



# STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF VOSAROXIN HYDROCHLORIDE AND CYTARABINE HYDROCHLORIDE IN SYNTHETIC MIXTURE

**T Riya<sup>1</sup>, P Jaymin<sup>2</sup>, P Bhumi<sup>3</sup>, P Ronak<sup>4</sup>, P Janki<sup>5</sup>, P Divyakant<sup>6</sup>**

Student<sup>1</sup>, Associate professor<sup>2</sup>, Associate professor<sup>3</sup>, Associate professor<sup>4</sup>, Associate professor<sup>5</sup>, Principal<sup>6</sup>

Department of Quality Assurance

Address: Sharda School of pharmacy, Pethapur, Gandhinagar, Gujarat 382610.

**ABSTRACT:** Rapid, precise, and accurate RP-HPLC method developed for simultaneous estimation of Vosaroxin hydrochloride and Cytarabine hydrochloride in synthetic mixture. Combination of Vosaroxin hydrochloride and cytarabine hydrochloride was studied under clinical trial phase III. It was proved that the combination is effective in Cancer patient. The combination of Vosaroxin with cytarabine produced synergistic cytotoxic effects in human leukemia cell lines and primary AML blasts. Safe and effective treatments are urgently needed for patients with relapsed or refractory acute myeloid leukemia. The efficacy and safety of Vosaroxin, a first-in-class anticancer quinolone derivative, plus cytarabine in patients with relapsed or refractory acute myeloid leukaemia. From the literature survey, it was found that even though individually both drugs have been analysed by few methods, but no analytical method has been developed and validated for analysis of Vosaroxin hydrochloride and Cytarabine hydrochloride in combination and no Single Patent has been found on Analytical Method Development and Validation for Simultaneous Estimation of Vosaroxin hydrochloride and Cytarabine hydrochloride in Synthetic Mixture. Therefore, it was thought of interest to develop and validate Stability Indicating RP- HPLC method for Simultaneous estimation of Vosaroxin hydrochloride and Cytarabine hydrochloride in synthetic mixture.

## KEYWORDS:

Vosaroxin hydrochloride, Cytarabine hydrochloride, Stability indicating RP- HPLC Method, Validation.

## I. INTRODUCTION:

AML is a cancer of the blood that starts inside the bone marrow and progresses rapidly. AML is a cancer of the blood that starts inside the bone marrow and progresses rapidly.[1] Vosaroxin is a replication-dependent DNA damaging agent that leads to G2 arrest and cell death (apoptosis).[2] Cytarabine is used alone or with other chemotherapy drugs to treat certain types of leukemia (cancer of the white blood cells), including acute myeloid leukemia (AML), acute lymphocytic leukemia (ALL), and chronic myelogenous leukemia (CML).[3] Leukemia is different from most other types of cancer. Leukemia is cancer that starts in the bone marrow. This is where new blood cells are made. The bone marrow is a thick, sponge-like tissue in the centre of certain bones in your body. Leukemia cells are early or immature forms of blood cells, most often white blood cells. [4-6] A stability indicating method accurately measures the active ingredients, without interference from degradation products, process impurities, excipients, or other potential impurities. [7] Combination of Vosaroxin hydrochloride and cytarabine hydrochloride was studied under clinical trial phase III. It was proved that the combination is effective in Cancer patient. [8] Identification of pure API (Vosaroxin HCL and Cytarabine HCL) was carried out by M.P., Solubility, IR and UV study.[9] Literature review reveals that number of individual analytical methods available for estimation of Vosaroxin hydrochloride and Cytarabine hydrochloride in their individual dosage forms. But no methods have been reported for analytical method development of simultaneous estimation of Vosaroxin hydrochloride and Cytarabine hydrochloride in synthetic mixture. So, the aim of present work is Stability Indicating RP-HPLC Method for simultaneous estimation of Vosaroxin hydrochloride and Cytarabine hydrochloride in synthetic mixture. [10-11]

## II .MATERIALS AND METHODS

A Systronic RP-HPLC (LC-20-AD) (SPD- 20 A) model and Clarify software was used. Acetonitrile, Methanol and water of HPLC grade from Finar Chemicals Pvt. Ltd, Ortho Phosphoric acid, Formic acid of AR grade from Astron Chemicals was used.

### IR identification and wavelength selection

FT-IR Spectra of Vosaroxin hydrochloride and Cytarabine hydrochloride drug standards were obtained by FT-IR spectrophotometer. Small quantities of standard were kept directly in the sample compartment of FT-IR and they were scanned in the range of 400-4000  $\text{cm}^{-1}$ . And then FTIR spectra were interpreted, and results were co-related with M.P., UV spectra and solubility to confirm identity of individual drugs. Wavelength was selected from the overlay spectra of above solutions.

Figure 1. Structure of Vosaroxin HCL

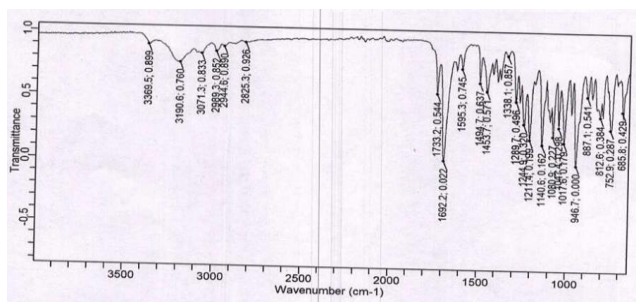


Figure 3: IR spectrum of Vosaroxin HCL (API)

Figure 2. Structure of Cytarabine HCL

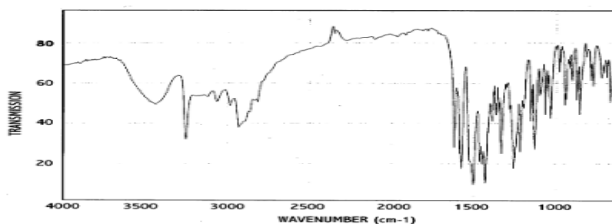


Figure 4: IR Spectrum of Vosaroxin HCL (Std.)

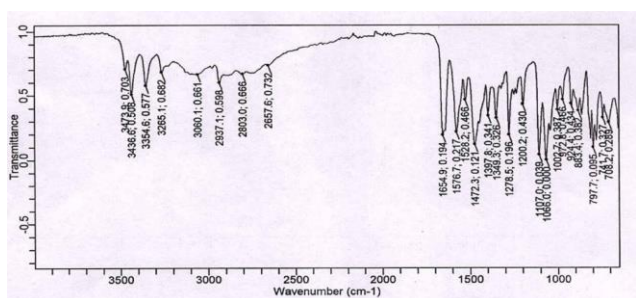


Figure 5: IR spectrum of Cytarabine HCL (API)

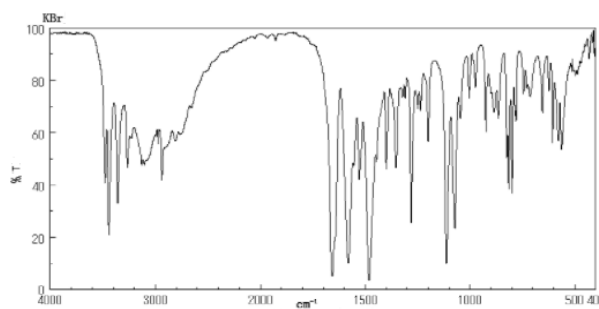


Figure 6: IR Spectrum of Cytarabine HCL (Std.)

