RESEARCH ARTICLE

Development and Validation of RP-HPLC Method for the Simultaneous Determination of Cisplatin, Capecitabine and Trastuzumab

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ABSTRACT

The reverse phase high performance liquid chromatography (RP-HPLC) technology was used to produce a simple, predictable, and accurate method for estimating Cisplatin, Capecitabine, and Trastuzumab. The following chromatographic conditions were used: phase of inactivity C18, 150 mm x 4.6 mm, 3.5 m., Waters symmetry Adjust the pH to 2.5 using ortho phosphoric acid and a 2.5 mL buffer of Hexane Sulphonic Acid in 1 L of water: The diluent was mobile phase, with acetonitrile (40:60v/v) and a flow rate of 1.0-mL/min. The detection wavelength was set at 232 nm, the column temperature was set to ambient, and the detection wavelength was set at 232 nm. As an optimized procedure, the conditions were finalized. By injecting the standard five times, system suitability parameters were investigated, and the results were considerably below the acceptance criteria. Between 10% and 15% levels, a linearity analysis was conducted, and the R2 value was found to be 0.999. For repeatability, precision was found to be 0.2, 0.24, and 1.79, and for intermediate precision, 0.47, 0.32, and 1.73. LOQs are 0.001, 0.5, and 0.15 g/mL, as well as 0.01, 5, and 1.5 g/mL. Using the foregoing procedure, an analysis of marketed formulation revealed that 100.02, 100.4, and 100.6% were present. Cisplatin, Capecitabine, and Trastuzumab have all been studied. The purity threshold was greater than the purity angle in all cases and within the permitted range.

Keywords: Capecitabine and Trastuzumab, HPLC Cisplatin, ICH Guidelines Method development.

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Conflict of interest: None

INTRODUCTION

Cisplatin (Figure 1) is a type of chemotherapy that is used to treat a variety of malignancies.[1-8] Testicular cancer, ovarian cancer, cervical cancer, breast cancer, bladder cancer, head and neck cancer, esophageal cancer, and lung cancer are some of the most common cancers.[8-19] It is administered through a venous injection. Bone marrow suppression, hearing problems, and kidney impairment are all common side effects. Capecitabine (Figure 2) is a chemotherapeutic drug used to treat cancers of the breast, stomach, and colon.[20-31] It's frequently combined with docetaxel in the treatment of breast cancer. Diarrhea, vomiting, weakness, and rashes are all common adverse effects.[32-38] Trastuzumab (Figure 3) is a monoclonal antibody that is used to treat cancers of the breast and stomach. It is used to treat cancers that have the HER2 receptor.

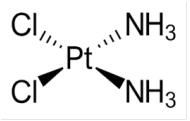

Figure 1: Structure of cisplatin

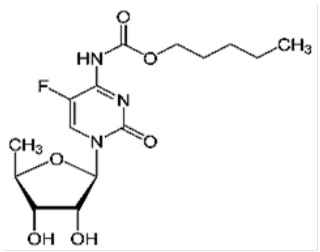

Figure 2: Structure of capecitabine

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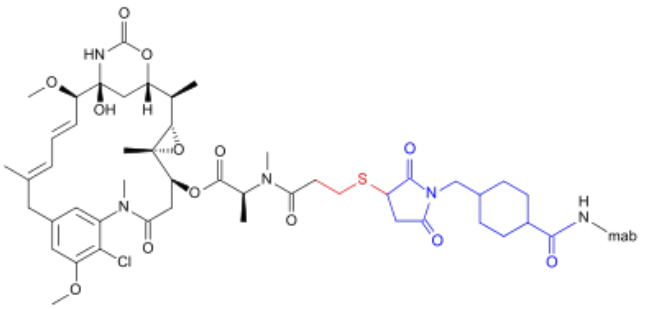

