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Method validation and development of analysis of Drotaverine and Mefenamic acid by RP-HPLC method

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ABSTRACT

A fast, sensitive, and reliable RP-HPLC method involving Waters HPLC System Model NO.2690/5 with PDA detector was developed and validated for the determination and quantification of Drotaverine and Mefenamic Acid . Chromatography was performed on the Inertsil -ODS C18 (250 x 4.6 mm, 5 µ) column using filtered and mixed Degassed Methanol : Acetonitrile (80:20) as a mobile phase with a flow rate of 1.0 ml / min and selected injection volume 20µl, an effluent of 280nm. Retention times for Drotaverine 2.955min, and Mefenamic Acid 3.538 min. The proposed method is accurate, precise, specific and rapid for estimation of Drotaverine and Mefenamic Acid in bulk and pharmaceutical dosage form.

Keywords: Acetonitrile; Drotaverine; Mefenamic Acid; RP-HPLC; ODS; Methanol.

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INTRODUCTION

Drotaverine hydrochloride and mefenamic acid are two commonly prescribed drugs used in combination for the management of pain associated with smooth muscle spasm, dysmenorrhea, and gastrointestinal disorders. Drotaverine is an antispasmodic drug, used to enhance cervical dilation during childbirth and to relieve smooth muscle spasms in the gastrointestinal tract, urinary system, and gall bladder. It is structurally related to papaverine, is a phosphodiesterase inhibitor and leading to an increase in cyclic adenosine monophosphate (cAMP) levels and subsequent smooth muscle relaxation. Drotaverine hydrochloride has a spasmolytic, myotropic, vasodilation, hypotensive action.

Mefenamic acid, a derivative of anthranilic acid, belongs to the non-steroidal anti-inflammatory drug (NSAID), and is used to treat mild to moderate pain. Mefenamic acid is used to treat pain and inflammation in rheumatoid arthritis and osteoarthritis, postoperative pain, acute pain including muscle and back pain, toothache and menstrual pain, as well as being prescribed for menorrhagia. Its pharmacological action through inhibition of cyclooxygenase (COX) enzymes, thereby reducing prostaglandin synthesis and providing analgesic and anti-inflammatory effects.

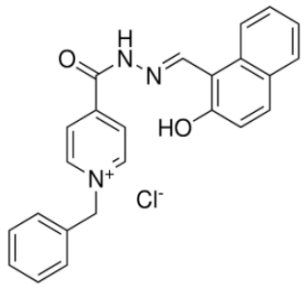
The combination of these two drugs provides synergistic relief from pain and spasm, making it a preferred formulation in clinical practice. However, simultaneous determination of drotaverine and mefenamic acid in combined dosage forms poses an analytical challenge due to their differing chemical properties and absorption characteristics. Hence, a reliable, accurate, and reproducible analytical method is essential for quality control and routine analysis.

Several analytical methods have been reported for the individual estimation of drotaverine hydrochloride and mefenamic acid, including UV spectrophotometric, HPLC, HPTLC, and derivative spectroscopic techniques. For instance, Patil et al. (2010) reported a UV spectrophotometric method for drotaverine estimation in tablet formulations, while

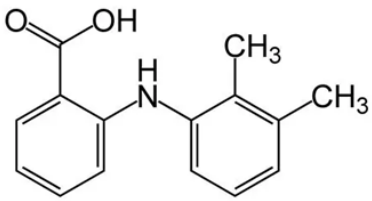
Singh et al. (2013) described an HPLC method for mefenamic acid quantification in plasma samples.

Reverse Phase High performance liquid chromatography (RP-HPLC) is the fastest growing analytical technique for the analysis of drugs. The technique of RP- HPLC is developed from advances made in column chromatography. The RP-HPLC method was performed by waters HPLC with PDA detector. It differs from conventional column chromatography in the sense that the mobile phase is pumped through the packed column under high pressure. Because of the relatively high pressure necessary to perform this type of chromatography, a more elaborate experimental set up is required.

Therefore, the present study aims to develop and validate a simple, precise, accurate, and reproducible RP- HPLC method for the simultaneous estimation of drotaverine hydrochloride and mefenamic acid in tablet dosage form and bulk form in accordance with ICH guidelines.