Table 1: IR spectrum of Vosaroxin HCL

Sr. No.	Functional group	Observed value	Standard value
1	O-H	3190.6	3650-3100
2	N-H	3369.5	3500-3300
3	C-H (Aromatic)	3071.3	3150-3020
4	C-H	2944.6	2960-2850
5	C=O	1733.2	1780-1650
6	C=C	1692.2	1600-1450
7	C-O	1017.6	1300-1000
8	C-N	1140.6	1230-1030
9	C-S	946.7	1300-800

Table 2: IR spectrum of Cytarabine HCL

sr. No.	Functional group	Observed value	Standard value
1	O-H	3473.9	3650-3400
2	N-H	3354.6	3500-3300
3	C-H	2803.0	2850-2690
4	C=O	1654.9	1780-1650
5	C=C	1472.3	1600-1450
6	C-C	1278.5	1300-800
7	C-N	1066.0	1230-1030
8	C=N	1576.7	1690-1540

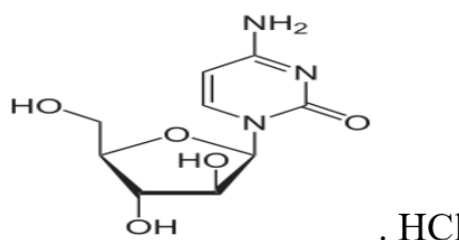
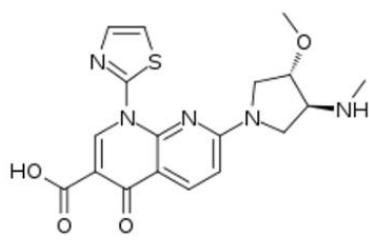
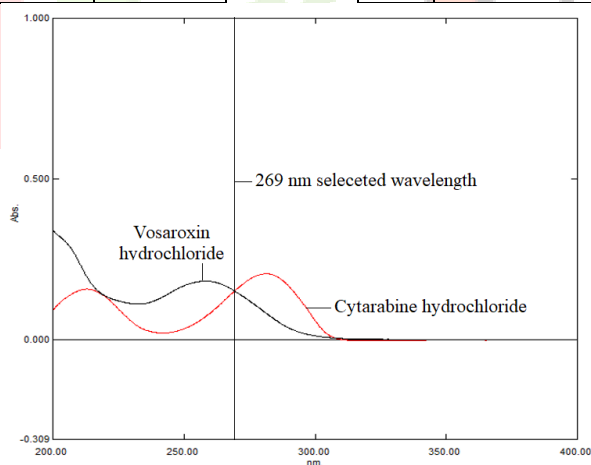


Figure 7: Determination of wavelength maximum (269 nm)

### Selection of Mobile phase

The composition and flow rate of mobile phase were changed to optimize the separation condition using combined solution. After number of trial experiments, it was established that the mobile phase 0.1% formic acid in Acetonitrile: Water (20:80 %v /v) shows good peak shape and resolution.

### Optimization of chromatographic conditions:

Various mobile phases, such as Acetonitrile: Water, Methanol: Water, in different proportion was tried. The combination of 0.1% formic acid in Acetonitrile: Water (20:80 %v /v) provided optimum polarity for proper migration, separation and resolution of Vosaroxin HCl and Cytarabine HCl. Under these conditions, the eluted peaks were well defined and resolved.

### METHOD DEVELOPMENT

#### Trial-1

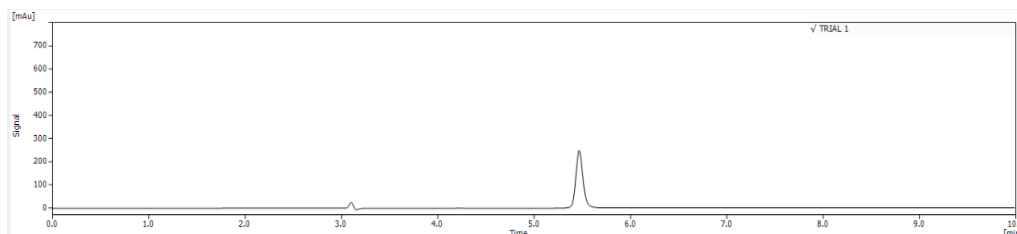


Figure 8: RP-HPLC Chromatogram of Vosaroxin hydrochloride (1.8 µg/ml) and Cytarabine hydrochloride (20 µg/ml) in Methanol: Water (90:10%v/v) at 258 nm {Run time: 10 min, Flow rate: 1 ml/min}

#### Trial-2

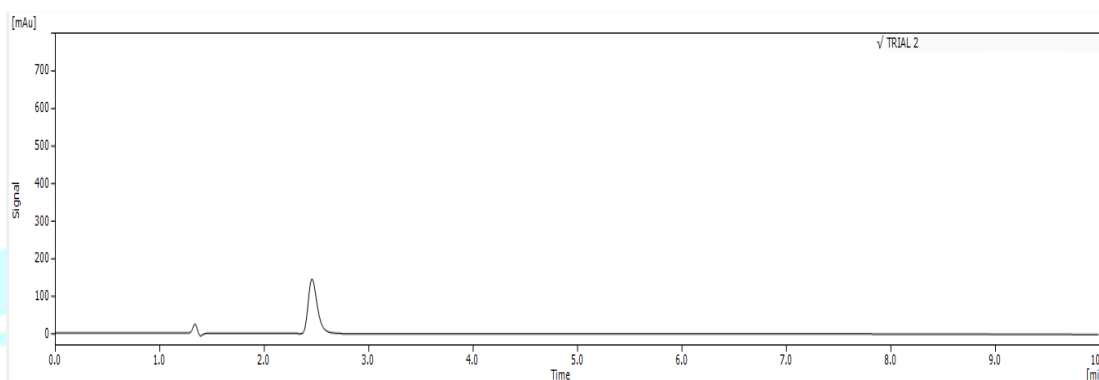


Figure 9: RP-HPLC Chromatogram of Vosaroxin hydrochloride (1.8 µg/ml) and Cytarabine hydrochloride (20 µg/ml) in ACN: Water (50:50%v/v) at 281 nm {Run time: 10 min, Flow rate: 1 ml/min}

#### Trial-3

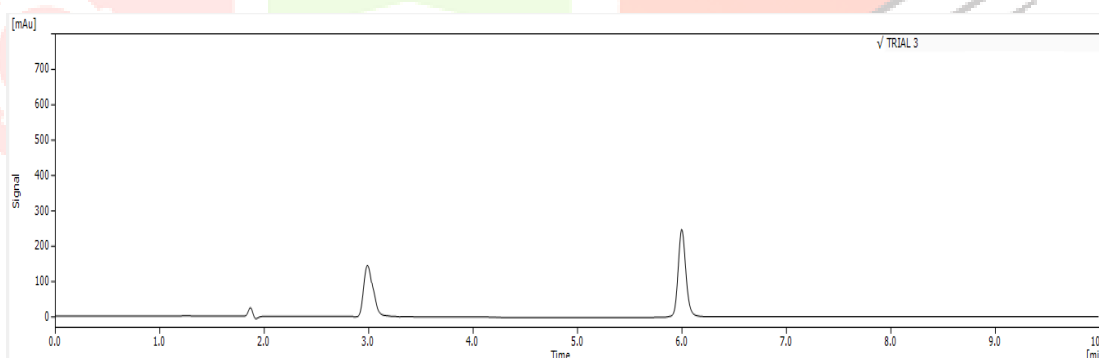


Figure 10: RP-HPLC Chromatogram of Vosaroxin hydrochloride (1.8 µg/ml) and Cytarabine hydrochloride (20 µg/ml) in ACN: Water (pH 3.4 adjusted with OPA) (30:70% v/v) at 269 nm {Run time: 10 min, Flow rate: 1 ml/min}

## Trial-4

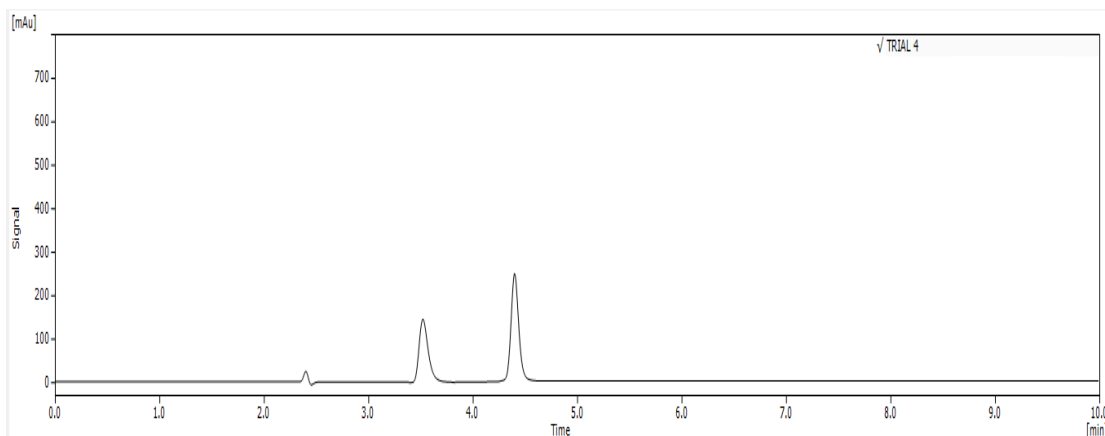


Figure 11: RP-HPLC Chromatogram of Vosaroxin hydrochloride (1.8 µg/ml) and Cytarabine hydrochloride (20 µg/ml) in 0.1% formic acid in Acetonitrile: Water (40:60%v/v) at 269 nm {Run time: 10 min, Flow rate: 1 ml/min}

