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Development & Validation of HPLC method for simultaneous estimation of Drotaverine & Omeprazole

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Abstract : A simple and accurate method was developed for simultaneous estimation of Drotaverine & Omeprazole. The chromatography parameters includes stainless steel column Develosil ODS HG-5 RP C₁₈, 5 μ m, 15cmx4.6mm i.d and acetonitrile: potassium dihydrogen phosphate buffer (0.02M, pH 3.0) (55:45v/v) as mobile phase at a flow rate (1.0 ml/minute). The determinations were performed using UV-Vis detector set at 273 nm. The developed HPLC method showed specificity and selectivity with good precision and accuracy, which makes it very suitable for quantification of Drotaverine & Omeprazole.

Key words: HPLC, simultaneous estimation, Drotaverine ,Omeprazole.

Introduction

Pharmaceutical analysis [1-3] plays a very significant and vital role in the evaluation of pharmaceutical formulations and bulk drugs with respect to the assurance and quality control [1-3]. The improvements in analytical method developments and instruments have reduced the time and cost of analysis and enhanced precision and accuracy.[4]Drotaverine (DRO) is has IUPAC name 1-[(3,4-[diethoxyphenyl) methylene]-6,7-diethoxy-1,2,3,4-tetrahydroisoquinoline and used as an antispasmodic and smooth-muscle relaxant in pain related with biliary colic, and postsurgical spasms. [5,6]. It affects by inhibiting phosphodiesterase enzyme IV which is particular utilized for smooth muscles [7,8]. Omeprazole drug is a used as an antiulcer drug and also used against other acid-related diseases [9]. Omeprazole acts as the proton pump inhibitor which in the acidic PH of the stomach, reacts with a cysteine group in H⁺/K⁺-ATPase, thus impede the ability of the parietal cells to generate gastric acid (Tripathi, 2008) [10] . The present work describes a new precise, simple, rapid and accurate method for simultaneous estimation of drotaverine hydrochloride and omeprazole in combined dosage form.

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Results & Discussion

Solubility

Drotaverine was found to be insoluble in water and soluble in acetonitrile & methanol. Omeprazole was found to be soluble in water and freely free soluble in methanol & acetonitrile.

Selection of wavelength

The λ_{\max} of the two ingredients i.e. Drotaverine & Omeprazole, were found to be 277 nm and 307 nm respectively in methanol as solvent system. The isobestic point for the drugs were found at 284 nm.

Preparation of standard solution of Drotaverine

Accurately weighed 10 mg of Drotaverine was weighed accurately and transferred into 100 ml volumetric flask. About 10 ml of HPLC grade methanol was added and sonicate to dissolve. The volume was made up to the mark with same solvent. The final solution contained about 100 $\mu\text{g/ml}$ of Drotaverine.

Preparation of standard solution of Omeprazole

Accurately weighed 10 mg of Omeprazole was weighed accurately and transferred into 100 ml volumetric flask. About 10 ml of HPLC grade methanol was added and sonicate to dissolve. The volume was made up to the mark with same solvent. The final solution contained about 100 $\mu\text{g/ml}$ of Omeprazole.