Structure-1 Drotaverine Hydrochloride



Structure-2 Mefenamic acid

MATERIALS AND METHOD

Drotaverine and mefenamic acid reference standard drugs were procured from Reddy's Laboratories, Hyderabad. Tablets were purchased from local pharmacy. Ultrapure water was obtained from a Millipore system. HPLC grade Acetonitrile and methanol were procured from Merck India Pvt Ltd. The other reagents used in the analytical method were of AR grade.

The separation of drotaverine and mefanamic acid was evaluated in different proportions of these solvents and each condition and the chromatographic parameters were evaluated.

Preparation of standard solution: The solution was prepared by dissolving 20.0 mg of precisely weighed Drotaverine and 25.0 mg of Mefenamic Acid in the mobile process, separately in two volumetric flasks of 100.0 mL and sonicating for 20min. Further dilutions were made to get the final concentrations of 40ppm of Drotaverine and 50 ppm of Mefenamic Acid respectively. The prepared solutions were sonicated and filtered through 0.45µmembrane filter.

Preparation of sample solution: Weigh approximately 10 tablets of Drofem and grind them in a motor to form a fine powder . Transfer the powder equivalent to 20.0 mg of Drotaverine and 25.0 mg of Mefenamic Acid into a clean dry volumetric flask dilute with solvent upto the mark to obtain concentration of 200ppm and 250ppm. Further dilution were carried with the solvent to obtain concentrations of 20ppm and 25ppm of both the drugs.

Method validation: The method was validated according to ICH guidelines. The validation parameters include Specificity, linearity, Accuracy, precison, ruggedness, robusteness, LOD and LOQ.

Linearity: Linearity was evaluated by analyzing standard solutions of drotaverine and mefenamic acid across a suitable concentration range of (20-80) ppm. A calibration curve was constructed by plotting peak area Vs Concentration, and the method showed excellent linearity with a correlation coefficient R^2 > 0.999. The slope and the intercept values for drotaverine and mefenamic acid were found to be confirmed a strong linear relationship demonstrating that the method is linear.

Table 1: Linearity

S.NO	Concentration (ppm)	Drotaverine	Mefenamic acid
		Peak Area	Peak Area
1	20	560960	2110652
2	30	1602034	3250149
3	40	2164732	4307216
4	50	3171457	5320468
5	60	4138838	6427385
6	70	5276830	7452108
7	80	6523097	8769527
Slope		10310	10893
Intercept		540.63	69769
Correlation Coefficient		0.999	0.999

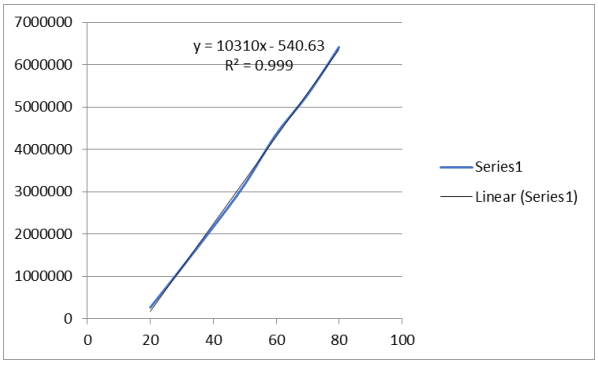


Figure 1: Linearity Plot (Concentration Vs Response) of Drotaverine and Mefenamic acid.

System suitability: As per the test procedure, standard solutions of both the drugs were prepared and injected into the HPLC device five times. The system suitability parameters such as retention time, theoretical plates, tailing factor, resolution and % RSD of peak areas were evaluated. The results demonstrated that the system was performing adequately and suitable for routine analysis of drugs.

Table 2: System Suitability for Drotaverine

Injection	RT	Peak Area	USP Plate count	USP Tailing
1	2.955	2164732	6648.722084	1.119216
2	2.956	2161848	6673.911816	1.142210
3	2.955	2198427	6630.743655	1.167058
4	2.954	2231236	6778.292084	1.140170
5	2.954	2254490	6687.924039	1.132946
Mean	2.955318	2202147	6683.919	1.14032
SD	0.000837	40693.03	-------	-------
% RSD	0.026462	1.84788	-------	-------

Table 3: System Suitability for Mefenamic Acid

Injection	RT	Peak Area	USP Plate count	USP Tailing
1	3.538	4037216	5368.357111	1.496148
2	3.532	4063397	5441.422408	1.471228
3	3.529	4115511	5413.470928	1.484832
4	3.527	4126557	5357.317249	1.466473
5	3.526	4195611	5398.478881	1.506952
Mean	3.52736	4107658	5395.809	1.485127
SD	0.00114	61391.54	-------	-------
% RSD	0.041257	1.494563	-------	-------

Specificity: It is the ability of the method to assess the analyte in the presence of formulation excipients, potential impurities and degradation products as per ICH guidelines. Blank, placebo,standard and sample solutions were analyzed to assess any interference at the analyte retention time. the chromatograms of blank and placebo show no peaks near the analyte retention time confirming the absence of matrix induced interference. Peak purity value confirms the homogeneity of the analyte peak.