## Trial-5 (Final)

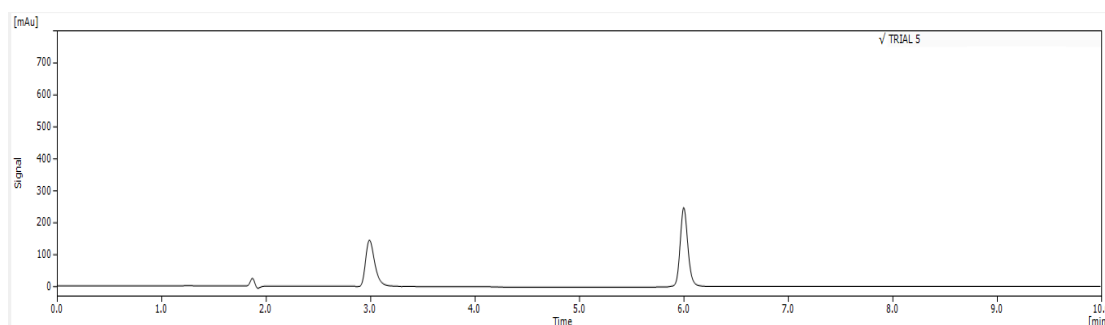


Figure 12: RP-HPLC Chromatogram of Vosaroxin hydrochloride (1.8 µg/ml) and Cytarabine hydrochloride (20 µg/ml) in 0.1% formic acid in Acetonitrile: Water (20:80%v/v) at 269 nm {Run time: 10 min, Flow rate: 1 ml/min} (pH 3.0)

Table 3: Mobile phase optimization trials for cytarabine HCL and Vosaroxin HCL

Trial	Mobile Phase	Ratio (%v/v)	Remark
1	Methanol: Water at 258 nm	90:10	Only one peak was obtained at 5.5 min (Maybe it was peak of Cytarabine hydrochloride)
2	ACN: Water at 281 nm	50:50	One peak was obtained at 2.5 min (Maybe it was peak of Vosaroxin hydrochloride)
3	ACN: Water (pH 3.4 adjusted with OPA) at 269 nm	30:70	Two peaks were obtained but one peak shape was not good
4	0.1% formic acid in Acetonitrile: Water at 269 nm	40:60	Two Peaks were observed, Peak shape was good but peak separation was not proper
5	0.1% formic acid in Acetonitrile: Water at 269 nm (pH 3.0)	20:80	<b>Good resolution and Sharp peaks were observed</b> <b>Rt: VOSA: 3 min</b> <b>CYTA: 6 min</b>

## IV. METHOD VALIDATION

## Linearity

The linearity of Vosaroxin hydrochloride and Cytarabine hydrochloride was found to be 0.9-4.5 µg/ml and 10-50 µg/ml, respectively.

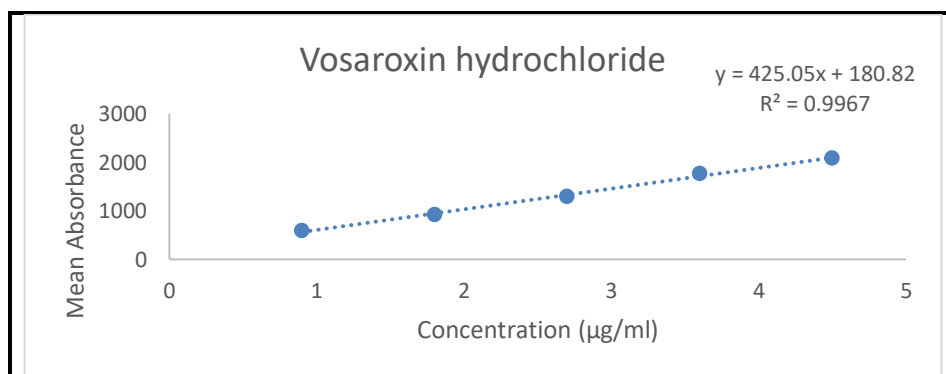


Figure 13: Calibration curve of Vosaroxin hydrochloride (0.9-4.5 µg/ml)

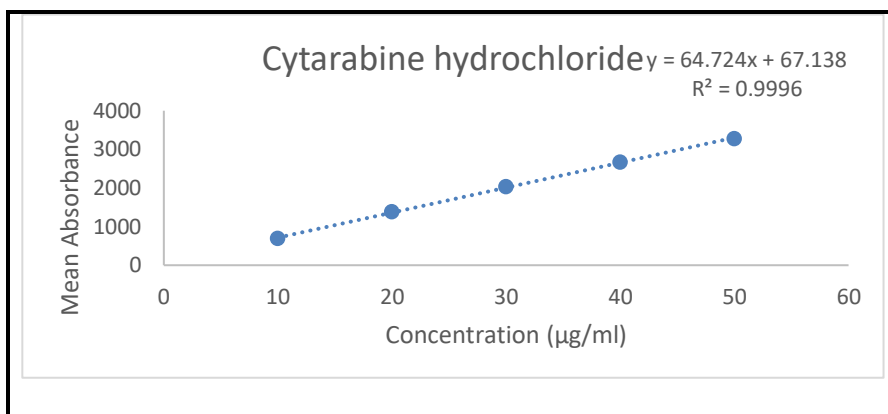


Figure 14: Calibration curve of Cytarabine hydrochloride (10-50 µg/ml)

Table 4: Calibration data for Vosaroxin HCl (0.9-4.5 µg/ml) and Cytarabine HCl (10-50 µg/ml)

Sr. No	Concentration (µg/ml)		Mean Peak area (mAu*sec)±S. D. (n=6)		% RSD	
	VOSA	CYTA	VOSA	CYTA	VOSA	CYTA
1	0.9	10	588.623±7.7109	690.235±8.8350	1.31	1.28
2	1.8	20	920.379±10.6763	1377.6±15.0158	1.16	1.09
3	2.7	30	1292.9±10.7310	2027.1±19.0547	0.83	0.94
4	3.6	40	1757.7±10.1946	2668.3±16.5434	0.58	0.62
5	4.5	50	2082.7±7.0811	3281.1±12.4681	0.34	0.38

