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Development and Validation of Simultaneous RP-HPLC Method for Estimation of DROTAVERINE HCl and MEFENAMIC Acid in bulk and Pharmaceutical Formulations

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ABSTRACT

A simple, specific, accurate and precise RP HPLC method has been developed for the simultaneous determination of Drotaverine HCl and Mefenamic Acid from combined dosage form by reverse phase C18 column (Younglin (S.K) Gradient System UV (250mm x 4.6mm) 5µ). The sample was analysed using Methanol : Water in the ratio of 80:20(0.1% triethylamine at pH 3) as a mobile phase at a flow rate of 1.0ml/min and detection at 250nm. The retention time for Drotaverine HCl and Mefenamic Acid was found to be 5.500 min and 9.550 min respectively. The stability assay was performed for this combination and was validated for accuracy, precision, linearity, specificity and sensitivity in accordance with ICH guidelines. Validation revealed the method is specific, rapid, accurate, precise, reliable, and reproducible. Calibration plots were linear over the2-10 µg/mL for Drotaverine Hcl and 6-30 µg/mL for Mefenamic Acid, respectively, and recoveries from combined dosage form were between 98 and 102%. The method can be used for estimation of combination of these drugs in combined dosage form.

Keywords: *Drotaverine Hydrochloride , Mefenamic Acid , RP-HPLC.*

INTRODUCTION

Drotaverine Hcl [Figure 1] is chemically known as 1-(3,4-diethoxybenzylidene)-6,7-diethoxy- 1,2, 3,4-tetrahydroisoquinoline. Drotaverine hydro chloride is a highly potent spasmolytic agent. It acts as an antispasmodic agent by inhibiting the phosphodiesterase IV enzyme, specific for smooth muscle spasms and pain, and used to reduce excessive labor pains.

Mefenamic acid [N-(2,3-xylyl)anthranilic acid] is an Aminobenzoate, a subclass of analgesic with Non steroidal anti-Inflammatory properties1. It acts by binding the prostaglandin synthetase receptors COX-1 and COX-2, inhibiting the action of prostaglandin synthetase2, 3. It is used for the treatment of rheumatoid arthritis, osteoarthritis, dysmenorrhea, and mild to moderate pain, inflammation, and fever4.

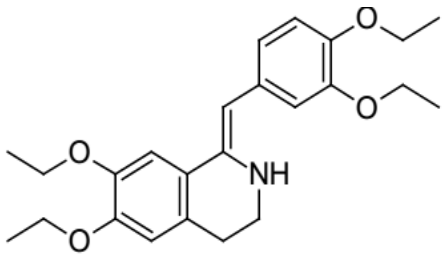

Figure 1: Chemical structure of Drotaverine Hcl{ (1Z)-1-(3,4-Diethoxybenzylidene)-6,7-diethoxy-1,2,3,4- tetrahydroisoquinoline. }

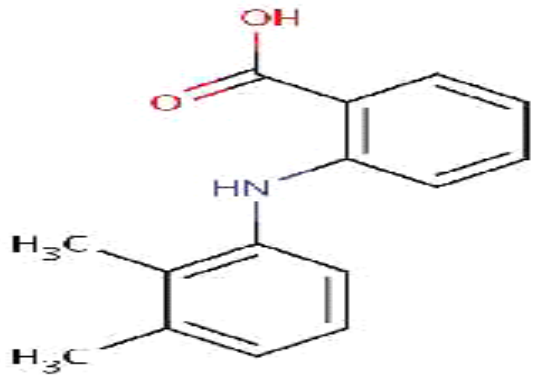

Figure 2: Chemical structure of Mefenamic Acid [N-(2,3-xylyl)anthranilic acid]

Experimentals

Instrument

A High Performance Liquid Chromatograph-system, the purity determination performed on a stainles ssteel column 250mm long, 4.6mm internal diameter filled with Octadecyl silane chemically bonded to porous silica particles of 5µm diameter reverse phase C18 column (*Younglin (S.K) Gradient System UV* (250mm x 4.6mm) 5µ). Optimized chromatographic conditions are listed in Table 1.

MATERIALS AND CHEMICALS

Drota HCl supplied as a gift sample by Wockheardt Pharamceutical Ltd, Aurangabad MA Avestia Pharma Ltd. Kandivali East. All the chemicals used of HPLC Grade (Merk Ltd., Mumbai) and double distilled water was used for mobile phase preparation.

Preparation of Standard Stock Solution Solution A:.Weigh accurately about 10 mg of Drotaverine HCl working standard in a 10.0 ml volumetric flask. Dissolve and dilute up to mark with diluent. Solution B: Weigh accurately about 30.0 mg of Mefenamic Acid working standard in a 10.0 ml volumetric flask. Dissolve and dilute up to mark with diluent and that give concentration 400 and 1200 µg/mL for Drota HCl and MA respectively.