Precision: It was evaluated at the repeatability and intermediate precision levels as per ICH Q2 guidelines. Repeatability was assessed by analyzing 6 replicate sample preparations at the target concentration, and the percentage RSD of peak areas was found to be within acceptable limits. Intermediate precision was established by performing the analysis on a different day and by different analyst. The results showed excellent agreement, with percentage RSD values consistently below the regulatory threshold, confirming that the method is precise.

Table 4: Data of Repeatability (Method precision) for Drotaverine

	Injection	Peak Areas of Drotaverine	%Assay
Concentration 40ppm	1	2245703	99.55
	2	2291408	99.88
	3	2278639	99.40
	4	2239286	100.30
	5	2267407	100.53
	6	2278639	99.28
Statistical Analysis	Mean	2266847	99.82333
	SD	20436.91	0.505754
	% RSD	0.901557	0.506649

Table 5: Data of Repeatability (Method precision) for Mefenamic Acid

	Injection	Peak Areas of Mefenamic Acid	%Assay
Concentration 40ppm	1	4196762	98.54
	2	4237539	99.58
	3	4219201	98.86
	4	4278401	99.56
	5	4235847	99.86
	6	4219201	99.06
Statistical Analysis	Mean	4231159	99.24333
	SD	27435.85	0.503812
	% RSD	0.648424	0.507653

Table 6: Data of Intermediate precision (Analyst 2) for Drotaverine

	Injection	Peak Areas of Drotaverine	%Assay
Concentration 40ppm	1	2278639	99.99
	2	224732	99.66
	3	2267407	101.53
	4	2254490	99.98
	5	2231236	99.97
	6	2267407	101.10
Statistical Analysis	Mean	2260652	100.37
	SD	16338.36	0.753536
	% RSD	0.722728	0.75

Table 7: Data of Intermediate precision (Analyst 2) for Mefenamic Acid

	Injection	Peak Areas of Mefenamic Acid	%Assay
Concentration 40ppm	1	4219201	99.78
	2	4237216	99.95
	3	4235847	100.00
	4	4195611	98.55
	5	4226557	101.50
	6	4237216	101.37
Statistical Analysis	Mean	4225275	100.19
	SD	16219.94	1.100898
	% RSD	0.383879	1.09

Accuracy: It was determined using the recovery study by spiking the pre-analyzed sample with known amounts of analyte at 50%, 100% and 150% of target concentration. Each level was analyzed in triplicate, and the mean percentage recovery was within the acceptable limits. These results confirmed that the method is accurate and free from matrix interference.

Ruggedness: It was assessed by evaluating the small effect, deliberate variations in analytical conditions, including different analysts, instruments, and day of analysis. The results showed minimal variation in peak area and retention time, with percentage RSD values with acceptable range. This confirms that the method is rugged and provides consistent performance under varied operational conditions.

Robusteness: It was evaluated by introducing small deliberate variations in method parameters such as mobile phase composition, flow rate, detection wavelength and column temperature. These changes did not affect peak area, retention time or the system suitability parameters and % RSD values were within acceptable range.

Limit of detection and limit of quantification: The LOD and LOQ were determined based on the signal to noise ratio. The calculated LOD and LOQ values indicate that the method is sufficiently sensitive for detecting and quantifying low levels of the analyte.

