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Development and validation of new analytical method for the simultaneous estimation of daunorubicin and cytarabine in bulk and pharmaceutical dosage form

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Abstract

A simple, Accurate, precise method was developed for the simultaneous estimation of the Daunorubicin and Cytarabine inhalation dosage form. Chromatogram was run through BDS C18 150 x 4.6 mm, 5µ. Mobile phase containing 0.1% OPA: Acetonitrile taken in the ratio 60:40 was pumped through column at a flow rate of 1.0 ml/min. Temperature was maintained at 30°C. Optimized wavelength selected was 240nm. Retention time of Daunorubicin and Cytarabine were found to be 2.245 min and 2.813. %RSD of the Daunorubicin and Cytarabine were and found to be 0.6and 0.3 respectively. %Recovery was obtained as 99.39% and 99.26% for Daunorubicin and Cytarabine respectively. LOD, LOQ values obtained from regression equations of Daunorubicin and Cytarabine were 0.03, 0.10 and 0.31, 0.94 respectively. Regression equation of Daunorubicin is y = 2677x + 703.5, y = 2524x + 104.7 of Cytarabine.Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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Introduction

Daunorubicin , also known as Daunomycin, is a chemotherapy medication used to treat cancer. Specifically it is used for acute myeloid leukemia (AML), acute lymphocytic leukemia (ALL), chronic myelogenous leukemia (CML), and Kaposi's sarcoma. It is used by injection into a vein. Chemically called as 8S,10S)-8-acetyl-10-{[(2R,4S,5S,6S)-4-amino-5-hydroxy-6-methyloxan-2-yl]oxy}-6,8,11-trihydroxy-1-methoxy-5,7,8,9,10,12-hexahydrotetracene-5,12-dione. It has antimitotic and cytotoxic activity through a number of proposed mechanisms of action: Daunorubicin forms complexes with DNA by intercalation between base pairs, and it inhibits topoisomerase II activity by stabilizing the DNA-topoisomerase II complex, preventing the religation portion of the ligation-religation reaction that topoisomerase II catalyzes.the chemical structure is given in figure1

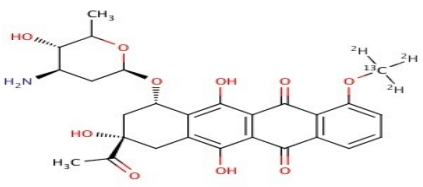

Figure 01: chemical structure of Daunorubicin

Cytarabine(cytosine arabinoside, 1-b-D-arabinofuranosyl cytosine, ara-C) is a pyrimidine nucleoside analogue that is used mainly in the treatment of leukemia, especially acute non-lymphoblastic leukemia. It also has antiviral and immunosuppressant properties.it is chemically called as 4-amino-1-[(2R,3S,4S,5R)-3,4-dihydroxy-5- (hydroxymethyl) oxolan-2-yl]-1,2-dihydropyrimidin-2-one. Cytarabine acts through direct DNA damage and incorporation into DNA. Cytarabine is cytotoxic to a wide variety of proliferating mammalian cells in culture. It exhibits cell phase specificity, primarily killing cells undergoing DNA synthesis (S-phase) and under certain conditions blocking the progression of cells from the G1 phase to the S-phase. Although the mechanism of action is not completely understood, it appears that cytarabine acts through the inhibition of DNA polymerase. A limited, but significant, incorporation of cytarabine into both DNA and RNA has also been reported. The chemical structure figure 2

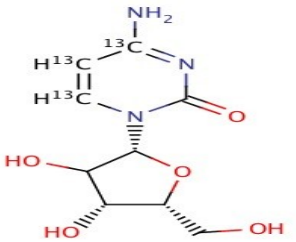

Figure 02: chemical structure of Cytarabine

Daunorubicin and Cytarabine (I.V injection) is a liposomal combination of that is FDA approved for the treatment of adults with newly-diagnosed therapy-related acute myeloid leukemia (t-AML) or AML with myelodysplasia-related changes (AML-MRC) [1-3]. Literature review reveals estimation of Daunorubicin by RP-HPLC4 and Cytarabine by RP-HPLC [5, 6] and by Spectroscopy method [7] individually. In combination, Doxorubicin and Cytarabine only one method was published [8,] but yet there is a need to develop new stability indicating RP-HPLC method with more sensitivity, accuracy and precision.