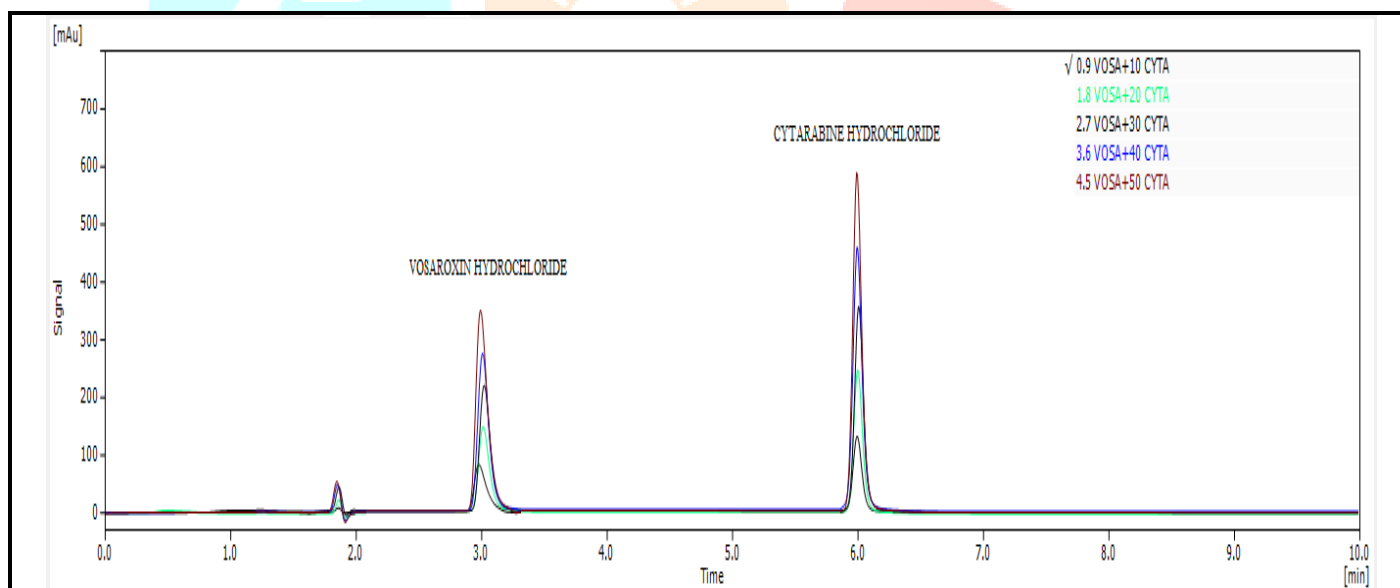


Figure 15: Overlain RP-HPLC chromatogram of Vosaroxin hydrochloride (0.9-4.5 µg/ml) and Cytarabine hydrochloride (10-50 µg/ml) at 269 nm {Run time: 10 min, Flow rate: 1 ml/min}

## Precision

Table 5: Precision study of Vosaroxin hydrochloride

Vosaroxin hydrochloride		
Intraday precision of Vosaroxin hydrochloride		
Conc. (µg/ml)	Mean peak area (mAu*sec) ± S.D (n=3)	% RSD
0.9	586.412±8.0924	1.38
1.8	918.256±9.6416	1.05
2.7	1291.13±8.5214	0.66
Interday precision of Vosaroxin hydrochloride		
Conc. (µg/ml)	Mean peak area (mAu *sec) ± S.D (n=3)	% RSD
0.9	591.764±8.6397	1.46
1.8	923.548±10.3437	1.12
2.7	1295.82±12.1807	0.94
Repeatability of Vosaroxin hydrochloride		
Conc. (µg/ml)	Mean peak area (mAu *sec) ±SD (n=6)	% RSD
1.8	920.582±6.2599	0.68

Table 6: Precision study of Cytarabine hydrochloride

Cytarabine hydrochloride		
Intraday precision of Cytarabine hydrochloride		
Conc. (µg/ml)	Mean peak area (mAu*sec) ±SD (n=3)	% RSD
10	688.014±8.8065	1.28
20	1375.34±15.2662	1.11
30	2025.73±17.0161	0.84
Interday precision of Cytarabine hydrochloride		
Conc. (µg/ml)	Mean peak area (mAu *sec) ±SD (n=3)	% RSD
10	693.647±10.8208	1.56
20	1381.59±18.0988	1.31
30	2029.74±23.5449	1.16
Repeatability of Cytarabine hydrochloride		
Conc. (µg/ml)	Mean peak area (mAu *sec) ±SD (n=6)	% RSD
20	1377.52± 7.8518	0.57

## Accuracy:

Table 7: Recovery study for Vosaroxin HCL and Cytarabine HCL

Name of Drug	% Level of recovery	Test Amount (µg/ml)	Amount of drug taken (µg/ml)	Total Std Amt (µg/ml)	Total amount Recovered (µg/ml)	% Mean Recovery± SD(n=3)
Vosaroxin hydrochloride	50	1.8	0.9	2.7	2.69	99.76±0.5465
	100	1.8	1.8	3.6	3.59	99.95±0.6631
	150	1.8	2.7	4.5	4.49	99.98±0.8243
CYTARABINE hydrochloride	50	20	10	30	29.95	99.86±0.6246
	100	20	20	40	39.97	99.94±0.7564
	150	20	30	50	50.03	100.06±0.8436

## Robustness

Table 8: Robustness data for Vosaroxin HCL and Cytarabine HCL

Condition	Variation	Vosaroxin HCL	Cytarabine HCL
		%Assay ± SD (n=3)	%Assay ± SD (n=3)
Flow rate (1 ml ± 0.2 ml/ min)	0.8 ml/min	99.42±3.5166	98.45±1.3730
	1.0 ml/min	99.65±5.2691	99.75±2.5545
	1.2 ml/min	99.76±7.4770	99.95±4.0286
Detection wavelength (269 nm ± 2 nm)	267	98.85±4.1268	99.65±2.9454
	269	99.82±4.4267	99.85±2.5055
	271	100.04±6.0256	99.99±5.3762
Mobile Phase (0.1% formic acid in Acetonitrile: Water (20:80 ± 2 % v/v))	18:82	99.23±3.0784	98.84±4.1116
	20: 80	99.83±4.9421	99.65±1.0552
	22: 78	98.44±5.0143	100.05±4.1845

## LOD and LOQ

Table 9: Limit of Detection and Limit of Quantitation Data of Vosaroxin HCL and Cytarabine HCL

Parameter	Vosaroxin hydrochloride	Cytarabine hydrochloride
LOD ( $\mu\text{g/ml}$ )	0.059	0.450
LOQ ( $\mu\text{g/ml}$ )	0.181	1.365

## V. Forced Degradation Condition

## 1. Acid Degradation

After heating the drug solution with 0.1 N Hydrochloric acid in 1 and 2 hour at 60°C 4.25 – 10.00 % degradation was observed in Cytarabine HCl and 3.82 – 12.49 % degradation was observed in Vosaroxin HCl.