RESULTS AND DISCUSSION

The present RP-HPLC method for the quantification of Drotaverine and Mefenamic acid in bulk and pharmaceutical dosage forms, revealed as simple, rapid, accurate and precise method with significant shorter retention time of 10 min. The linearity for the detection of Drotaverine and Mefenamic acid was 20 -80 ppm with the coefficients of variation based on mean peak area for three replicate injections were found to be 0.999.The Precision (Repeatability and Intermediate Precision) of the method were determined using Six replicate injections analyzed on the same day and next day over a period of 24 hours. The result revealed the precision with Repeatability of Drotaverine and Mefenamic acid the % RSD is 0.901557 and 0.648424 and the precision with intermediate precision of both drotaverine and mefenamic acid is 0.722728 and 0.383879 respectively for intraday and inter day. To ensure the reliability and accuracy of the method, the recovery studies were carried accuracy was evaluated by injecting the solution about three times, at three different concentrations equivalent to 50%, 100% and 150% of the active ingredients,. the mean % recoveries were in between 100.08%. The assay for the marketed tablets of Drotaverine and Mefenamic acid tablets was established with present chromatographic condition developed and it was found to be more accurate and reliable. The average drug content was found to be 100.37% of the labeled claim and no interfering peaks were found in chromatogram, indicating that the estimation of drug free from inference of excipients. To know reproducibility of the method system suitability test

Table 8: Accuracy data (Recovery)

Drotaverine				Mefenamic acid			
Concentration	Amount recovered	% Amount recovery	%RSD	Concentration	Amount recovered	% Amount recovery	%RSD
50%	20.01	100.06	0.18	50%	19.93	99.69	0.92
100%	40.01333	100.04	0.091	100%	39.93	99.83	0.41
150%	60.01	100.02	0.09	150%	59.98	99.97	0.31

Table 9: Effect of variation in the flow rate

Drotaverine	Flow rate	0.8ml	1.0ml	1.2ml
	Peak area	2264489	2266847	2221693
	% RSD	0.96787	0.901557	1.345138
	Tailing factor	1.14032	1.116323	1.115199
	%RSD	1.530435	0.248825	0.237999
Mefenamic acid	Flow rate	0.8ml	1.0ml	1.2ml
	Peak area	4107658	4231159	4225275
	% RSD	1.494563	0.648424	0.383879
	Tailing factor	1.482517	1.47122	1.495022
	%RSD	0.99	1.86	0.378877

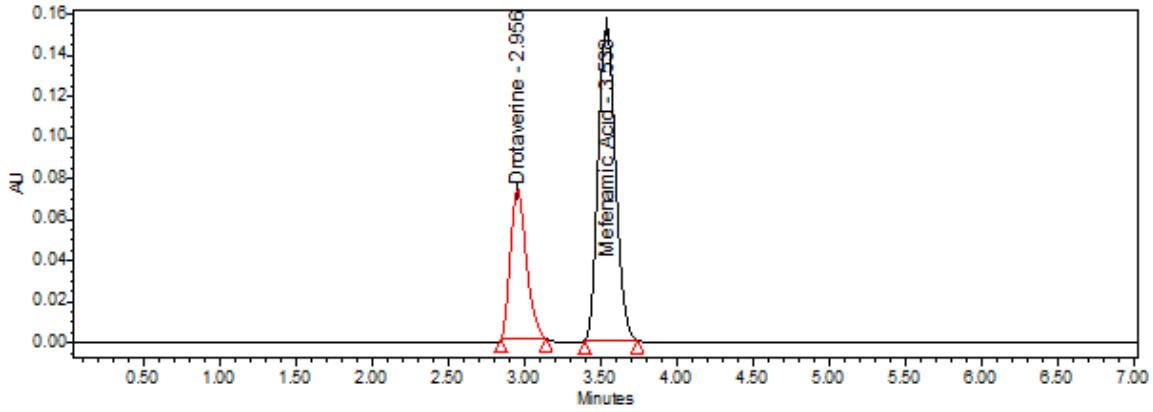


Figure 2: System Suitability Chromatogram of Dtrotaverine and Mefenamic Acid (Standard)