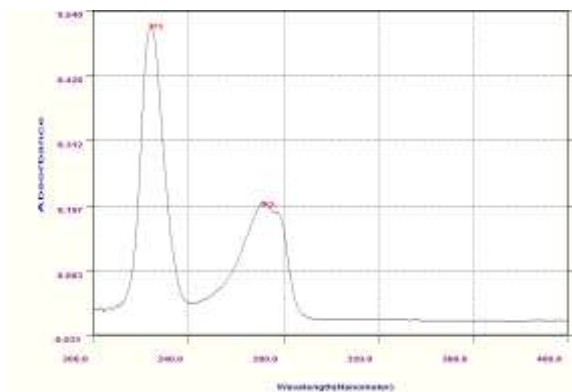


Fig:1 Drotaverine

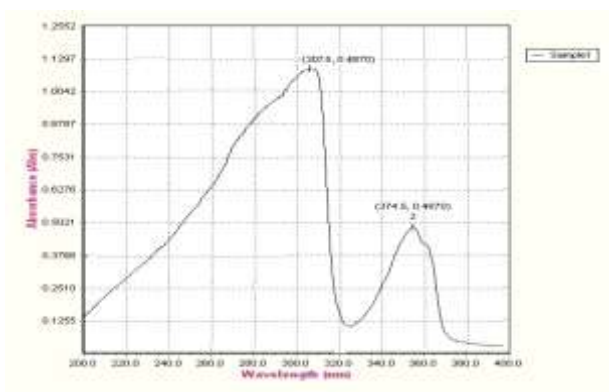


Fig:2 Omeprazole

Preparation of mix. Standard solution of Drotaverine & Omeprazole

Accurately weighed 100 mg of Drotaverine and 100 mg of Omeprazole were transferred to 100 ml volumetric flask. About 40 ml of mobile phase was added and sonicated to dissolve. The volume was made up to mark with same solvent. Then 2 ml of the above solution was diluted to 100 ml with the solvent. The resultant solution was filtered through a 0.45 μm membrane filter and degassed under ultrasonic bath prior to use. From the above standard solution several working standard solutions are prepared by serial dilution technique.

Initialization of the instrument

The HPLC instrument was switched on. The column was washed with HPLC water for 45 minutes. The column was then saturated with mobile phase for 45 minute. The mobile phase was run to find the peaks. After 20 minutes the standard drug solution was injected in HPLC.

Different chromatographic conditions used and their Optimizations

The different HPLC chromatographic conditions were used to find out the optimum chromatographic condition for best elution of drugs such use of various mobile phases such as Water:ACN(20:80), Water: Methanol (20:80), Buffer : acetonitrile (40:60) etc., at different wavelength but the selected and optimized mobile phase was acetonitrile: potassium dihydrogen phosphate buffer (0.02M, pH 3.0) (55:45v/v) and conditions optimized were: flow rate (1.0 ml/minute), wavelength (273 nm), Run time was 20 min. Here the

peaks were separated and showed better resolution, theoretical plate count and symmetry. The proposed chromatographic conditions were found appropriate for the quantitative determination of the drugs. Here resolution was good, theoretical plate count and symmetry was appropriate. Also no unwanted little peaks were seen between two peaks. Hence it was acceptable.

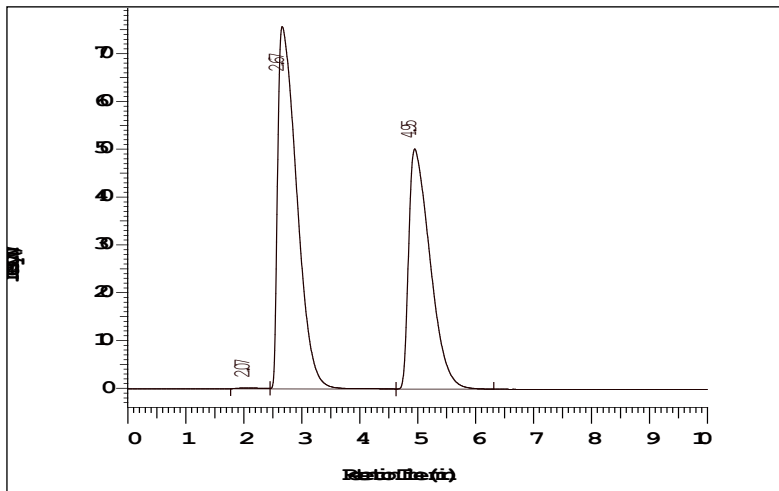


Fig:3the chromatogram obtained after condition, Typical chromatogram of DROTAVERINE (RT=2.67 min) and OMEPRAZOLE (RT= 4.95 min).

Preparation of mobile phase

Mobile phase was prepared by taking acetonitrile: potassium dihydrogen phosphate buffer (0.02M, pH 3.0) (55:45v/v). Mobile phase was filtered through 0.45 μm membrane filter and degassed under ultrasonic bath prior to use. The mobile phase was pumped through the column at a flow rate of 1.0 ml/min.

Running the standard solution of Drotaverine

Transferred 2 ml of stock solution prepared as mentioned under standard solution of Drotaverine was pipette out into a 10 ml volumetric flask. The volume was made up to the mark with mobile phase. The solution was filtered through the 0.45 μm membrane filter and degassed under ultrasonic bath prior to use. The solution was injected into the HPLC system.

