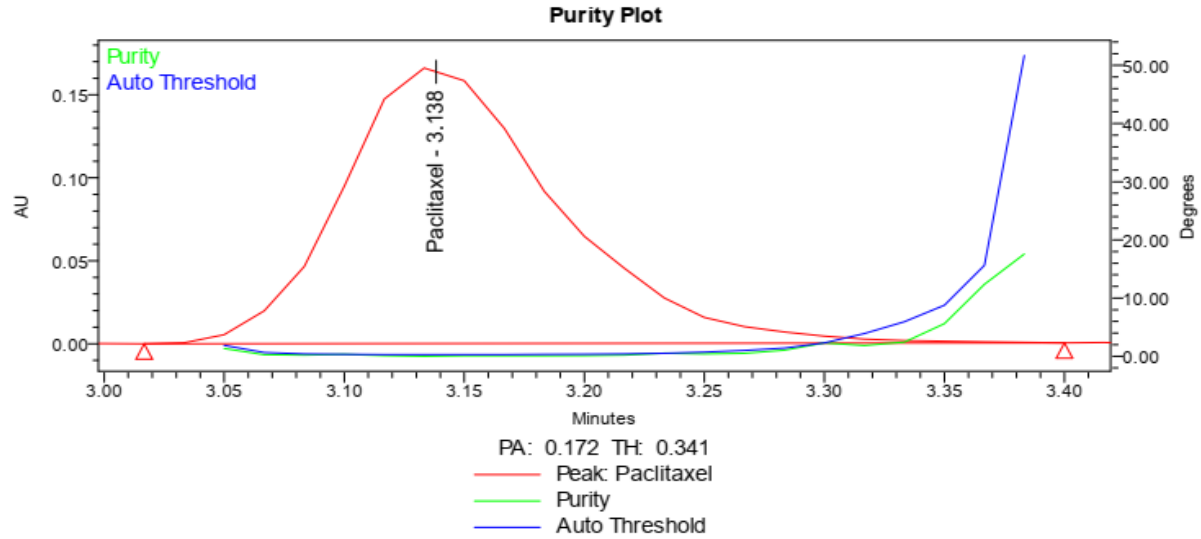


Figure 9: Purity plots for Acid Condition for Paclitaxel

Assay: Gemcitabine 15mg, Paclitaxel 6mg. Assay was performed with the above formulation. Average % Assay for Gemcitabine and Paclitaxel obtained was 100.68% and 100.31% respectively.

Table 11: assay data

	Gemcitabine			Paclitaxel		
S.no	**Std Area**	**Sample area**	**% Assay**	**Std Area**	**Sample area**	**% Assay**
1	2624141	2647498	100.41	1050947	1062561	100.28
2	2636171	2654655	100.68	1064651	1065414	100.55
3	2621425	2649206	100.48	1055562	1058795	99.92
4	2634743	2666802	101.15	1059850	1063070	100.32
5	2632248	2658287	100.82	1054778	1059199	99.96
6	2639258	2651537	100.57	1059278	1068304	100.82
Avg	**2631331**	**2654664**	**100.68**	**1057511**	**1062891**	**100.31**
Std ev	**7050.8**	**7090.5**	**0.27**	**4773.4**	**3639.1**	**0.34**
%RSD	**0.3**	**0.3**	**0.3**	**0.5**	**0.3**	**0.3**

Assay was calculated by the formula:

$$\% \text{ Assay} = \frac{AT}{AS} \times \frac{WS}{100} \times \frac{1}{10} \times \frac{100}{1} \times \frac{10}{1} \times \frac{P}{100} \times \frac{FV}{L.C} \times 100$$

AT	Average Peak area of sample in test solution
AS	Mean peak area of sample in standard solution
WS	Weight of drug working standard taken in mg
P	Assay of drug working standard in % on dried basis
L.C	Label Claim

Figure 10. formula

CONCLUSION:

The cost-effective technique created for the high-performance liquid chromatography (HPLC) simultaneous quantification of gemcitabine and paclitaxel turned out to be reliable, accurate, and precise. Excellent linearity, sensitivity, and reproducibility were displayed by this approach, guaranteeing accurate quantification of both medications in pharmaceutical formulations. It is perfect for routine quality control and batch release in industrial settings because of its lower cost and simpler process. Its appropriateness for the intended uses was validated in accordance with ICH requirements.

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