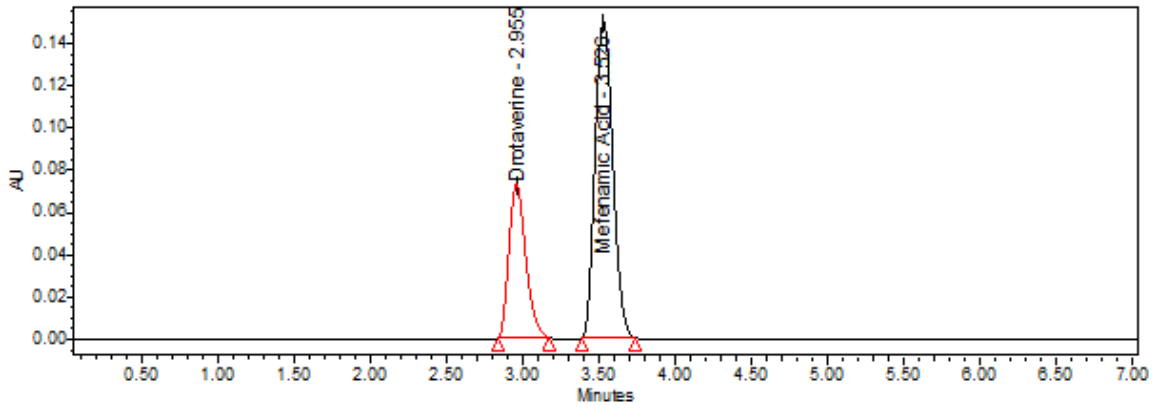


Figure 3: Standard Chromatogram

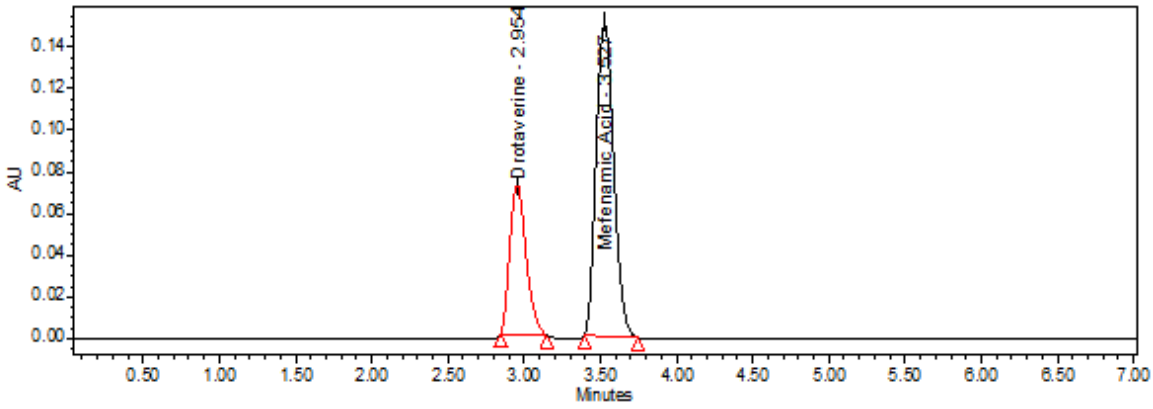


Figure 4: Chromatogram of Sample

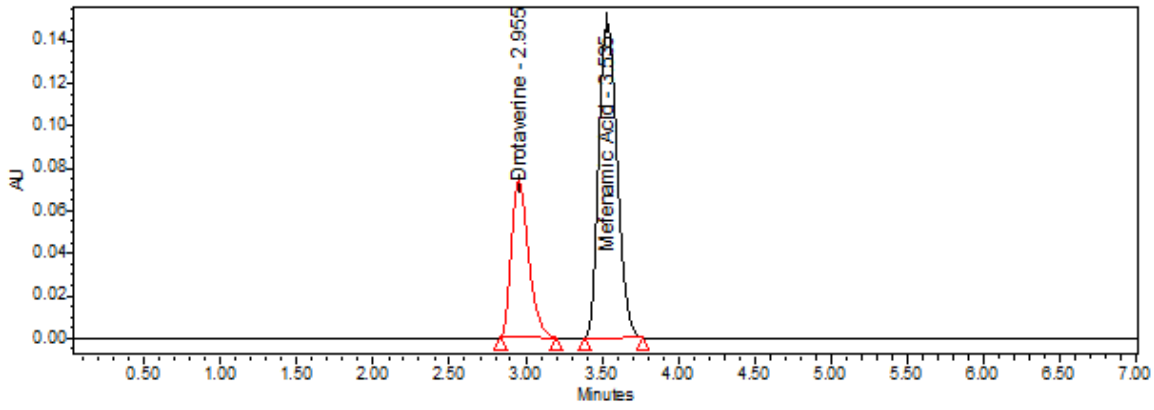


Figure 5: Repeatability Chromatogram