Figure 3: Structure of trastuzumab

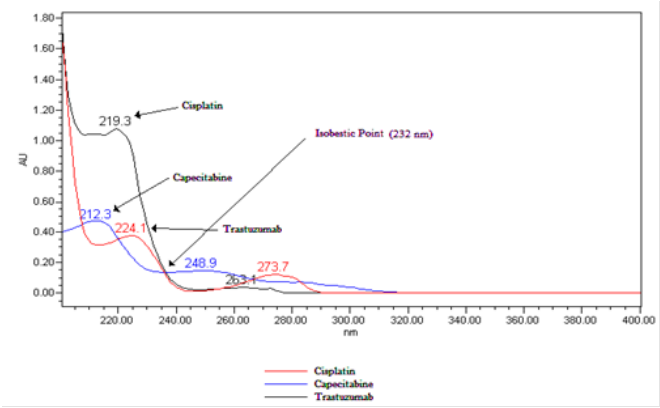

Figure 4: UV scan report

Table 1: Optimized method conditions

Parameters	*Method*
Stationary phase (column)	Symmetry C_{18} 150x4.6 mm, 3.5µ
Mobile Phase	Buffer: Acetonitrile (40:60)
Flow rate (mL/min)	1.0 mL/min
Run time (minutes)	8 min
Column temperature (°C)	Ambient
Volume of injection loop (µL)	10 µL
Detection wavelength (nm)	232 nm
Drug RT (min)	Cisplatin, Capecitabine and Trastuzumab were about 2.491, 5.159and 6.227 respectively

It can be used alone or in combination with other chemotherapeutic drugs.

MATERIALS AND METHODS

All of the chemicals and reagents utilised were of excellent quality and purity, and were obtained from a variety of sources. Waters 2695 HPLC with PDA Detector, Waters Symmetry C18, 150 mm x 4.6 mm, 3.5 m, Detector wavelength 232 nm, Colum Temperature is ambient. Acetonitrile, Methanol, water, Merck (HPLC-Grade), Cisplatin, Capecitabine, and Trastuzumab Waters Symmetry C18, 150 mm x 4.6 mm, 3.5 m, Detector Table 1 lists the optimised chromatographic conditions.

Preparation of Sample Solution

1-mg of Cisplatin, 500 mg of Capecitabine, and 150 mg of Trastuzumab were accurately weighed and transferred to 100 mL volumetric flasks. The compounds were then dissolved in buffer, and the volumes were filled with solvent to the required levels. Pipette 5mL of the aforementioned solution into a 50mL volumetric flask, then add diluents to make up the difference.

Preparation of Standard Solution

Solution A:

(a) Cisplatin

Fill a 100 mL volumetric flask with around 1-mg of Cisplatin working standard. 70 mL diluent, sonicate to dissolve, and dilute with diluent to volume.

Solution B

(b) Capecitabine

In a 100 mL volumetric flask, weigh accurately about 500 mg Capecitabine working standard. 70 mL diluent, sonicate to dissolve, and dilute with diluent to volume.

Solution C

(C) Trastuzumab

In a 100 mL volumetric flask, weigh accurately about 150 mg Trastuzumab working standard. 70 mL diluent, sonicate to dissolve, and dilute with diluent to volume.

With the diluent, dilute each 5 mL Solution-A, Solution-B, and Solution-C to 50 mL.

Preparation of Sample Solution

One milligrams of Cisplatin, 500 mg of Capecitabine, and 150 mg of Trastuzumab are transferred to a 100 mL volumetric flask. Add 70 mL diluents, sonicate to dissolve fully, then top up with diluents to the mark. Pipette 5 mL of the aforementioned solution into a 50 mL volumetric flask and dilute to the desired concentration with diluents.

Assay Procedure

In the chromatographic apparatus, inject 10 L of Standard solution five times and Sample solution once. For Cisplatin, Capecitabine, and Trastuzumab, chromatograms were recorded and peak responses were assessed (Figures 4 and 5, Table 1).

ASSAY CALCULATION

%Assay of was carried out in tablet formulation with results were calculated by using the formula given below and reported

$$= \frac{\text{Test area x STD weight x Test dilution x Avg. Weight x Potency x 100}}{\text{STD area x test weight x STD dilution x label claim x 100}}$$

Preparation of Mobile Phase

2.5 g of Hexane sulphonic acid in 1 L water and adjust pH=2.5 with 0.1% OPA, acetonitrile in the ratio (40:60 v/v)

UV Scan

The UV spectroscopy was used to scan the prepared samples. A wavelength was chosen from the drug spectra below at which the medicines showed greatest absorption. 232 nm was chosen as the wavelength.