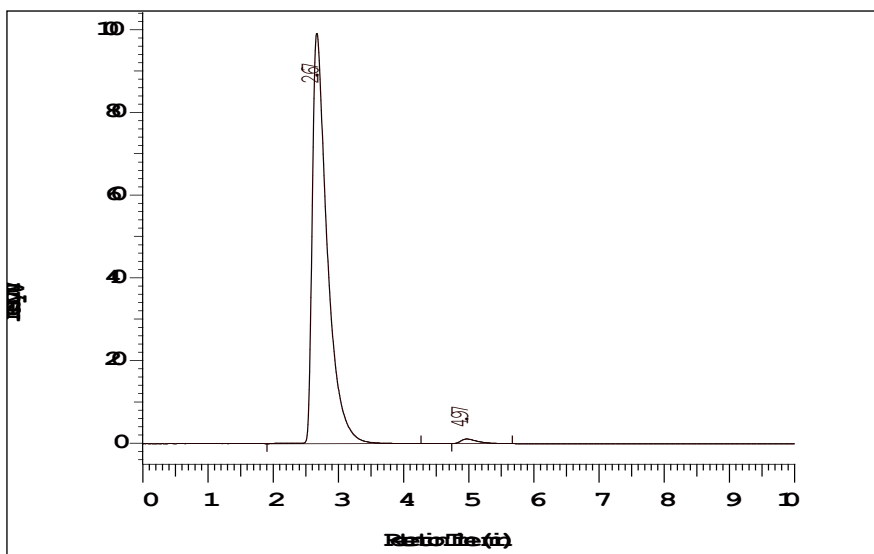


Fig:4Chromatogram of Drotaverine

Running the standard solution of Omeprazole

Transferred 4 ml of stock solution prepared as mentioned under standard solution of Omeprazole was pipetted into a 10 ml volumetric flask. The volume was made up to the mark with methanol. The solution was filtered through the 0.45 µm membrane filter and degassed under ultrasonic bath prior to use. The solution was injected into the HPLC system. The chromatogram obtained is shown in figure 5.