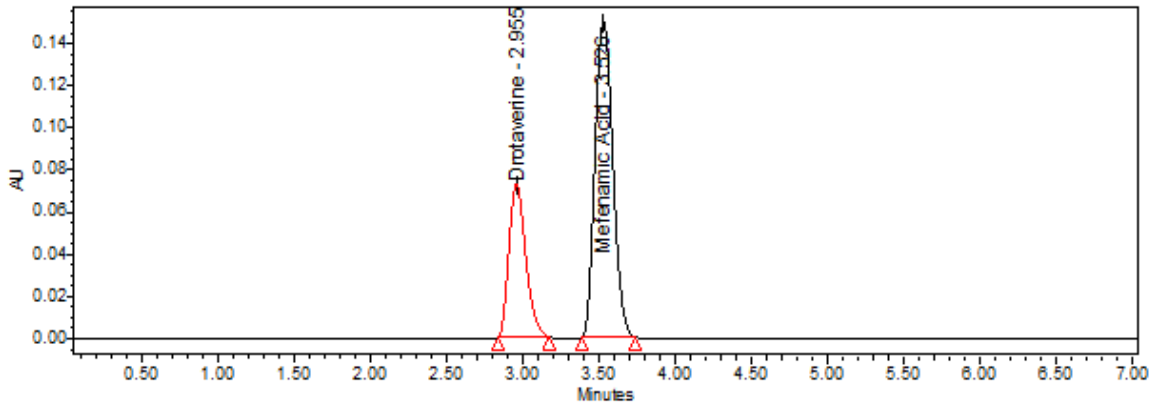


Figure 6: Intermediate precision chromatogram

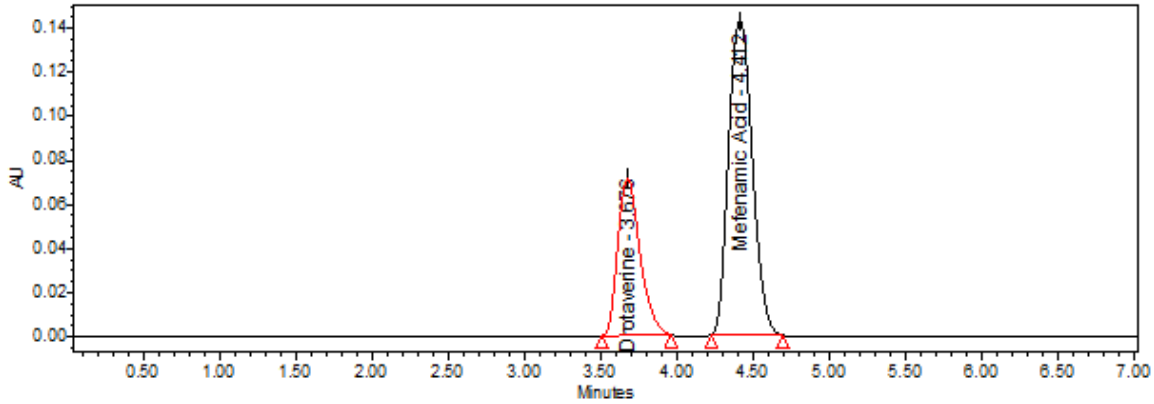


Figure 7: Impact of flow rate variability (range 0.8 ml / min)