Mixtured Standard Preparation From the standard stock solution, the mixed standard solutions were prepared using Methanol to contain 10µg/mL of Drota HCl and 30µg/mL of MA.

Preparation of test sample from Sample Stock Preparation: Take 10 µgm/ml+ 30 µgm/ml sample for assay (0.25 ml from tab stock and makeup 10 ml with 0.25 ml from tab stock and makeup 10 ml with mobile phase.

Selection of analytical wavelength

Each solution was scanned using double beam UV visible spectrophotometer in the spectrum mode

between the wavelength range of 400 nm to 200 nm and their spectra was overlaid. The wavelength selected was 250 nm**.**

Selection of detection wavelength: UV detector was selected, as it is reliable and easy to set at constant wavelength. A fix concentration of analyte were analysed at different wavelengths. As per the response of analyte, 250 nm was selected

Linearity Study: From the standard stock solution of Drota HCl and MA 0.25 mL were taken in 10 mL volumetric flask diluted up to the with Methanol such that final concentration of Drota HCl and MA in the range 2-10 µg/mL of Drota HCl and 6-30 µg/mL of MA respectively. Volume of 20µl of each sample was injected with the help of Hamilton Syringe. All measurements were repeated five times for each concentration and calibration curve was constructed by plotting the peak area versus the drug concentration.

Method Validation: The proposed method was validated as per ICH guidelines. The solutions of the drugs were prepared as per the earlier adopted procedure given in the experiment.

Accuracy: It was done by recovery study using standard addition method at 80%, 100% and 120% level; known amount of Drorta HCl and MA standard was added to preanalysed sample and subjected to the proposed HPLC method.

Precision: Precision of the method was studied as intra-day and inter- day variation and also repeatability of sample injections. Intra- day precision was determined by analyzing, the three different concentration 4 µg/mL, 6 µg/mL and 8µg/mL of Drota HCl and 12µg/mL, 18 µg/mL and 24 µg/mL of MA respectively, for three times in the same day. Inter day variability was assessed using above mentioned three concentration analysed on two different days, over a period of one week.

Repeatability: It was performed by injecting sample 10µg/mL of Drota HCl and 30 µg/mL of MA into the system and measuring the peak area. It was repeated for six times.

Ruggedness: Ruggedness of the method was studied by two different analyst using same operational and environmental condition. An appropriate concentration 10µg/mL of Drota HCl and 30 µg/mL of MA was analysed and concentration were determined. The procedure was repeated for six times.

Robustness: Robustness of the method was studied by making deliberate variation in parameters such as flow rate (± 0.1mL), % of Methanol in the mobile phase composition (± 10%), and change in detection wavelength (±2 nm) and the effect on the results were examined. It was performed using 10µg/mL and 30 µg/mL solution of Drota HCl and MA in triplicate.

Limit of Detection and Limit of Quantification

The limit of detection (LOD) and the limit of quantification (LOQ) for Drotaverine HCl and Mefenamic Acid were determined from standard deviation of the response and the slope.

$$\mathbf{LOD = \sigma/S \times 3.3;}$$

$$\mathbf{LOQ = \sigma/S \times 10}$$

RESULTS AND DISCUSSION

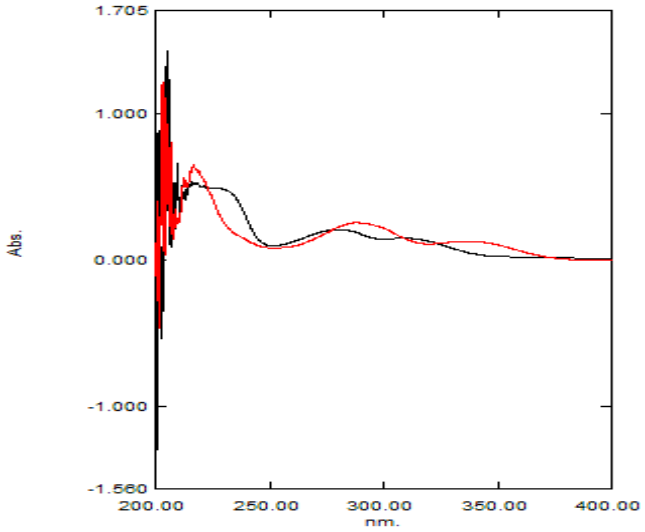

Fig.3 Overlain spectra of Drotaverine HCl and Mefenamic Acid

HPLC Method Development and Optimization: The finally optimized chromatographic conditions are.