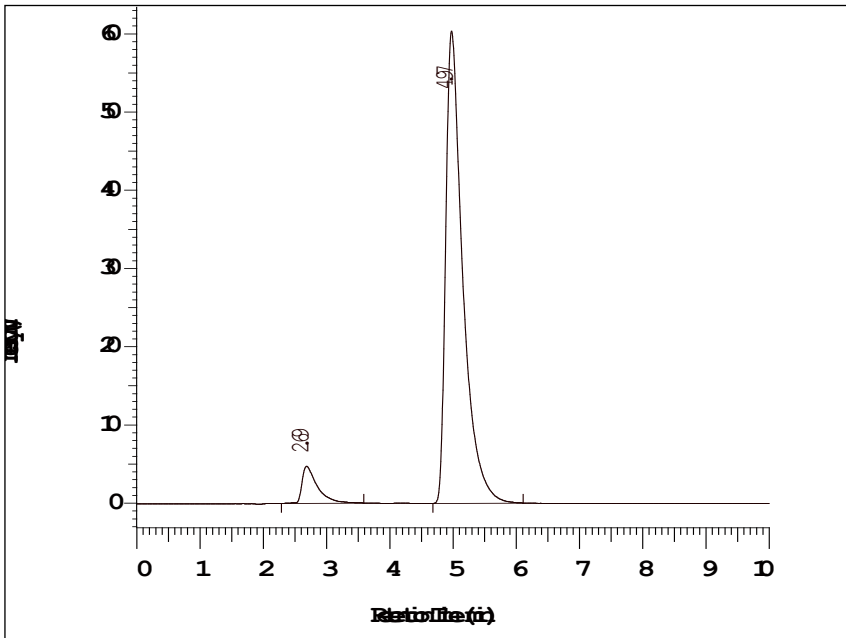


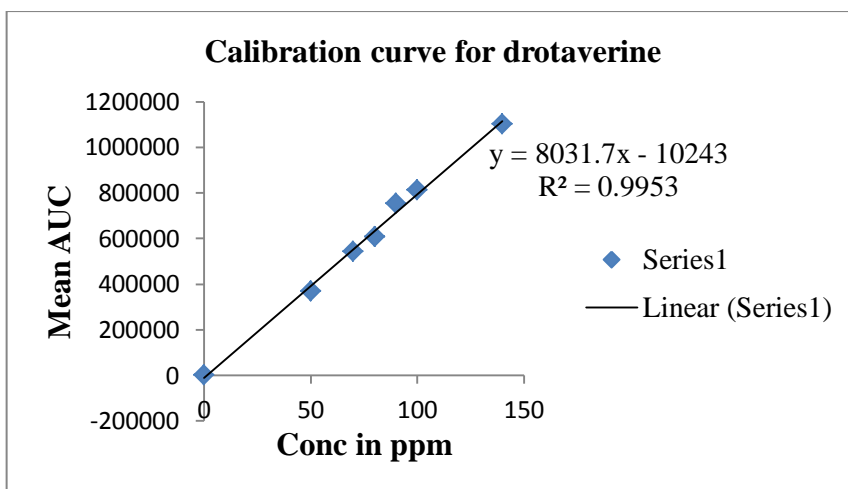
Fig : 5 Chromatogram of Omeprazole

Retention time was found to be 4.97 min. The HPLC system was set with the optimized chromatographic conditions to run the standard solution of Drotaverine and Omeprazole for 10 min. The retention time were found to be 2.69 min and 4.97 min respectively.

Method Validation.

Linearity and Range

Method : As per assessed different concentrations of drotaverine & omeprazole were prepared for linearity.



Fig;6 Standard curve for Drotaverine

Table: 1 Standard curve for Drotaverine

CONC.	AUC
0	0
50	368528
70	542580
80	607574
90	753125
100	811256
140	1102010

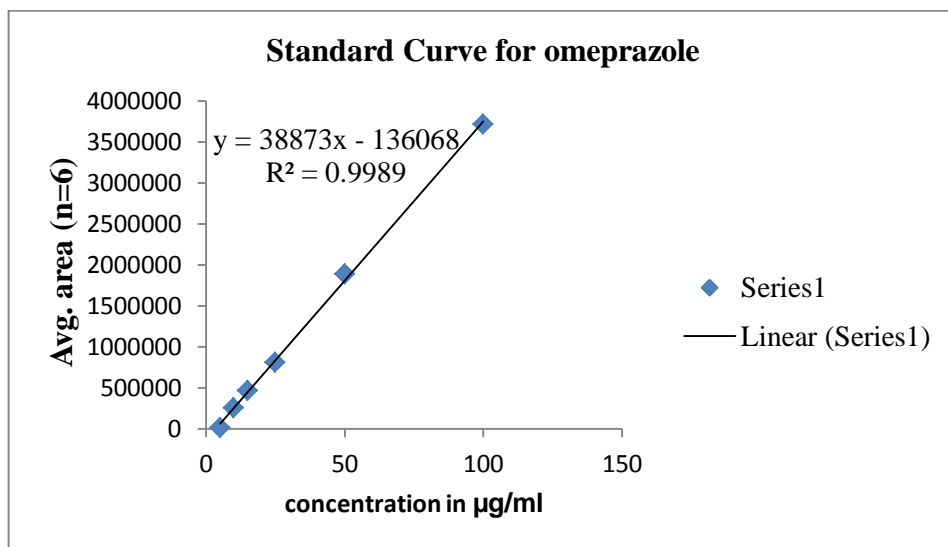


Fig:7 Standard curve for Omeprazole

Table: 2 Standard curve for Omeprazole

CONC.(µg/ml)	MEAN AUC (n=6)
5	12586
10	258963
15	468722
25	808830
50	1886594
100	3716852

Linearity range was found to be 0-140 µg/ml for Drotaverine. The correlation coefficient was found to be 0.995, the slope was found to be 8031 and intercept was found to be 10243 for Drotaverine. Linearity range was found to be 5-100 µg/ml for Omeprazole. The correlation coefficient was found to be 0.998, the slope was found to be 38873 and intercept was found to be 13606 for Omeprazole.