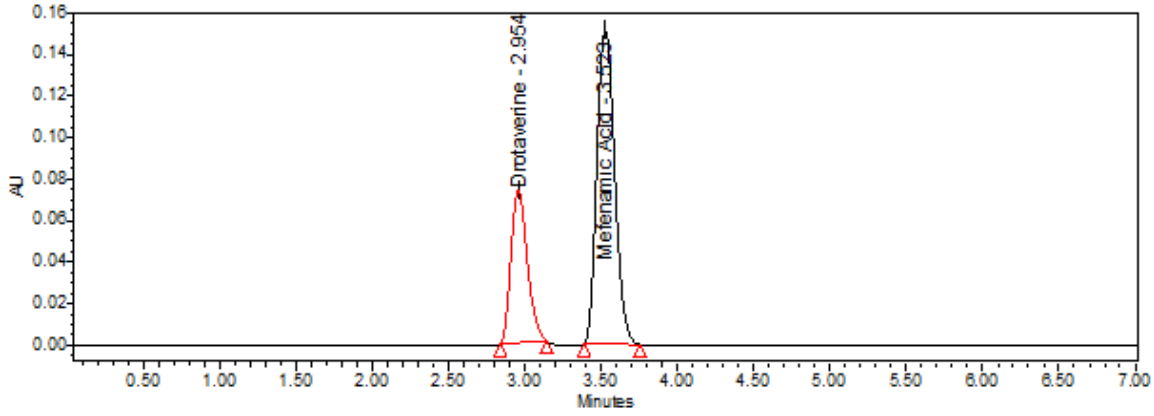


Figure 8: Impact of flow rate variability 1ml / min of chromatogram

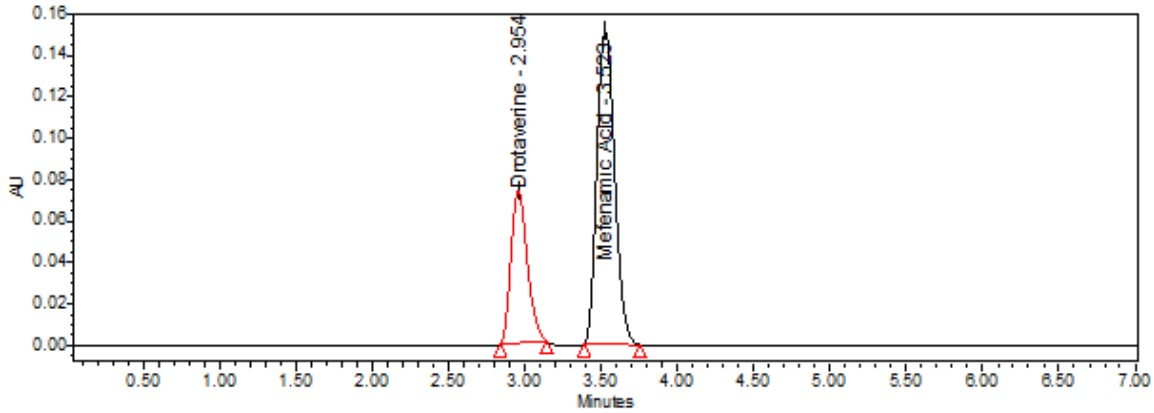


Figure 9: Impact of flow rate variability 1.2ml / min Chromatogram

was employed to establish the parameters such as tailing factors, theoretical plates, limit of detection and limit of quantification were give good results and good valuses as well as meet the standard chromatogram. Robustness of the proposed method was estimated by changing the flow rate from 1ml to 0.8 ml/min and 1.2 ml/min, The parameters of suitability for the device were tested and found to be within the 1.0ml / min and 1.2ml / min flow limits. Drotaverineand Mefenamic Acid were resolved from all other peaks and the retention times were comparable to those obtained for the 1.0ml / min mobile process. indicating that the test method was robust for all variable conditions. Hence the method was sufficiently robust for normally expected variations in chromatographic conditions. Limit of detection (LOD) and quantification (LOQ), the limits of detection and quantification were calculated by the method based on the standard deviation (σ) and the slope (S) of the calibration plot, using the formulae LOD = 3.3σ/s LOQ=10 σ/s the LOQ value of both drugs are 1.58 and 1.48 and the LOD value of both drugs are 0.522 And 0.491.The specificity test of the proposed method demonstrated that the excipients from tablets do not interfere in the drug peak. Further more, well shaped peaks indicate the specificity of the method.

Table 10: LOQ and LOD

Drotaverine		Mefenamic acid	
LOQ	1.58	**LOQ**	1.48
LOD	0.522	**LOD**	0.491

CONCLUSION

The empirical method was developed by analyzing diverse parameters. First of all, maximum absorbance was observed at 226 nm for Drotaverine, and 280 nm for Mefenamic Acid. Typical wavelength would be 280 nm, and peak purity was superb. Injection volume was selected as 20µl which gave a good peak area. Inertsil C18, a nice peak type chosen by ODS, was the column used for analysis. Ambient temperature has been found to match the essence of a drug solution. The flow rate was set at 1.0ml / min due to good peak range, sufficient hold time and good resolution. Different mobile phase ratios were tested, 80:20 Mobile phase ratio methanol : Acetonitrile was set because of good symmetric peaks and good resolution.The new recovery over the same range as 98.0-101.50 was found to be linear and reliable. It has been found that accuracy of both the tool and the procedure is accurate and within range. Detection limit of Drotaverine is 0.522 and 0.491 for Mefenamic acid. The linearity test was, it was found to be correlation coefficient and curve fitting. The analytical method observed linearity of both the drugs over the target concentration range of 20-80ppm. The analytical has passed sturdiness as well as robustness tests. For both cases the relative standard deviation was very satisfying.

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