Experimental work

Materials and Methods

Daunorubicin and Cytarabine pure drugs (API), Combination Daunorubicin and Cytarabine. VYXEOS (Cytarabine 100mg, Daunorubicin 44mg.) received from spectrum labs,Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer, Ortho-phosphoric acid. Alltheabovechemicalsandsolvents are from Rankem

Instruments

Electronics Balance-Denver ,p^{H} meter -BVK enterprises, India, Ultrasonicator-BVK enterprises, WATERS HPLC 2695 SYSTEM equipped with quaternary pumps, Photo Diode Array detector and Auto sampler integrated with Empower 2 Software , UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2 mm and 10mm and matched quartz cells integrated with UV win 6 Software was used for measuring absorbances of Daunorubicin and Cytarabine solutions.

Methods

Diluent

Based up on the solubility of the drugs, diluent was selected, Methanol and Water taken in the ratio of 50:50

Preparation of Standard stock solutions

Accurately weighed 11 mg of Daunorubicin, 25 mg of Cytarabine and transferred to 25ml volumetric flasks and 3/4 th of diluents was added to these flask and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution. 440µg/ml of Daunorubicin and 1000µg/ml Cytarabine)

Preparation of Standard working solutions (100% solution)

1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (44µg/ml of Daunorubicin and 100µg/ml of Cytarabine)

Preparation of Sample stock solutions

1 vial equivalent to 44 mg Daunorubicin& 100mg Cytarabine was transferred into a 100ml volumetric flask, 50ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (440µg/ml of Daunorubicin and 1000µg/ml of Cytarabine)

Preparation of Sample working solutions (100% solution)

1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent.(44µg/ml of Daunorubicin and 100µg/ml of Cytarabine)

Preparation of buffer

Buffer:0.1N Potassium dihyrogen Ortho phosphate

Accurately weighed 1.36gm of Potassium dihyrogen Ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then added 1ml of Triethylamine then PH adjusted to 3.8 with dil. Orthophosphoric acid solution

Method Validation [9-12]

System suitability parameters

The system suitability parameters were determined by preparing standard solutions of Daunorubicin (44ppm) and Cytarabine (100ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined. The % RSD for the area of six standard injections results should not be more than 2%.

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.

Precision:

Preparation of Standard stock solutions

Accurately weighed 11 mg of Daunorubicin, 25 mg of Cytarabine and transferred to 25ml volumetric flasks and 3/4 th of diluents was added to these flask and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution. 440µg/ml of Daunorubicin and 1000µg/ml Cytarabine)

Preparation of Standard working solutions (100% solution)

1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (44µg/ml of Daunorubicin and 100µg/ml of Cytarabine)

Linearity

25% Standard solution

0.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (11µg/ml of Daunorubicin and 25µg/ml of Cytarabine)

50% Standard solution

0.5ml each from two standard stock solutions was pipetted out and made up to 10ml. (22µg/ml of Daunorubicin and 50µg/ml of Cytarabine)

75% Standard solution

0.75ml each from two standard stock solutions was pipetted out and made up to 10ml. (33µg/ml of Daunorubicin and 75µg/ml of Cytarabine)

100% Standard solution

1.0ml each from two standard stock solutions was pipetted out and made up to 10ml. (44µg/ml of Daunorubicin and 100µg/ml of Cytarabine)

125% Standard solution

1.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (55µg/ml of Daunorubicin and 8125µg/ml of Cytarabine)

150% Standard solution

1.5ml each from two standard stock solutions was pipettede out and made up to 10ml (66µg/ml of Daunorubicin and 150µg/ml of Cytarabine)

Accuracy

Preparation of Standard stock solutions

Accurately weighed 11 mg of Daunorubicin, 25 mg of Cytarabine and transferred to 25ml volumetric flasks and 3/4 th of diluents was added to these flask and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution. 440µg/ml of Daunorubicin and 1000µg/ml Cytarabine)

Preparation of 50% Spiked Solution

0.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 100% Spiked Solution

1.0ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 150% Spiked Solution

1.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Acceptance Criteria

The % Recovery for each level should be between 98.0 to 102

Robustness

Small deliberatechanges in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines. Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus, mobile phase plus, temperature minus (25°C) and temperature plus(35°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.