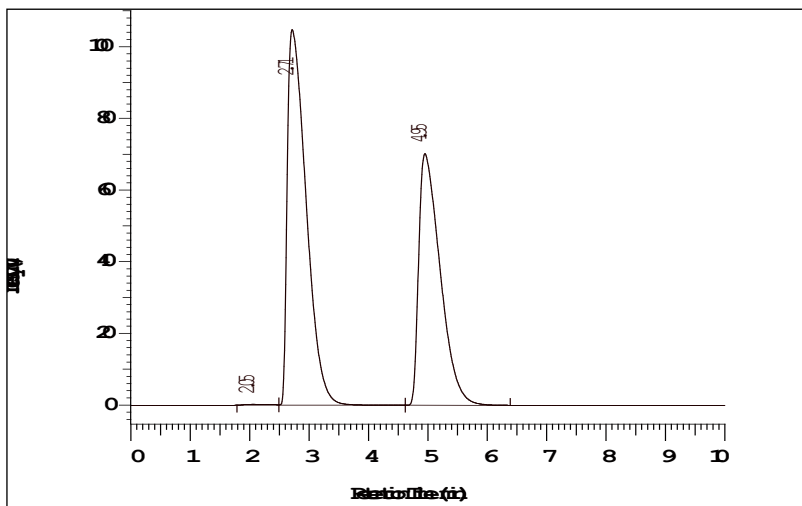


Fig:8 Chromatogram for linearity

Accuracy

Recovery study : Drotaverine

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of pure drug of Drotaverine were taken and added to the pre-analyzed formulation of concentration 10µg/ml. From that percentage recovery values were calculated. The results were shown in table-3.

Table: 3Data showing Recovery study analysis for Drotaverine

Sample ID	Concentration (µg/ml)		%Recovery of Pure drug	Statistical Analysis
	Pure drug	Formulation		
S ₁ : 80 %	16	20	99.13	Mean= 98.94667% S.D. = 0.171561 % R.S.D.= 0.1733
S ₂ : 80 %	16	20	98.79	
S ₃ : 80 %	16	20	98.92	
S ₄ : 100 %	20	20	99.72	Mean= 99.76% S.D. = 0.045826 % R.S.D.= 0.0459
S ₅ : 100 %	20	20	99.81	
S ₆ : 100 %	20	20	99.75	
S ₇ : 120 %	24	20	99.36	Mean= 99.37667% S.D. = 0.105987 % R.S.D. = 0.1066
S ₈ : 120 %	24	20	99.28	
S ₉ : 120 %	24	20	99.49	

Recovery study: Omeprazole

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of pure drug of Omeprazole were taken and added to the pre-analyzed formulation of concentration 10µg/ml. From that percentage recovery values were calculated. The results were shown in table-4.

Table:4 Data showing Recovery study analysis for Omeprazole

Sample ID	Concentration (µg/ml)		%Recovery of Pure drug	Statistical Analysis
	Pure drug	Formulation		
S ₁ : 80 %	32	40	99.18	Mean= 98.97667% S.D. = 0.200083 % R.S.D.= 0.202152
S ₂ : 80 %	32	40	98.78	
S ₃ : 80 %	32	40	98.97	
S ₄ : 100 %	40	40	99.87	Mean= 99.54% S.D. = 0.33 % R.S.D.= 0.331525
S ₅ : 100 %	40	40	99.54	
S ₆ : 100 %	40	40	99.21	
S ₇ : 120 %	48	40	99.32	Mean= 99.567% S.D. = 0.33 % R.S.D. = 0.331159
S ₈ : 120 %	48	40	99.65	
S ₉ : 120 %	48	40	99.98	

The mean recoveries were found to be 98.976, 99.54, 99.57 % for Omeprazole and 98.94, 99.76, 99.31% for Drotaverine The limit for mean % recovery is 95-105% and as both the values are within the limit, hence it can be said that the proposed method was accurate.

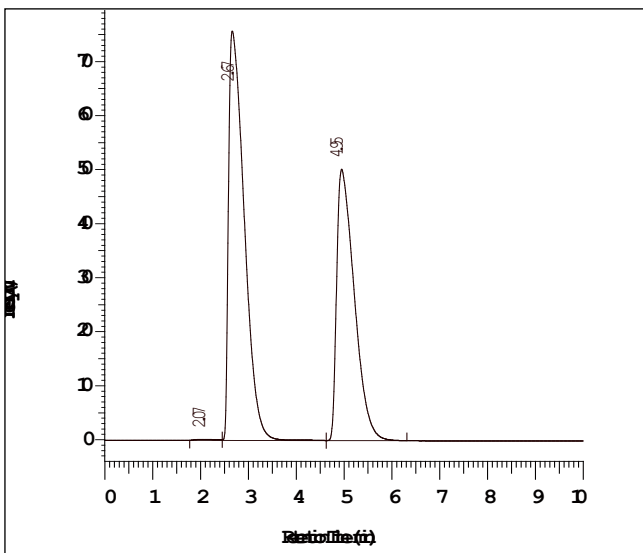


Fig:9 Chromatogram for spiked

Precision

Repeatability

The precision of each method was ascertained separately from the peak areas obtained by actual determination of five replicates of a fixed amount of drug. Omeprazole &Drotaverine .