Mobila Phase	Mixture of Methanol & HPLC Grade water (0.1%Triethylamine)in the ratio of 80:20%v/v
Column	Hi Q C 18 W,4.6*250mm,5
Flow Rate	1.0ml/min
Injection Volume	20µl
Column Oven Temp	Ambient

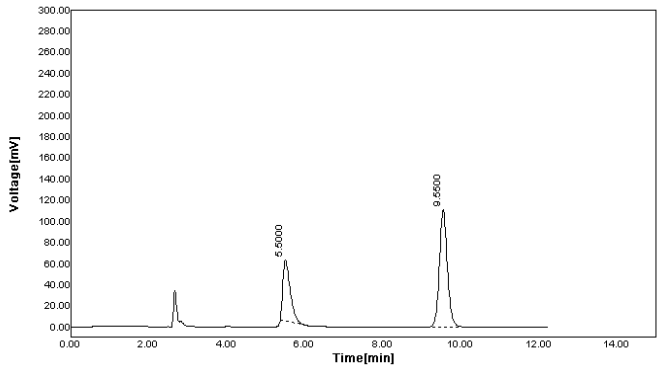

Figure 4: optimized chromatogram of Drota HCl and MA.

2. Linearity:

Table 1: Linearity studies of Drota HCl

Concentration of Drota HCl [µg/mL]	Peak Area	± SD	%RSD
2	85.14	0.76	0.89
4	171.76	1.33	0.77
6	259.25	3.36	1.30
8	322.43	0.66	0.20
10	415.65	1.71	0.41

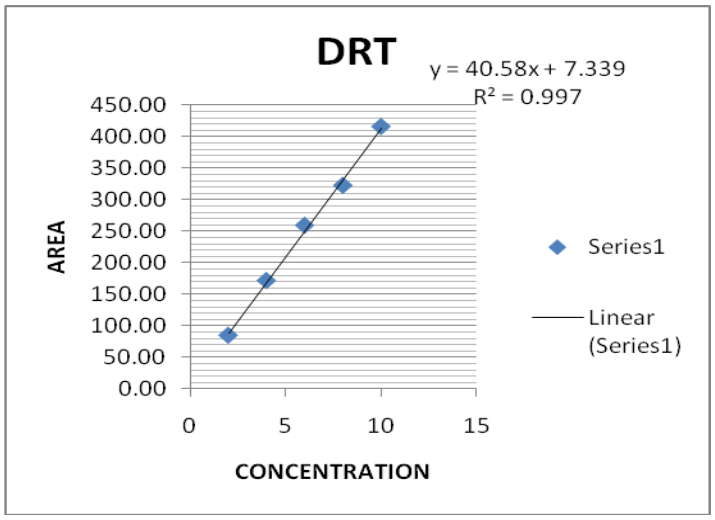

Figure 5: Linearity studies of Drota HCl

Table 2: Linearity studies of MA

Concentration of MA[µg/mL]	Peak Area	± SD	%RSD
6	195.85	0.76	0.39
12	373.94	0.49	0.13
18	527.52	0.92	1.31
24	727.75	5.74	0.79
30	872.15	7.81	0.90

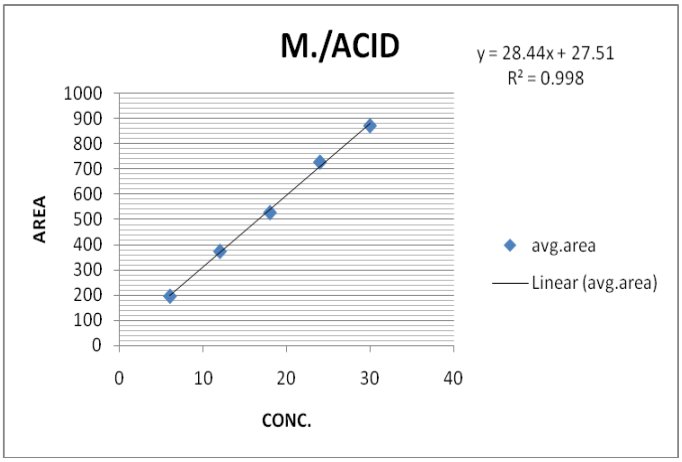

Figure 6: Linearity studies of MA

Analysis of marketed formulation

Brand Name: DROTIN-M (MARTIN AND HARRIES LAB.LTD. (HARIDAWAR)

Total weight of 20 tab wt. . = 11.80 Gms

Avgr Weight = 0.590.2 Gms./Tab

Eq.wt for 30 mg= 30 X 590.2 /250 =70.824 mg

Method Validation:

Accuracy:

It was ascertained by recovery studies based on standard addition method at level of 80%, 100%, 120%. The average percentage recoveries for Drota HCl was found to be 103.30, 104.35, 103.38 and for MA it was found to be 98.42, 96.04, 100.35. which was in good agreement with labeled amount of Pharmaceutical formulation.