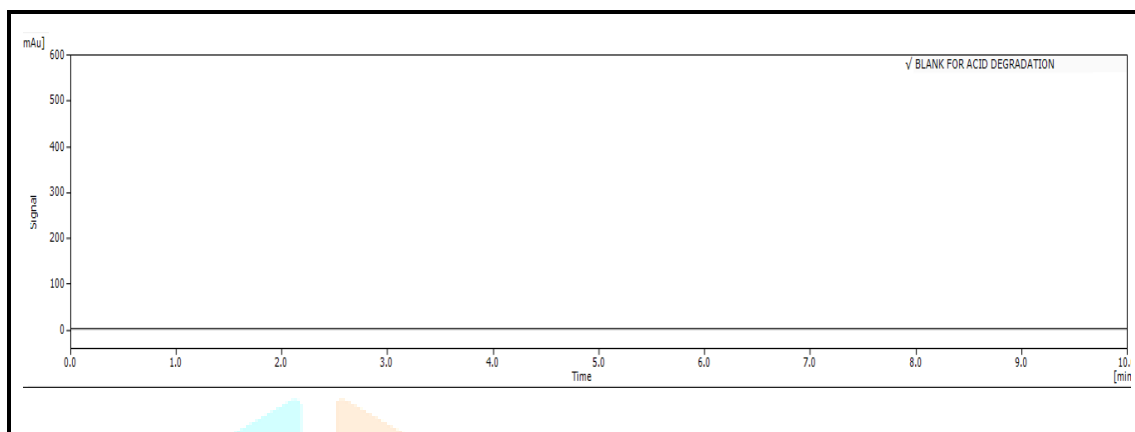
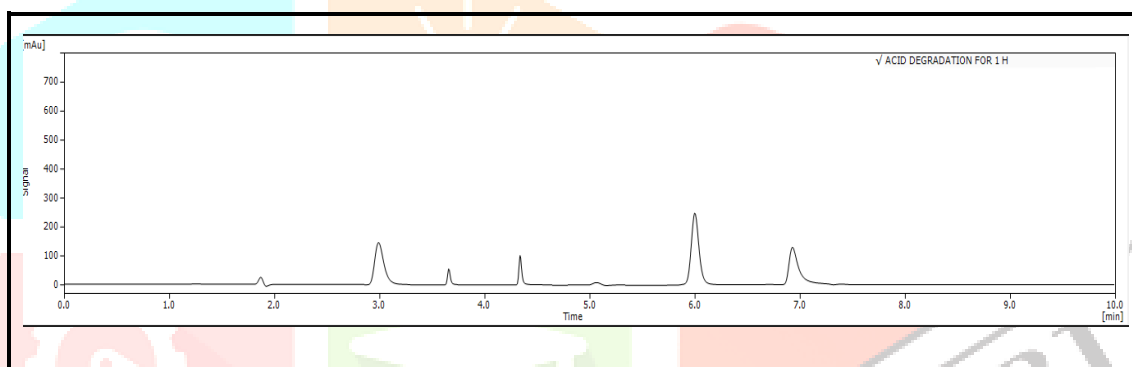
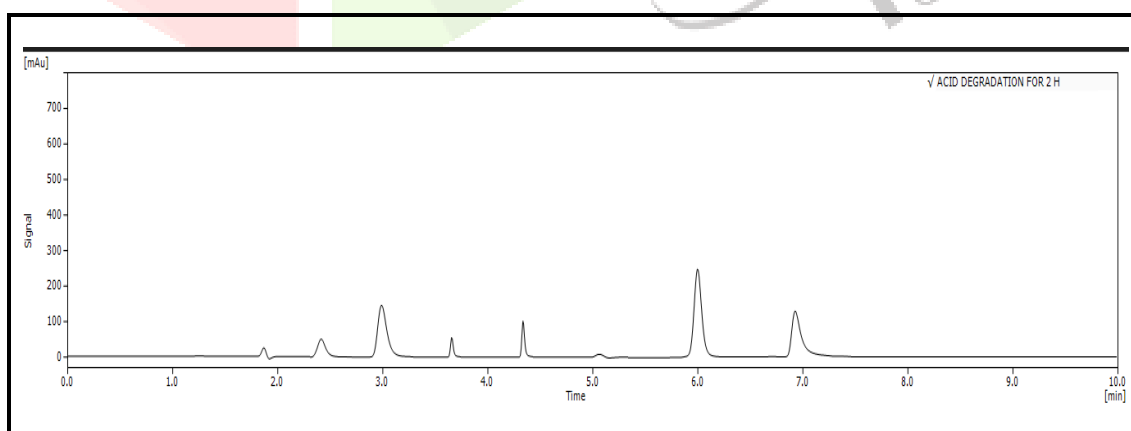


Figure 16: RP-HPLC Chromatogram of Acid Degradation for Blank at 269 nm in 0.1 N HCl {Run time: 10 min, Flow rate: 1ml/min}

Figure 17: RP-HPLC Chromatogram of Acid Degradation for Cytarabine HCl (20  $\mu\text{g/ml}$ ) and Vosaroxin HCl (1.8  $\mu\text{g/ml}$ ) Sample at 1 hr 269 nm in 0.1 N HCl {Run time: 10 min, Flow rate: 1ml/min}Figure 18: RP-HPLC Chromatogram of Acid Degradation Cytarabine HCl (20  $\mu\text{g/ml}$ ) and Vosaroxin HCl (1.8  $\mu\text{g/ml}$ ) Sample at 2 hr 269 nm in 0.1 N HCl {Run time: 10 min, Flow rate: 1ml/min}

## 2. Base Degradation

After heating the drug solution with 0.1 N NaOH at 60°C for 1, 2 and 3 hour, 1.33 – 8.94 % degradation was observed in Cytarabine HCl and 2.22 – 9.49 % degradation was observed in Vosaroxin HCl.

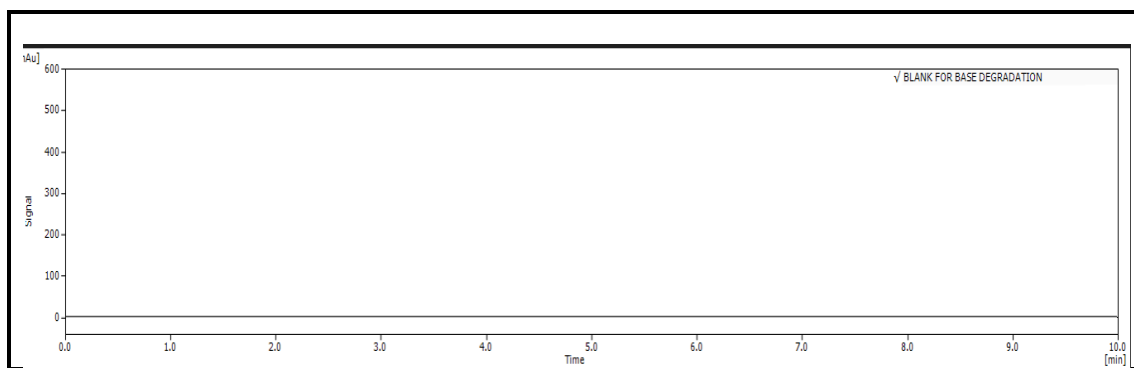


Figure 19: RP-HPLC Chromatogram of Base Degradation for Blank at 269 nm in 0.1 N NaOH {Run time: 10 min, Flow rate: 1ml/min}

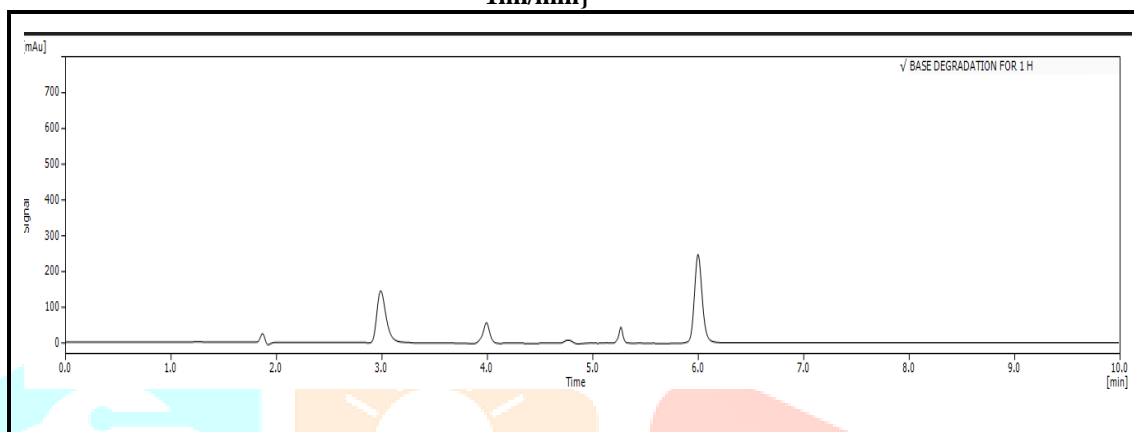


Figure 20: RP-HPLC Chromatogram of Base Degradation for Cytarabine HCl (20 µg/ml) and Vosaroxin HCl (1.8 µg/ml) Sample at 1 hr 269 nm in 0.1 N NaOH {Run time: 10 min, Flow rate: 1ml/min}