Table: 5 Data showing repeatability analysis for Drotaverine & Omeprazole

HPLC Injection Replicates	AUC for Drotaverine	AUC for Omeprazole
Replicate – 1	811256	482414
Replicate – 2	810248	483451
Replicate – 3	811563	472415
Replicate – 4	811248	487569
Replicate – 5	810236	485120
Average	810910	482193.8
Standard Deviation	623.075	5803.219
% RSD	0.07684	1.203503

The repeatability study which was conducted on the solution having the concentration of about 20 $\mu\text{g/ml}$ for Drotaverine and 40 $\mu\text{g/ml}$ for Omeprazole ($n = 5$) showed a RSD of 0.07684% for Drotaverine and 1.203503% for Omeprazole. It was concluded that the analytical technique showed good repeatability.

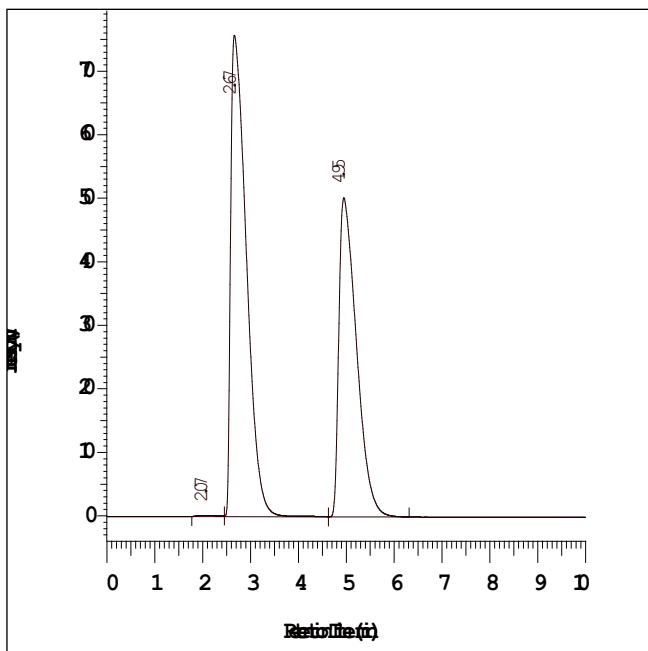


Fig:10 Chromatogram for repeatability

Intermediate precision

For intra-day studies the drug having concentration value 80%, 100 % & 120% of the target concentration ($n = 3$), were injected in triplicate into the HPLC system and for inter-day studies the drug at above three concentrations were injected in triplicate into the HPLC system for three days. Data were subjected to statistical treatment for the calculation of SD and RSD.

Table: 6 Data for Drotaverine analysis

Conc.OfDrotaverine (API) ($\mu\text{g/ml}$)	Observed Conc. Of Drotaverine ($\mu\text{g/ml}$) by the proposed method			
	Intra-Day		Inter-Day	
	Mean ($n=3$)	% RSD	Mean ($n=3$)	% RSD
10	9.95	1.05	10.01	0.24
20	20.98	0.55	20.051	0.41
40	39.84	0.18	39.95	0.18

Table: 7 Data for Omeprazole analysis

Conc. Of Omeprazole (API) ($\mu\text{g/ml}$)	Observed Conc. Of Omeprazole ($\mu\text{g/ml}$) by the proposed method			
	Intra-Day		Inter-Day	
	Mean ($n=3$)	% RSD	Mean ($n=3$)	% RSD
20	20.01	0.86	20.03	0.87
40	40.02	0.30	40.03	0.32
60	59.97	0.13	59.95	0.11

Intraday and interday studies show that the mean RSD (%) was found to be within acceptance limit ($\leq 2\%$), so it was concluded that there was no significant difference for the assay, which was tested within day and between days. Hence, method at selected wavelength was found to be precise.