LOD sample Preparation

0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flasks and made up with diluents. From the above solutions 0.1ml each of Daunorubicin, Cytarabine, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluents

LOQ sample Preparation

0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flask and made up with diluent. From the above solutions 0.3ml each of Daunorubicin, Cytarabine, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluent.

Degradation studies [13-14]

Oxidation

To 1 ml of stock solution ofDaunorubicin and Cytarabine, 1 ml of 20% hydrogen peroxide (H2O2)was added separately. The solutions were kept for 30 min at 60^{0}c. For HPLC study,theresultantsolutionwasdilutedto obtain 44µg/ml&100µg/ml solutionand 10µl were injected into the system and the chromatograms were recorded to assessthe stability of sample.

Acid Degradation Studies

To 1 ml of stock ssolutionDaunorubicin and Cytarabine, 1 ml of 2N Hydrochloricacid was added and refluxed for 30mins at 60^{0}c.The resultant solution was diluted to obtain 44µg/ml & 100µg/ml solution and10µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

AlkaliDegradationStudies

To 1 ml of stock solutionDaunorubicin and Cytarabine, 1 ml of 2N sodium hydroxidewasadded and refluxed for 30mins at 60^{0}c. Theresultantsolutionwasdiluted to obtain 44µg/ml&100µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry Heat Degradation Studies

Thestandarddrug solution was placedinovenat105°C for1h tostudydryheatdegradation.ForHPLCstudy,theresultant solution was diluted to 44µg/ml&100µg/ml solution and10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

PhotoStabilitystudies

The photochemical stability of the drug was also studied by exposing the 440µg/ml Daunorubicin& 1000µg/ml Cytarabineµg/ml solution to UV Light by keeping the beaker in UV Chamber for 1days or 200 Watt hours/m^2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 44µg/ml&100µg/ml solutions and 10µl were injected into the system and the chromatograms were recordedtoassessthestabilityofsample.

Neutral Degradation Studies

Stress testing under neutral conditions was studied by refluxingthedruginwaterfor1hrs atatemperatureof 60º. For HPLC study, the resultant solution was diluted to 44µg/ml&100µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess thes tability of the sample**.**

Results and discussion

Optimized method

Chromatographic conditions

Mobile phase	: 0.1% OPA: Acetonitrile(60:40)
Flow rate	: 1.0ml/min
Column	: BDS C8 (4.6 x 150mm, 5µm)
Detector wave length	: 240nm
Column temperature	: 30°C
Injection volume	: 10µL
Run time	: 10min
Diluent	: Water and Acetonitrile in the ratio 50:50
Results	: Both peaks have good resolution, tailing Factor, theoretical plate count and resolution.

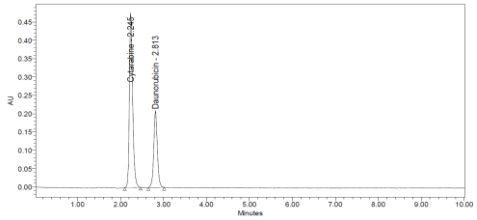

Fig :03 Optimized Chromatogram

Observation

Cytarabine and Daunorubicin were eluted at 2.245 min and 2.813 min respectively with good resolution. Plate count and tailing factor was very satisfactory, so this method was optimized and to be validated.

System suitability

All the system suitability parameters were within the range and satisfactory as per ICH guidelines

Table 01: Systemsuitability parameters for Daunorubicin and Cytarabine

Sn	Cytarabine			Daunorubicin			
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	Resoluton
1	2.256	4131	1.29	2.806	5264	1.04	3.5
2	2.257	4355	1.29	2.808	5484	1.07	3.6
3	2.257	4284	1.27	2.809	5575	1.06	3.7
4	2.258	4410	1.28	2.812	5241	1.03	3.6
5	2.260	4330	1.27	2.813	5242	1.05	3.7
6	2.262	4285	1.25	2.813	5339	1.03	3.6

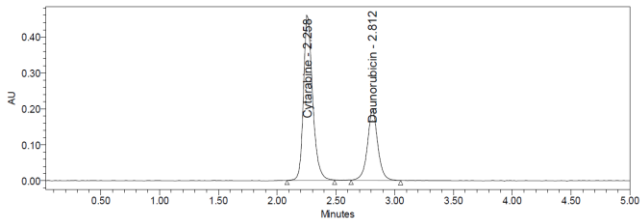

Fig 04: Systemsuitability Chromatogram

Discussion

According to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2. All the system suitable parameters were passed and were within the limits.