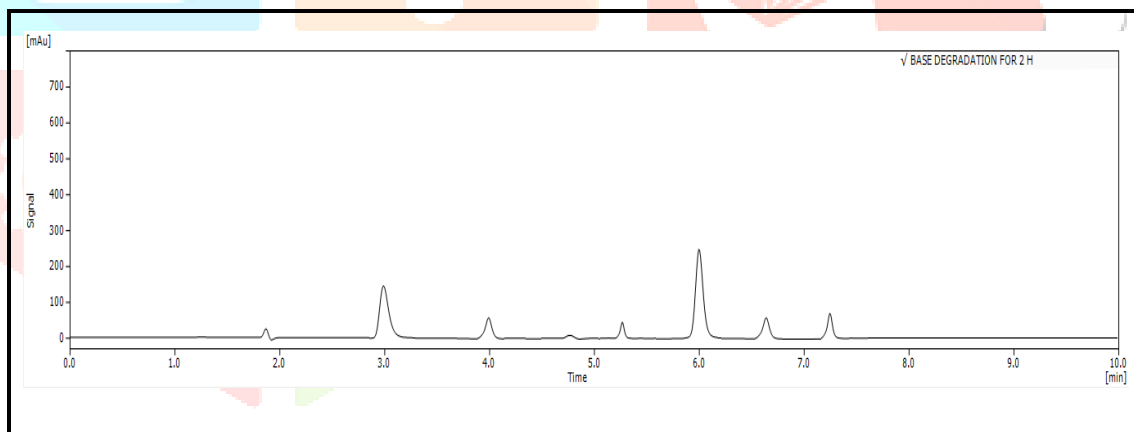


Figure 21: RP-HPLC Chromatogram of Base Degradation Cytarabine HCl (20 µg/ml) and Vosaroxin HCl (1.8 µg/ml) Sample at 2 hr 269 nm in 0.1 N NaOH {Run time: 10 min, Flow rate: 1ml/min}

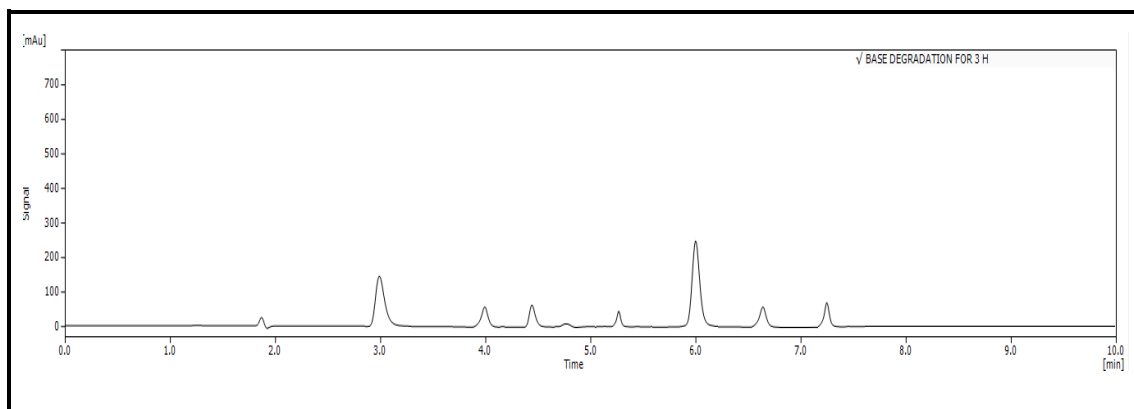


Figure 22: RP-HPLC Chromatogram of Base Degradation for Cytarabine HCl (20 µg/ml) and Vosaroxin HCl (1.8 µg/ml) Sample at 3 hr 269 nm in 0.1 N NaOH {Run time: 10 min, Flow rate: 1ml/min}



### 3. Oxidative Degradation

After heating the drug solution with 3% Hydrogen peroxide at room temperature for 1, 2 hours 1.14 – 7.18 % degradation was observed in Cytarabine HCl and 4.58 – 8.09 % degradation was observed in Vosaroxin HCl.

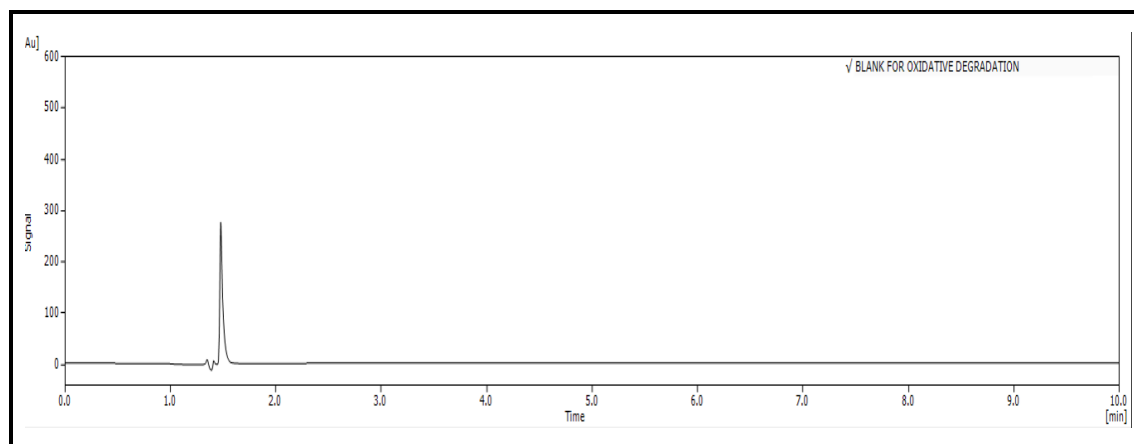


Figure 23: RP-HPLC Chromatogram of Oxidative Degradation for Blank at 269 nm in 3% H<sub>2</sub>O<sub>2</sub> {Run time: 10 min, Flow rate: 1ml/min}

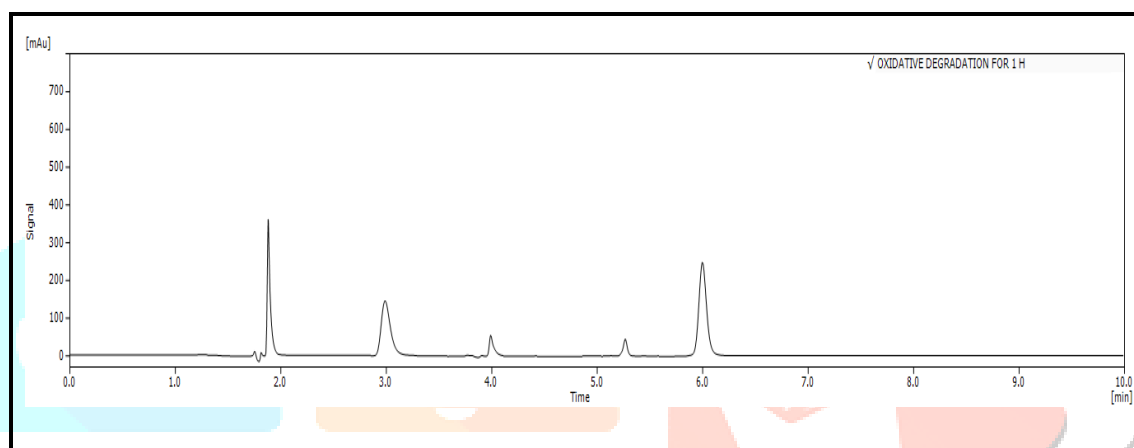


Figure 24: RP-HPLC Chromatogram of Oxidative Degradation for Cytarabine HCl (20 µg/ml) and Vosaroxin HCl (1.8 µg/ml) Sample at 1 hr 269 nm in 3% H<sub>2</sub>O<sub>2</sub> {Run time: 10 min, Flow rate: 1ml/min}

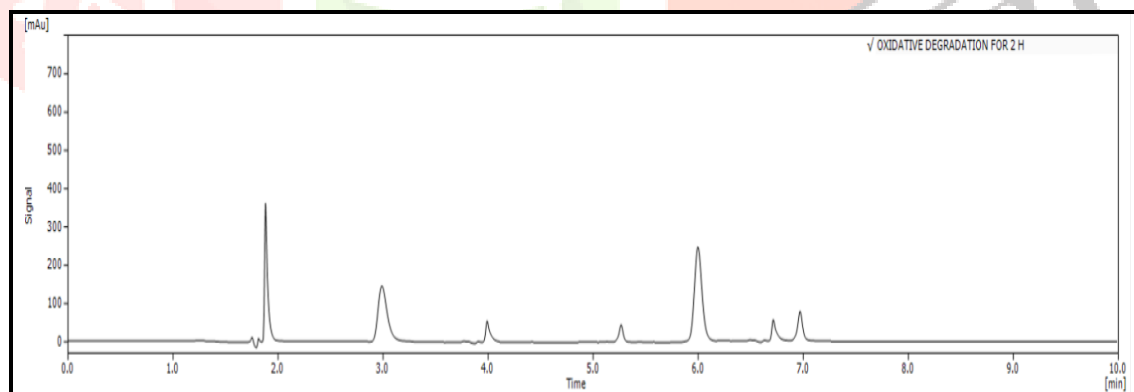


Figure 25: RP-HPLC Chromatogram of Oxidative Degradation for Cytarabine HCl (20 µg/ml) and Vosaroxin HCl (1.8 µg/ml) Sample at 2 hr 269 nm in 3% H<sub>2</sub>O<sub>2</sub> {Run time: 10 min, Flow rate: 1ml/min}

#### 4. Photo Degradation

When drug solution was exposed to direct UV light for 1, 2 hour, 1.79 – 4.19 % degradation was observed in Cytarabine HCl and 4.19 – 6.64 % degradation was observed in Vosaroxin HCl.