Assay Results

Acceptance Criteria:

Assay observations of Cisplatin, Capecitabine and Trastuzumab

Drug	*Label claim for sample taken (mg)*	*Calculated value (mg±SD)*	*% of Assay*
Cisplatin	1	1.25	100.2
Capecitabine	500	500.5	100.6
Trastuzumab	150	151	100.4

The %assay should be within range of 98–102%

Observation: The %assay was found to be within the range.

Table 2: Cisplatin data for linearity

S.No.	*Conc (µg/mL)*	*Peak Area*
1	0.1	15947
2	0.25	38404
3	0.5	76176
4	1	153464
5	1.25	181978
6	1.5	224306

Table 3: Capecitabine data for linearity

S.No.	*Conc (µg/mL)*	*Peak Area*
1	50	214501
2	125	498237
3	250	1039993
4	500	2077387
5	625	2487304
6	750	3061858

Table 4: Trastuzumab data for linearity

S.No.	*Conc (µg/mL)*	*Peak Area*
1	15	76772
2	37.5	192712
3	75	388507
4	150	776429
5	187.5	960719
6	225	1151180

Validation of the Method[15-31]

In terms of specificity, linearity, precision, accuracy, limit of detection (LoD), limit of quantification (LoQ), and sample resilience, the method was validated according to ICH criteria.

RESULTS

Specificity

The injection of a blank was used to test specificity. It was done to see if there were any contaminants interfering with the retention time of the analytical peak. There are no contaminants that affect the retention time of the analytical peak.

Linearity

The linearity approach was proved for Cisplatin concentrations of 0.1–1.5 g/mL (Table 2 and Figure 7), Capecitabine concentrations of 50–750 g/mL(Table 3 and Figure 8), and Trastuzumab concentrations of 15–225 g/mL (Table 3 and Figure 9). Aliquots of the above solutions were produced from stock solution and labelled solution 1, 2, 3, 4, 5, 6 and injected into the HPLC system according to the test procedure. The concentration Vs peak area calibration curve for Cisplatin, Capecitabine, and Trastuzumab was plotted appropriately. The findings can be seen in the Tables 2 to 4. The correlation coefficient should be 0.999 or above.

Precision

Repeatability

Cisplatin, Capecitabine, and Trastuzumab had percent relative standard deviations of 1.63, 0.20, and 0.40, respectively, for repeatability. As a result, the percent RSD figures show a high

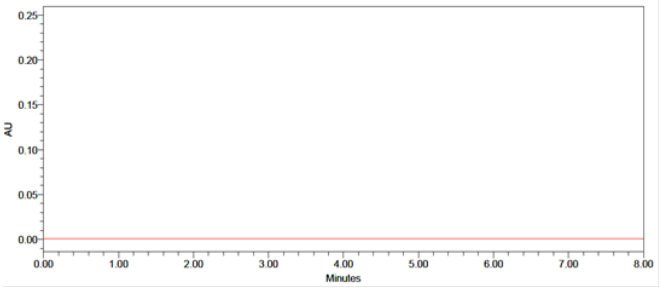

Figure 5: Blank chromatogram

Table 5: Data of repeatability for cisplatin, capecitabine and trastuzumab

Concentration 10 µg/mL		*Cisplatin*	*Capecitabine*	*Trastuzumab*
	Injection	*Peak area*	*Peak area*	*Peak area*
	1	157013	2025701	773266
	2	153689	2022592	771824
	3	159027	2023408	774252
	4	153466	2030822	778945
	5	156748	2025312	779502
	6	152588	2018499	776966
Statistical Analysis	Mean	155422	2024389	775793
	SD	2535.515	4070.352	3149
	%RSD	1.63	0.20	0.40

level of precision within the given range. Table 5 and Table 6 summarizes the findings.

Accuracy

A study of accuracy for the Cisplatin, Capecitabine, and Trastuzumab assay in triplicate (50, 100, and 150%) as per the test method with equivalent amounts of drug containing cisplatin, capecitabine, and trastuzumab into each volumetric flask for