Validation

Specificity

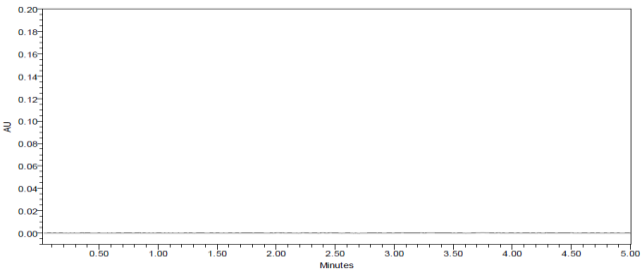

Fig 05: Chromatogram of blank

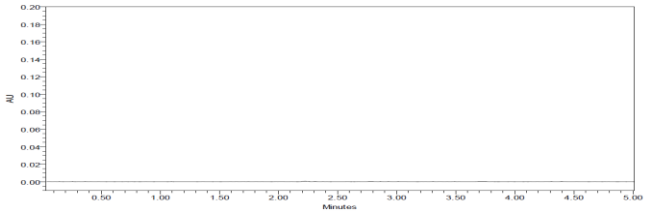

Fig 06: Chromatogramof placebo

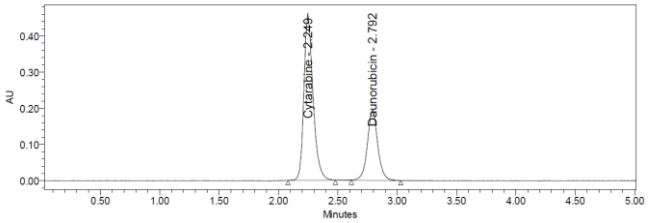

Fig 07: Typical Chromatogram

Discussion

Retention times of Daunorubicin and Cytarabine were 2.249 min and 2.792 min respectively. We did not found and interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

Linearity

Table 02: Linearity table for Daunorubicin and Cytarabine

Daunorubicin		Cytarabine.	
Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area
0	0	0	0
11	30323	25	63530
22	59595	50	130051
33	87814	75	183426
44	122107	100	252346
55	147962	125	318423
66	175627	150	378385