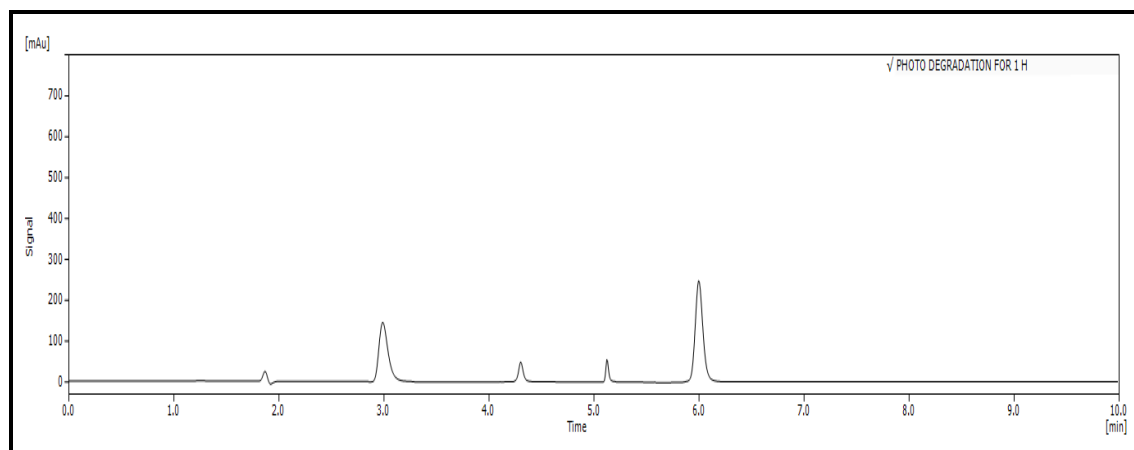


Figure 25: RP-HPLC Chromatogram of Photo Degradation for Cytarabine HCl (20 µg/ml) and Vosaroxin HCl (1.8 µg/ml) Sample at 1 hr 269 nm {Run time: 10 min, Flow rate: 1ml/min}

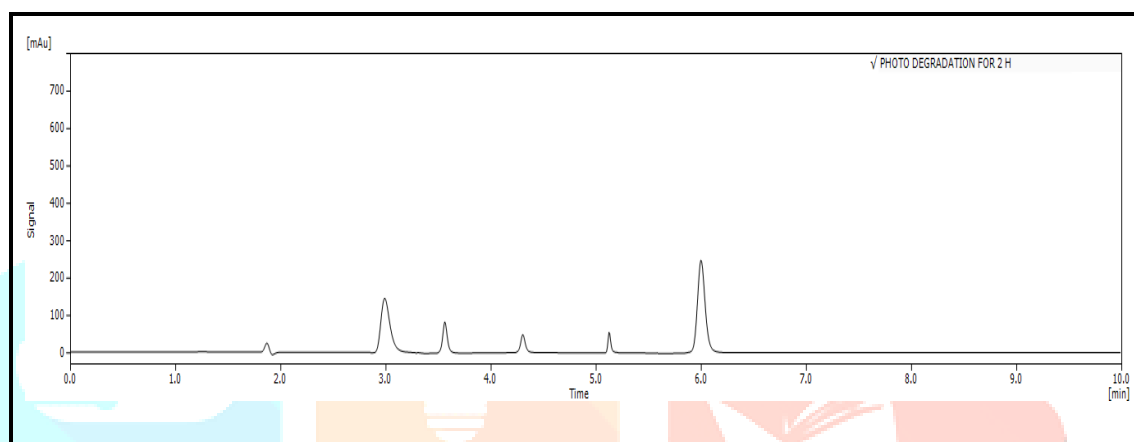


Figure 26: RP-HPLC Chromatogram of Photo Degradation for Cytarabine HCl (20 µg/ml) and Vosaroxin HCl (1.8 µg/ml) Sample at 2 hr 269 nm {Run time: 10 min, Flow rate: 1ml/min}

#### 5. Thermal Degradation

After heating the drug solution at 80 °C for 1, 2 hour, 4.91 – 7.37 % degradation was observed in Cytarabine HCl and 4.54 – 8.33 % degradation was observed in Vosaroxin HCl.

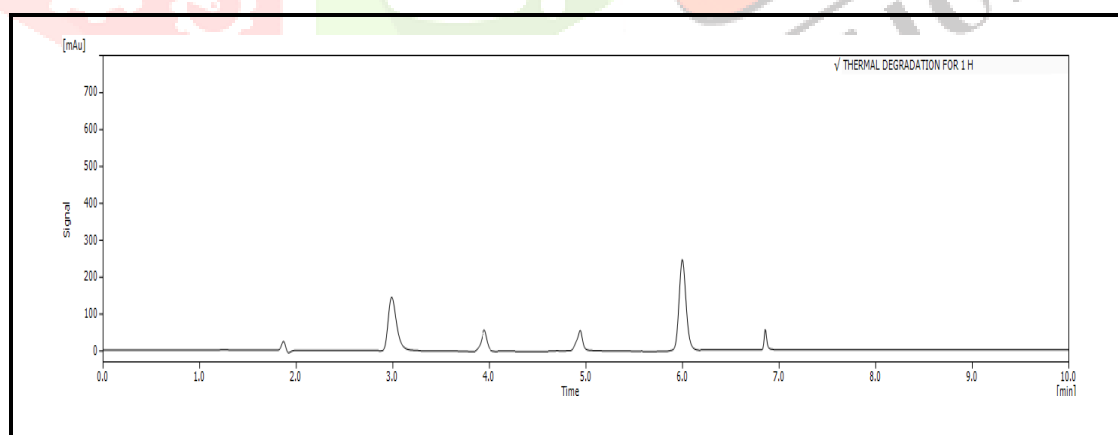


Figure 27: RP-HPLC Chromatogram of Thermal Degradation for Cytarabine HCl (20 µg/ml) and Vosaroxin HCl (1.8 µg/ml) Sample at 1 hr 269 nm 80 °C. {Run time: 10 min, Flow rate: 1ml/min}

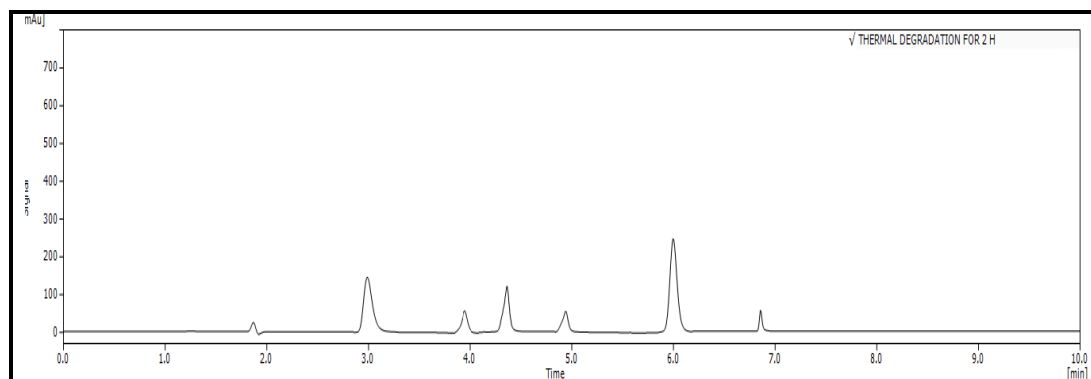


Figure 28: RP-HPLC Chromatogram of Thermal Degradation for Cytarabine HCl (20 µg/ml) and Vosaroxin HCl (1.8 µg/ml) Sample at 2 hr 269 nm 80 °C. {Run time: 10 min, Flow rate: 1ml/min}