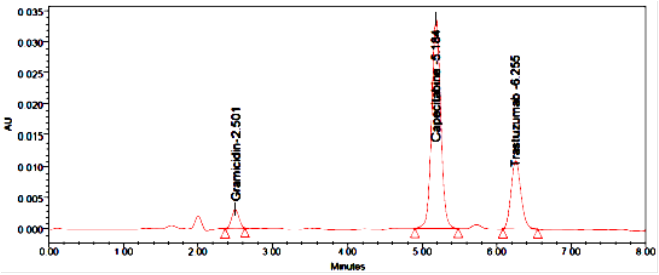

Figure 6: Sample chromatogram

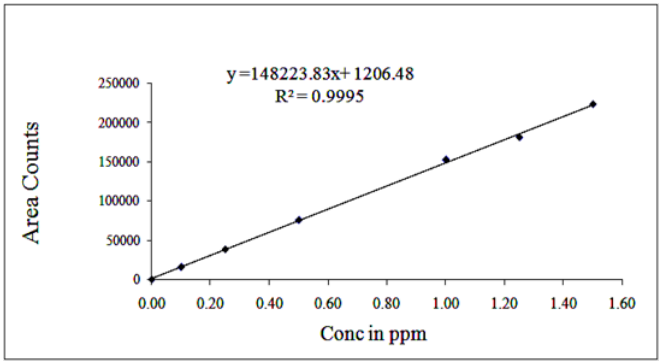

Figure 7: Cisplatin linearity graph

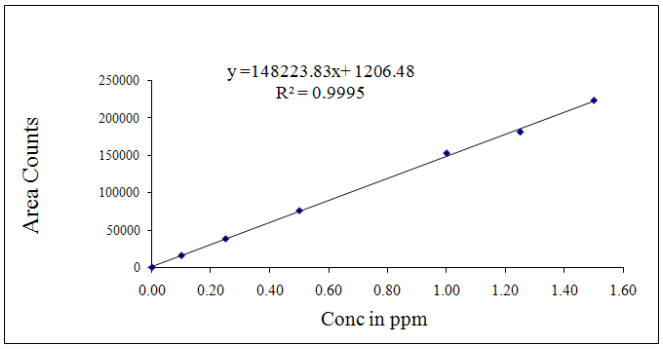

Figure 8: Capecitabine linearity graph

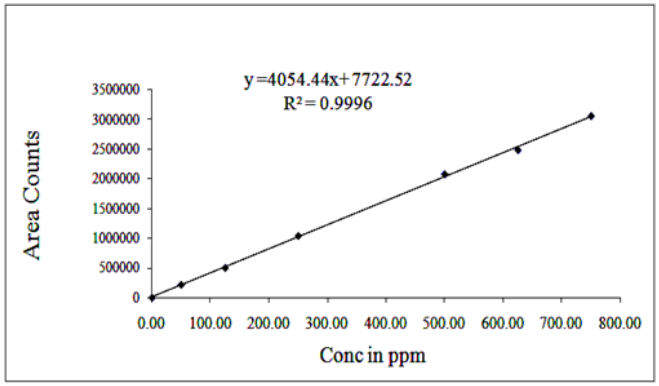

Figure 9: Trastuzumab linearity graph

Table 6: Data of method precision for cisplatin, capecitabine and trastuzumab

Concentration 10µg/ml		*Cisplatin*	*Capecitabine*	*Trastuzumab*
	Injection	*Peak area*	*Peak area*	*Peak area*
	1	154538	2027194	774245
	2	156309	2027149	777657
	3	150367	2027755	775615
	4	153466	2019468	772437
	5	153762	2021265	773738
	6	158523	2019468	773320
Statistical Analysis	Mean	154494	2023716	774502
	SD	2762.212	4057.086	1871.331
	%RSD	1.79	0.20	0.24

Table 7: Accuracy data for cisplatin

S.No.	*Spike Level*	*Amount of sample added (µg/mL)*	*Amount of API added (µg/mL)*	*% Recovery*	*Statistical analysis*
		337.2	0.5	100.0	Mean 99.3
1	50%	337.1	0.5	98.0	SD 1.16
		337.3	0.5	100.0	%RSD 1.160
		674.0	1	100.0	Mean 100.0
2	100%	674.2	1	101.0	SD 1.00
		674.1	1	99.0	%RSD 1.000
		1011.1	1.5	98.7	Mean 98.7
3	150%	1011.3	1.5	98.0	SD 0.67
		1011.4	1.5	99.3	%RSD 0.680

each spike level to obtain Cisplatin, Capecitabine, and Trastuzumab concentrations equivalent to 50, 100, and 150% of the It was determined what the average percent recovery was. Cisplatin, capecitabine, and trastuzumab should have a mean percent recovery of not less than 98 percent and not more than 102 percent at each level (Tables 7–9 and Figure 6).