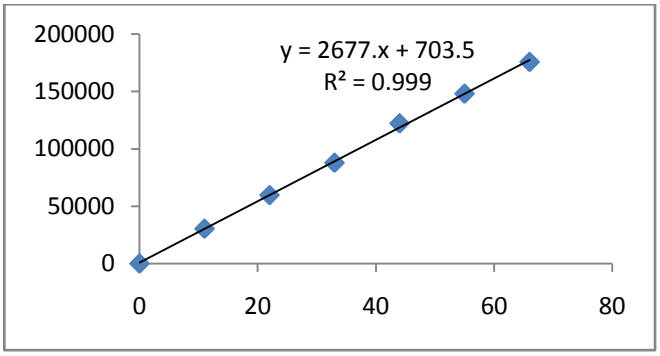

Fig 08: Calibrationcurve of Daunorubicin

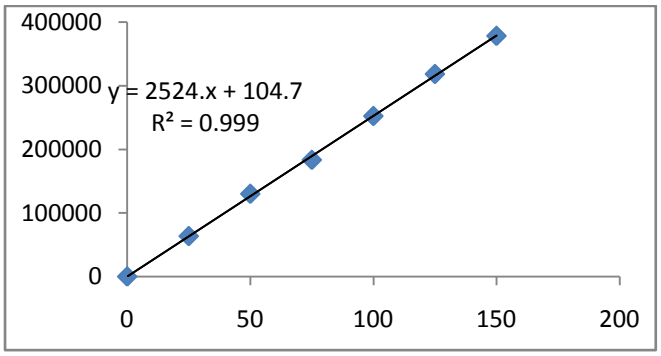

Fig 09: Calibrationcurve of Cytarabine

Discussion

Six linear concentrations of Daunorubicin(11-66µg/ml) and Cytarabine (25-150µg/ml) were injected in a duplicate manner. Average areas were mentioned above and linearity equations obtained for Daunorubicin was y = 2677x + 703.4 and of Cytarabine was y = 2524x + 104.7. Correlation coefficient obtained was 0.999 for the two drugs.

Table 03: System precision table of Daunorubicin and Cytarabine

S. No	Area of Daunorubicin	Area of Cytarabine
1.	119169	252676
2.	119165	252661
3.	118338	252638
4.	119901	252495
5.	118480	254357
6.	118065	254772
Mean	118853	253267
S.D	681.3	1016.0
%RSD	0.6	0.4

Discussion: From a single volumetric flask of working standard solution six injections were given and the obtained areas were mentioned above. Average area, standard deviation and % RSD were calculated for two drugs. % RSD obtained as 0.6% and 0.4% respectively for Daunorubicin and Cytarabine.As the limit of Precision was less than "2" the system precision was passed in this method.

Table 04: Repeatability table of Daunorubicin and Cytarabine

S. No	Area of Daunorubicin	Area of Cytarabine
1.	118516	251414
2.	118393	252509
3.	118673	252274
4.	118782	253096
5.	118796	251544
6.	118832	252751
Mean	118665	252265
S.D	176.1	667.9
%RSD	0.1	0.3

Discussion

Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given and obtained areas were mentioned in the above table. Average area, standard deviation and % RSD were calculated for two drugs and obtained as 0.1% and 0.3% respectively for Daunorubicin and Cytarabine. As the limit of Precision was less than "2" the system precision was passed in this method.

Intermediate precision (Day_ Day Precision)

Table 05: Intermediate precision table of Cytarabine andDaunorubicin

S. No	Area of Cytarabine	Area of Daunorubicin
1.	248551	110678
2.	249426	110488
3.	246627	110077
4.	247790	110417
5.	247881	111001
6.	248538	110191
Mean	248136	110475
S.D	944.4	334.8
%RSD	0.4	0.3

Discussion

Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given on the next day of the sample preparation and obtained areas were mentioned in the above table. Average area, standard deviation and % RSD were calculated for two drugs and obtained as 0.4% and 0.3% respectively for Cytarabine and Daunorubicin. As the limit of Precision was less than "2" the system precision was passed in this method.

Accuracy

Table 06: Accuracy table of Daunorubicin

% Level	Amount Spiked(µg/mL)	Amount recovered(µg/mL)	% Recovery	Mean %Recovery
50%	22	21.83	99.24	99.39%
	22	21.84	99.29	
	22	21.88	99.46	
100%	44	43.59	99.07	
	44	43.80	99.54	
	44	43.87	99.69	
150%	66	65.73	99.60	
	66	65.34	99.01	
	66	65.76	99.64	

Table 07: Accuracy table of Cytarabine

% Level	Amount Spiked(µg/mL)	Amount recovered(µg/mL)	% Recovery	Mean %Recovery
50%	50	49.31	98.62	99.26%
	50	49.83	99.66	
	50	49.91	99.81	
100%	100	99.05	99.05	
	100	99.48	99.48	
	100	98.12	98.12	
150%	150	149.31	99.54	
	150	149.61	99.74	
	150	148.98	99.32	

Discussion

Three levels of Accuracy samples were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean %Recovery was obtained as 99.39% and 99.26% for Daunorubicin and Cytarabine respectively.