Limit of detection and limit of quantification

The detection limit (LOD) and quantitation limit (LOQ) may be expressed as:

$$L.O.D. = 3.3(SD/S).$$

$$L.O.Q. = 10(SD/S)$$

Where, SD = Standard deviation of the response

S = Slope of the calibration curve

The LOD was found to be 0.32 $\mu\text{g/ml}$ and 1.44 $\mu\text{g/ml}$ and LOQ was found to be 0.96 $\mu\text{g/ml}$ and 4.32 $\mu\text{g/ml}$ for Drotaverine & Omeprazole respectively which represents that sensitivity of the method is high.

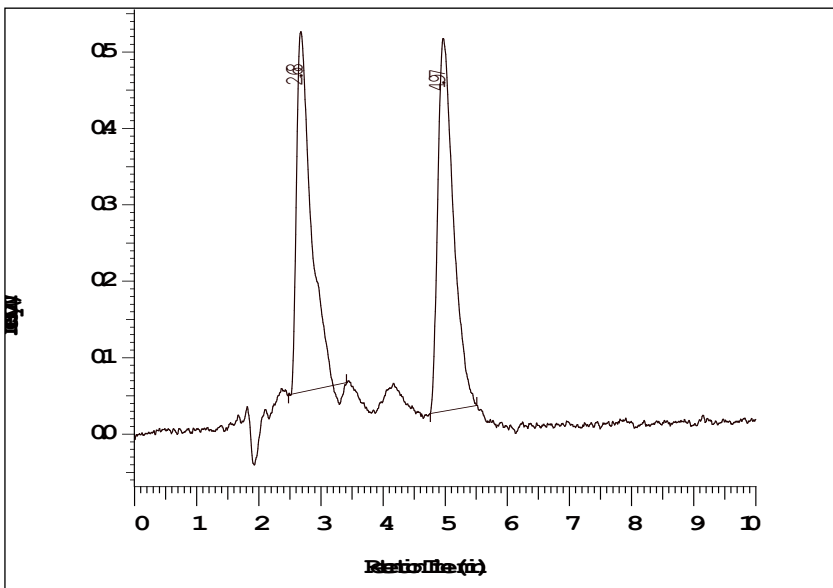


Fig:11 Chromatogram for LOD

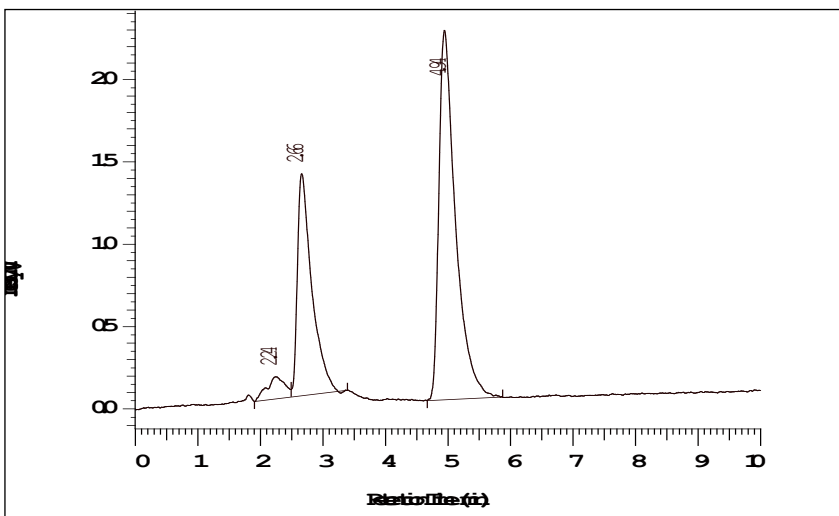


Fig:12 Chromatogram for LOQ

System Suitability Parameter

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. Following system suitability test parameters were established. The data are shown in Table 8.

Table: 8 Data of System Suitability Parameter

S.No.	Parameter	Limit	Result
1	Resolution	$R_s > 2$	3.15
2	Asymmetry	$T \leq 2$	Drotaverine =0.14 Omeprazole =0.19
3	Theoretical plate	$N > 2000$	Drotaverine =3971 Omeprazole= 4861

Conclusion

The results show that the HPLC method developed here can be considered suitable for the analytical determination of drotaverine & omeprazole. The run time was short (20 min) thus enables rapid quantification of the drugs. The proposed chromatographic conditions were found appropriate linear, precise, specific and accurate for the quantitative determination of of drotaverine & omeprazole.

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