Robustness

Small adjustments in flow rate, mobile phase composition, and temperature were used to test the method's resilience. Three replicates of a concentration level at 100 percent were used to test robustness. In the robustness investigation, the percent RSD was less than 2%, indicating that the procedure is exact, accurate, and robust; the findings are given in Tables 10–13.

LoD AND LoQ

LoD and LoQ were calculated by the method based on the standard deviation (Σ) and solpe of the calibration curve using the formula

$$LoD = 3.3\ \sigma / S$$

$$LoQ = 10\ \sigma / S$$

Where,

σ = the standard deviation of the response

S = the slope of the calibration curve

The LoD and LoQ were calculated as per formula and were shown in the Table 14.

DEGRADATION STUDIES

Acid Degradation

5 mL of the working standard solution was transferred to a 50 mL volumetric flask, 3 mL of 5 N HCl was added, the

Table 8: Accuracy data for Capecitabine

S.No.	*Spike level*	*Amount of sample added (µg/ml)*	*Amount of API added (µg/mL)*	*%Recovery*	*Statistical analysis*	
		337.2	250	101.2	Mean	100.4
1	50%	337.1	250	100.6	SD	0.91
		337.3	250	99.4	% RSD	0.900
		674.0	500	100.5	Mean	100.2
2	100%	674.2	500	100.1	SD	0.31
		674.1	500	99.9	% RSD	0.310
		1011.1	750	101.6	Mean	100.9
3	150%	1011.3	750	100.4	SD	0.61
		1011.4	750	100.8	% RSD	0.610

Table 9: Accuracy data for Trastuzumab

S.No.	*Spike Level*	*Amount of Sample added (µg/ml)*	*Amount of API added (µg/mL)*	*%Recovery*	*Statistical analysis*	
		337.2	75	98.1	Mean	98.6
1	50%	337.1	75	98.4	SD	0.62
		337.3	75	99.3	% RSD	0.630
		674.0	150	99.8	Mean	99.8
2	100%	674.2	150	99.5	SD	0.27
		674.1	150	100.1	% RSD	0.270
		1011.1	225	99.3	Mean	100.5
3	150%	1011.3	225	101.5	SD	1.13
		1011.4	225	100.7	% RSD	1.130

Table 10: Robustness data of Cisplatin, Capecitabine and Trastuzumab (Effect of variation of flow rate)

Cisplatin			*Capecitabine*			*Trastuzumab*		
	Retention time	*Peak area*		*Retention time*	*Peak area*		*Retention time*	*Peak area*
	3.104	197322		6.500	2556580		7.846	1002320
Flow 0.8 ml	3.112	196410	Flow 0.8 mL	6.514	2562544	Flow 0.8 mL	7.851	1027819
	3.108	190774		6.508	2549474		7.849	1017819
% RSD		1.82	% RSD		0.26	% RSD		1.26
	2.079	136436		4.351	1813578		5.252	723497
Flow 1.2 ml	2.083	136459	Flow 1.2 mL	4.359	1821817	Flow 1.2 mL	5.256	720363
	2.087	136928		4.355	1824689		5.257	724497
% RSD		0.20	% RSD		0.31	% RSD		0.29

Table 11: Robustness data of Cisplatin, Capecitabine and Trastuzumab (Effect of variation in mobile phase composition)

Cisplatin			*Capecitabine*			*Trastuzumab*		
	Retention time	*Peak area*		*Retention time*	*Peak area*		*Retention time*	*Peak area*
Organic phase ratio plus	2.079	146564	Organic phase ratio plus	4.351	1921472	Organic phase ratio plus	5.252	732218
	2.084	144662		4.356	1921164		5.250	732267
	2.081	141204		4.352	1917366		5.251	730575
%RSD	1.88		% RSD	0.11		%RSD	0.83	
Organic phase ratio minus	3.099	181853	Organic phase ratio minus	6.493	2454135	Organic phase ratio minus	7.838	919698
	3.096	183853		6.494	2433304		7.835	922707
	3.102	184232		6.499	2473908		7.833	904171
%RSD	0.69		% RSD	0.82		%RSD	1.02	