## RESULT AND DISCUSSION

Table 10: Summary of Validation parameters

Sr. No.	Parameters	Vosaroxin HCl	Cytarabine HCl
1	Detection wavelength (nm)	269 nm	
2	Linearity Range (µg/ml)	0.9-4.5	10-50
3	Regression equation (y = mx + c)	y = 425.05x + 180.82	y = 64.724x + 67.138
4	Correlation Coefficient (r <sup>2</sup> )	0.9967	0.9996
5	Intraday Precision (%RSD, n=3)	0.66-1.38	0.84-1.28
6	Interday Precision (% RSD, n=3)	0.94-1.46	1.16-1.56
7	Repeatability (% RSD, n=6)	0.68	0.57
8	Accuracy (% Recovery, n=3)	99.76-99.98%	99.86-100.06%
9	LOD (µg/ml)	0.059	0.450
10	LOQ (µg/ml)	0.181	1.365
11	% Assay	99.86%	99.97%

Table 11: Summary of forced degradation study of Cytarabine HCl by stability indicating RP-HPLC method.

Condition	Time (hr)	Sample		Area	% Degradation
		For Degradation	For Neutralization		
Acid	1	2 ml 0.1 N HCl	2ml 0.1 N NaOH	1318.96	4.25
	2	"	"	1239.76	10.0
Base	1	2 ml 0.1 N NaOH	2 ml 0.1 N HCl	1359.23	1.33
	2	"	"	1298.75	5.72
	3	"	"	1254.39	8.94
Oxidation	1	2 ml 3% H <sub>2</sub> O <sub>2</sub>	-	1361.89	1.14
	2	"	-	1278.56	7.18
Photo	1	-	-	1352.94	1.79
	2	-	-	1319.86	4.19
Thermal	1	-	-	1309.86	4.91
	2	-	-	1275.96	7.37

Table 12: Summary of forced degradation study of Vosaroxin HCl by stability indicating RP-HPLC method

Condition	Time (hr)	Sample		Area	% Degradation
		For Degradation	For Neutralization		
Acid	1	2 ml 0.1 N HCl	2ml 0.1 N NaOH	885.15	3.82
	2	"	"	805.38	12.49
Base	1	2 ml 0.1 N NaOH	2 ml 0.1 N HCl	899.86	2.22
	2	"	"	869.23	5.55
	3	"	"	833.01	9.49
Oxidation	1	2 ml 3% H <sub>2</sub> O <sub>2</sub>	-	878.14	4.58
	2	"	-	845.92	8.09
Photo	1	-	-	885.56	3.78
	2	-	-	859.23	6.64
Thermal	1	-	-	878.58	4.54
	2	-	-	843.65	8.33

## CONCLUSION

A rapid, sensitive, accurate and precise Stability Indicating RP-HPLC method has been developed and validated for routine analysis of Vosaroxin hydrochloride and Cytarabine hydrochloride in Synthetic mixture.

The RP-HPLC method is suitable for simultaneous estimation of Vosaroxin hydrochloride and Cytarabine hydrochloride in Synthetic mixture in without interference of each other. The developed method was successfully applied in Synthetic mixture.

The proposed method can be utilized for the routine analysis of Vosaroxin hydrochloride and Cytarabine hydrochloride in Synthetic mixture. Linearity Range of 0.9-4.5 µg/ml for Vosaroxin hydrochloride and 10-50 µg/ml for Cytarabine hydrochloride with Correlation Coefficient for Vosaroxin hydrochloride 0.9967 and for Cytarabine hydrochloride 0.9996, respectively and the Precision data was obtained with less than 2% of RSD.

Accuracy was carried out by the Recovery Studies and was obtained in range of 99.76-99.98% for Vosaroxin hydrochloride and 99.86-100.06% for Cytarabine hydrochloride.

LOD and LOQ Values were found to be 0.059 µg/ml and 0.181 µg/ml for Vosaroxin hydrochloride and 0.450 µg/ml and 1.365 µg/ml for Cytarabine hydrochloride, respectively. Percentage assay of Vosaroxin hydrochloride and Cytarabine hydrochloride was found to be 99.86 and 99.97, respectively. Comprehensive stress testing to Vosaroxin hydrochloride and Cytarabine hydrochloride in Synthetic mixture was carried out according to ICH guidelines Q1A (R2).

After acid degradation, 3.82 – 12.49 % degradation was observed in Vosaroxin HCl and 4.25 – 10.00 % degradation was observed in Cytarabine HCl.

After base degradation, 2.22 – 9.49 % degradation was observed in Vosaroxin HCl and 1.33 – 8.94 % degradation was observed in Cytarabine HCl. After oxidative degradation, 4.58 – 8.09 % degradation observed in Vosaroxin HCl and 1.14 – 7.18 % degradation was observed in Cytarabine HCl.

After photolytic degradation, 3.78 – 6.64 % degradation was observed in Vosaroxin HCl and 1.79 – 4.19 % degradation was observed in Cytarabine HCl. After thermal degradation, 4.54 – 8.33 % degradation was observed in Vosaroxin HCl 4.91 – 7.37 % degradation was observed in Cytarabine HCl.

There was no co-elution of any degradation with main peak and the results obtained were found within the acceptance criteria.

Hence, the proposed stability indicating RP-HPLC assay method can be applied for the estimation of Vosaroxin hydrochloride and Cytarabine hydrochloride in Synthetic Mixture.

## REFERENCES

1. Goyal RK., and Mehta AA., Balaraman R., Derasari and Gandhi's. elements of Pharmacology; 7th Edition; Shah BS., Prakashan, Ahmedabad, 2007-2008, pp 322, 330-333, 265-266, 269-272.
2. Vosaroxin hydrochloride: Drug profile" February 2022, [https://en.wikipedia.org/wiki/Vosaroxin\\_hydrochloride](https://en.wikipedia.org/wiki/Vosaroxin_hydrochloride)
3. Cytarabine hydrochloride: Drug Profile", February 2022, <http://www.drugbank.ca/drugs/DB00678>
4. Rang H., and Dale M., Rang & Dale's. pharmacology; 7th Edition; Elsevier Publication, Toronto, 2012, pp 377-378.
5. Tripathi KD., Essential of Medical pharmacology; 6th Edition; Jaypee Brother Medical publishers Limited, New Delhi, 2008, pp 254-270, 273.
6. Skoog DA., and Holler James F. Principle of Instrumental Analysis; 5th Edn; Thomson Asia Pvt. Ltd, Haryana, 2005, pp 300-322, 706-708.
7. ICH, Stability testing of new drug substances and drug products, Q1A (R2), International Conference On Harmonization, *Icpma*, Geneva 1996.
8. ICH, Q2 (R1) Validation of Analytical Procedures: Text and Methodology International Conference on Harmonization, IFPMA, Geneva, Switzerland, 2005.
9. Andel LV, Rosing H, Lubomirov R, Aviles P, Fudio S, Tibben MM, Nan-Offeringa L, Schellens JHM and Beijnen JH "Development and validation of a liquid chromatography-tandem mass spectrometry assay for the quantification of Vosaroxin hydrochloride in human plasma and urine" *J Pharm Biomed Anal.*, **2018**, 158, 160-165. doi: 10.1016/j.jpba.2018.05.053.
10. U.S. National Library of Medicine "Study of Vosaroxin or Placebo in Combination with Cytarabine in Patients with First Relapsed or Refractory AML (VALOR)" August 2018, <https://clinicaltrials.gov/ct2/show/NCT01191801>
11. U.S. National Library of Medicine "Vosaroxin and Infusional Cytarabine in Treating Patients with Untreated Acute Myeloid Leukemia (VITAL)" September 2021, <https://clinicaltrials.gov/ct2/show/NCT02658487> (ClinicalTrials.gov Identifier: NCT02658487)