Table 08: Sensitivity table of Daunorubicin and Cytarabine

Molecule	LOD	LOQ
Daunorubicin	0.03	0.10
Cytarabine	0.31	0.94

Robustness

Table 09: Robustness data for Daunorubicin and Cytarabine

S.no	Condition	%RSD of Daunorubicin	%RSD of Cytarabine
1	Flow rate (-) 0.90ml/min	0.2	0.2
2	Flow rate (+) 1.1ml/min	0.4	1.5
3	Mobile phase (-) 55B:45A	0.4	0.5
4	Mobile phase (+) 45B:55A	0.2	0.1
5	Temperature (-) 25°C	0.1	0.3
6	Temperature (+) 35°C	0.3	0.7

Discussion

Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus (55B:45A), mobile phase plus (45B:55A), temperature minus (25°C) and temperature plus (35°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.

Assay

VYXEOS, bearing the label claim Cytarabine 100mg, Daunorubicin 44mg. Assay was performed with the above formulation. Average % Assay for Daunorubicin and Cytarabine obtained was 99.83% and 100.60% respectively

Table 10: Assay Data of Daunorubicin

S.no	Standard Area	Sample area	% Assay
1	119169	118516	99.32
2	119165	118393	99.21
3	118338	118673	99.45
4	119901	118782	99.54
5	118480	118796	99.55
6	118065	118832	99.58
Avg	118759	118665	99.44
Stdev	681.3	176.1	0.15
%RSD	0.6	0.1	0.1

Table 11: Assay Data of Cytarabine

S.no	Standard Area	Sample area	% Assay
1	252676	251414	98.87
2	252661	252509	99.30
3	252638	252274	99.21
4	252495	253096	99.53
5	254357	251544	98.92
6	254772	252751	99.40
Avg	253267	252265	99.21
Stdev	1016.0	667.9	0.3
%RSD	0.4	0.3	0.3

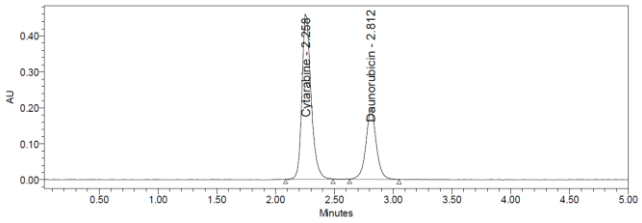

Fig 09: Chromatogram of working standard solution

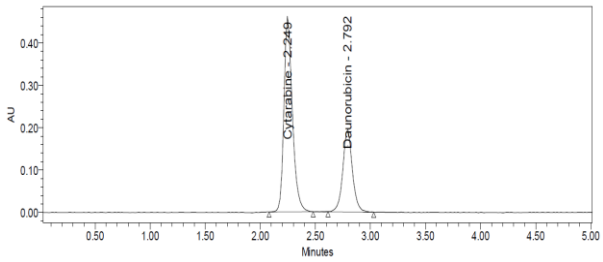

Fig 10: Chromatogram of working sample solution

Degradation

Degradation Studies

Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation

Table12: Degradation Data of Daunorubicin

S.NO	Degradation Condition	Aera	% Recover	% Drug Degraded
1	Acid	112076	93.92	6.08
2	Alkali	113067	94.75	5.25
3	Oxidation	115019	96.39	3.61
4	Thermal	116020	97.23	2.77
5	UV	117346	98.34	1.66
6	Water	118126	98.34	1.66

Table 13: Degradation Data of Cytarabine

S.NO	Degradation Condition	Aera	% Recover	% Drug Degraded
1	Acid	240493	94.58	5.42
2	Alkali	242104	95.21	4.79
3	Oxidation	243912	95.92	4.08
4	Thermal	246873	97.09	2.91
5	UV	249523	98.13	1.87
6	Water	251418	98.87	1.13

Conclusion

A simple, Accurate, precise method was developed for the simultaneous estimation of the Daunorubicin and Cytarabine in Tablet dosage form. Retention time of Daunorubicin and Cytarabine were found to be 2.245 min and 2.813. %RSD of the

Daunorubicin and Cytarabine were and found to be 0.6and 0.3 respectively. %Recovery was obtained as 99.39% and 99.26% for Daunorubicin and Cytarabine respectively. LOD, LOQ values obtained from regression equations of Daunorubicin and Cytarabine were 0.03, 0.10 and 0.31, 0.94 respectively. Regression equation of Daunorubicin is y = 2677x + 703.5, y = 2524x + 104.7 of Cytarabine.Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries

Author Contribution

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