Table 12: Solution stability data of Cisplatin, Capecitabine and Trastuzumab

Cisplatin				*Capecitabine*				*Trastuzumab*			
S.NO	*Stability (Hrs)*	*Retention time (min)*	*Peak area*	*S.NO*	*Stability (Hrs)*	*Retention time (min)*	*Peak area*	*S.NO*	*Stability (Hrs)*	*Retention time (min)*	*Peak area*
1	Initial	2.494	156038	1	Initial	5.177	2024660	1	Initial	6.236	774132
2	6	2.491	155538	2	6	5.172	2021620	2	6	6.233	773846
3	12	2.494	154523	3	12	5.183	2018188	3	12	6.243	772133
4	18	2.491	154185	4	18	5.172	2015185	4	18	6.233	771054
5	24	2.492	153903	5	24	5.176	2013438	5	24	6.238	770239
Mean		154837		Mean		2018618		Mean		772280	
SD		912.39		SD		4591.31		SD		1700.92	
%RSD		0.58		%RSD		0.22		%RSD		0.22	

Table 13: Ruggedness data (Effect of changes in the analyst)

Analyst	*Retention time of Cisplatin(min)*	*Peak area of Cisplatin*	*Retention time of CAP(min)*	*Peak area of CAP(min)*	*Retention time of TSZ(min)*	*Peak area of TSZ (min)*
Analyst 1	2.474	152757	5.135	2018669	6.206	777657
Analyst 2	2.471	152353	5.132	2026320	6.203	770554
Mean	-	152555	-	2022494	-	774105
SD	-	285.68	-	5410.08	-	5022.58
%RSD	-	0.19	-	0.27	-	0.65

Table 14: Limit of detection and limit of quantification

Sample	*LoD*	*LoQ*
Cisplatin	3	22
Capecitabine	8	29
Trastuzumab	5	24

contents were thoroughly mixed, and the flask was set aside for 1 hour. Then, using 3 mL of 5 N NaOH as a neutralizer, make up to volume with mobile phase. To obtain chromatograms, the solution was injected into an HPLC system.

Base Degradation

From the standard working solution 5 mL was placed in a 50 mL volumetric flask, 3 mL of 5N NaOH was added, the contents were thoroughly mixed, and the flask was set aside for 1 hour. After that, 3 mL of 5N HCl was used to neutralise the mixture before it was built up to volume with mobile phase. To obtain chromatograms, the solution was injected into an HPLC system.

Oxidation

From the standard working solution 5 mL was placed in a 50 mL volumetric flask, 1 mL of 30% v/v H2O2 was added, the contents were thoroughly mixed, and the flask was set aside for 1 hour. Then use the mobile phase to make up the difference in volume. To obtain chromatograms, the solution was injected into an HPLC apparatus.

Reduction Degradation

A total of 5 mL of the working standard solution was added to a 50 mL volumetric flask, along with 3 mL of 10% sodium bisulphite; the contents were thoroughly mixed and set aside for 1 hour. Then use the mobile phase to make up the difference in volume. To obtain chromatograms, the solution was injected into an HPLC system.

Temperature Stress Studies

In a 50 mL volumetric flask, 5 mL of the working standard solution was taken. The solution was heated to 45°C before being cooled to room temperature. The solution is then injected into an HPLC apparatus, which produces chromatograms.

Table 15: Results of forced degradation study for Cisplatin

Cisplatin								
	Sample weight in g	*Area counts Injections*	*Mean Area count*	*% Label claim*	*Purity angle*	*Purity threshold*	*%Degradation*	*Pass/Fail*
Control	674	155805	155805	100	2.383	8.476	0	Pass
Acid	674	139432	139432	89.5	0.092	5.043	10.5	Pass
Alkali	674	132343	132343	84.9	0.788	5.082	15.1	Pass
Peroxide	674	136587	136587	87.7	2.051	8.413	12.3	Pass
Thermal	674	137842	137842	86.1	1.02	7.075	13.9	Pass
Hydrolysis	674	134087	134087	88.5	1.02	7.075	11.5	Pass