Table 4: Recovery studies of Drota HCl

Label claim (mg/Tab)	Amount Added (mg)	Total Amount	Amount Recovered(mg)	% RSD	% Recovery
10	3.6(80%)	103.30	1.33	0.64	1.47
10	4(100%)	104.35	0.68	0.35	0.56
10	4.4(120%)	103.38	1.09	0.58	1.20

Table 5: Recovery studies of MA

Label claim (mg/Tab)	Amount Added (mg)	Total Amount	Amount Recovered(mg)	% RSD	% Recovery
30	9.6(80%)	98.42	6.56	0.56	1.31
30	12(100%)	96.04	0.37	0.18	0.38
30	14.4(120%)	100.35	1.13	0.61	1.13

Precision: Precision of the method was studied as intra-day and inter- day variation and also repeatability of sample injections. Intra- day precision was determined by analyzing, the three different concentration 4µg/mL,6 µg/mL and 8µg/mL of Drota HCl and 12 µg/mL, 18 µg/mL and 24 µg/mL of MA respectively, for three times in the same day. Inter day variability was assessed using above mentioned three concentration analysed on three different days, over a period of oF one week.

Table 6: Recovery studies of Drota HCL

Conc(mg/ml)	Intra-day Amount found(mg/ml)			Inter-day Amount Found(mg/ml)		
	Mean	+SD n=3	%RSD	Mean	+SD=3	%RSD
4	159.67	0.63	0.39	165.91	3.12	1.88
6	156.28	2.79	1.79	247.86	2.89	1.17
8	154.10	1.27	0.83	329.71	1.53	1.46

Table 7: Recovery studies of MA

Conc(mg/ml)	Intra-day Amount found(mg/ml)			Inter-day Amount Found(mg/ml)		
	Mean	+SD n=3	%RSD	Mean	+SD=3	%RSD
12	366.62	1.32	0.36	367.02	1.90	0.52
18	363.41	1.12	0.31	531.10	2.39	0.45
24	363.17	0.08	0.02	739.45	4.57	0.62

Repeatability:

Table 8: Repeatability studies on Drota HCl

Concentration of Drota HCl (mg/ml)	Peak Area	Amount found	%Amount found
6	242.25	14.96	99.76
6	239.52	14.97	249.50
6	245.35	15.26	254.33
	Mean	15.06	201.20
	SD	0.17	87.88
	%RSD	0.13	43.68

Table 9: Repeatability studies on MA

Concentration Of MA(mg/ml)	Peak Area	Amount found	%Amount found
18	529.96	17.66	99.76
18	532.71	17.76	98.67
18	527.15	17.58	97.67
	Mean	17.67	98.70
	SD	0.09	1.05
	%RSD	0.51	1.06

Ruggedness: Ruggedness of the method was studied by two different analyst using same operational and environmental condition. An appropriate concentration 10.0µg/mL of Drota HCl and 30.0µg/mL of MA was analysed and concentration were determined.

Table 10: Ruggedness studies on Drota HCl

Condition	Mean	± SD n=3	%RSD
Analyst1	169.17	1.49	0.88
Analyst2	243.66	2.05	0.84

Table 11: Ruggedness studies on MA

Condition	Mean	± SD n=3	%RSD
Analyst1	364.95	1.03	0.28
Analyst2	359.61	0.71	0.20

Robustness:

Table 12: Robustness studies on Drota HCl

Condition	Mean	± SD n=3	%RSD
Change in flow rate(±0.1ml)	201.54	1.07	0.53
Change in wavelength(±2nm)	209.48	1.61	0.77
Change in mobile phase	221.73	1.01	0.46

Table 13: Robustness studies on MA

Condition	Mean	± SD n=3	%RSD
Change in flow rate(±0.1ml)	267.35	0.30	0.11
Change in wavelength(±2nm)	234.40	1.44	0.56
Change in mobile phase	256.56	1.53	0.65

Limit of detection (LOD)

LOD is calculated from the formula = 3.3 σ /S

σ = Standard deviation of the response, S= slope of the calibration curve

Drotaverine HCl =0.2

Mefenamic Acid =0.34

Limit of quantification (LOQ)

LOQ is calculated from the formula = 10 σ / S

σ = Standard deviation of the response, S= slope of the calibration curve

Drotaverine HCl=0.61

Mefenamic Acid=0.01

CONCLUSION

The developed HPLC technique is precise, specific, accurate analysis proves that the method is suitable for the analysis of both Drotaverine HCl and Mefenamic Acid in bulk and pharmaceutical formulation without any interference from the excipients. The method has been found to be better than previously reported methods, because of use of a less economical and readily available mobile phase, lack of extraction procedures, no internal standard, and use of the same mobile phase for washing of the column. All these factors make this method suitable for quantification of Drotaverine HCl and Mefenamic Acid in bulk drugs and in pharmaceutical dosage forms.

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