Table 16: Results of forced degradation study for Capecitabine

Capecitabine								
	Sample Weight In g	*Area Counts Injections*	*Mean Area Count*	*% Label claim*	*Purity angle*	*Purity threshold*	*%Degradation*	*Pass/Fail*
Control	674	2023150	2023150	99.9	0.389	5.217	0.1	Pass
Acid	674	1782432	1782432	88	0.09	5.003	12	Pass
Alkali	674	1652343	1652343	81.6	0.09	5.006	18.4	Pass
Peroxide	674	1736587	1736587	85.7	0.408	5.230	14.3	Pass
Hydrolysis	674	1704087	1704087	84.1	0.291	5.143	15.9	Pass
Thermal	674	1667842	1667842	82.3	0.291	5.138	17.7	Pass

Table 17: Forced degradation study for Trastuzumab:

Trastuzumab								
	Sample Weight in g	*Area Counts Injections*	*Mean Area Count*	*% Label Claim*	*Purity Angle*	*Purity Threshold*	*%Degradation*	*Pass/Fail*
Control	674	774216	774216	100	0.878	5.98	0	Pass
Acid	674	692432	692432	89.4	0.122	5.014	10.6	Pass
Alkali	674	652343	652343	84.2	0.139	5.025	15.8	Pass
Peroxide	674	633255	633255	81.8	0.967	5.998	18.2	Pass
Hydrolysis	674	641121	641121	82.8	0.555	5.620	17.2	Pass
Thermal	674	623844	623844	80.5	0.523	5.620	19.5	Pass

Hydrolysis Degradation

A total of 5 mL of the working standard solution was placed in a 50 mL volumetric flask, 10 mL of diluent was added, 20 mL of water was added to disperse and dissolve, and the flask was heated on a water bath at 70°C for 3 hours. Remove the flask from the water bath and cool to room temperature before diluting to volume with diluent and mixing. The solution is then injected into an HPLC apparatus, which produces chromatograms.

Humidity Degradation

Sample was exposed at 25°C/92% RHfor 2 hours.andanalyzed the exposed sample are injected (Table 15, 16 and 17).

Acceptance Criteria for Forced Degradation

Purity angle should be less than purity threshold. Cisplatin, Capecitabine and Trastuzumab and its degraded substances should not have any flag in purity results table.

Observation: Purity angle is found to be less than threshold angle in all forced degradation studies without having signs of purity flags.

DISCUSSION AND CONCLUSION

Because of their relevance in quality control of medications and drug products, the development of an analytical method for determining drugs by HPLC has gotten a lot of attention in recent years. The goal of this work was to create a simple, fast, precise, accurate, and sensitive HPLC method for estimating Cisplatin, Capecitabine, and Trastuzumab in bulk and in pharmaceutical dose forms.

The developed method for simultaneous estimation of Cisplatin, Capecitabine, and Trastuzumab was carried out in an isocratic mode using a mobile phase composition of 2.5 gm Hexane-1-Sulphonic acid dissolved in 1lt water adjusted pH-2.5 with 0.1 percents OPA and acetonitrile in the ratio (40:60 v/v) with a flow rate of 1.0 mL/min in a waters symmetry

(150 mm x 4.6 mm, 3.5 µm). The effluents were monitored at 232 nm.

Cisplatin, Capecitabine, and Trastuzumab had percent test values of 100.2, 100.5, and 100.2%, respectively, according to the data.

Linearity was seen for Cisplatin concentrations of 0.1–1.5 g/mL, Capecitabine concentrations of 50–750 g/mL, and Trastuzumab concentrations of 15–225 g/mL. For both medications, the correlation coefficient was 0.999, indicating that the concentration provided good linearity.

Cisplatin, Capecitabine, and Trastuzumab had percent RSD values of 1.63 and 1.21 for System precision and 0.20 and 0.39 and 0.16 and 0.89 for Method precision, respectively. Because the results are within the acceptable range of less than 2%, the proposed method has strong repeatability. The results are excellent in terms of method and system precision. The mean percentage recovery values of pure medication were determined to be 99.3, 100.5, and 99.6% for Cisplatin, Capecitabine, and Trastuzumab, respectively, and these results were found to be within the acceptability limit of 98–102%, indicating that the procedure was accurate.

The developed method's resilience was tested by varying the flow rate and mobile phase composition. The approach was resilient since all of the parameters were within the bounds of all variable conditions.

Two separate analysts assessed the robustness of the suggested method. The %RSD was found to be within acceptable limits, i.e., it should not exceed 2.0. As a result, the proposed procedure is very repeatable.

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