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Development and validation of RP-HPLC and UV-Spectrophotometric method for Mefanamic acid and Drotaverin hydrochloride in combined dosage form

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ABSTRACT

*A reverse phase-high performance Liquid chromatography method is a simple, accurate, precise and reproducible one. UV-Spectrophotometric simultaneous equation method is adopted by official compendia for the stable substance that have reasonably broad absorption bands and which are practically unaffected by the variations of Instrumental parameters. The use of standard **A** (1%1cm) value avoids the need to prepare a standard solution of the reference substance in order to determine absorptivity. A reverse phase high performance liquid chromatography method has been developed for the simultaneous estimation of Drotaverine and Mefanamic acid in tablet dosage form using C18 column(LC 20 AT Isocratic) in Isocratic mode. The mobile phase consisted of Acetonitrile, methanol and 20 µl phosphate buffer adjusted to PH 3.5 in ratio of 50:15:35 v/v with ultraviolet visible detection at 240 nm. The method was linear over the concentration range for Mefanamic acid 0.5-50µg/ml and for Drotaverine 0.5-50µg/ml. The mean recovery was found to be in the range of 98% to 102%. The Validation method was carried out using International Conference on Hormonisation Guidelines. The described RP-HPLC method was successfully employed for the analysis of Pharmaceutical formulations containing combined dosage form.*

Key words: Simultaneous estimation, RP-HPLC, Mefanamic acid, Drotaverine, ICH-guidelines.

INTRODUCTION

Mefanamic acid{Dimethyl phenyl[amino]benzoic acid} is a N-(2,3-xylyl) anthranilic acid derivative.(STRUCTURE-1a) with the improved analgesic and is used for relief the pain and inflammation in rheumatoid arthritis. Drotaverine is chemically {1,2,3,4-tetrahydra-6,7-diethoxy-1-([3,4-diethoxy phenyl] methylene)iso quinoline hydrochloride}(STRUCTURE-1b) is Antispasmodic effect directly on the smooth muscles. A combination of these drugs containing

Mefanamic acid and Drotaverine hydrochloride is commercially available and more effective and had a high safety profile in the treatment of Analgesic and Antispasmodic.

Literature review revealed that various analytical methods like High Performance Thin Layer Chromatography, HPLC and Spectrophotometric methods were reported for the determination of Mefanamic acid and Drotaverine from their formulations individually and in combination with other drugs . The literature review indicates that no method is yet reported for the simultaneous estimation of both drugs in combination. This prompted us to develop a simple, accurate, precise and sensitive simultaneous estimation of mefanamic acid and Drotaverine by RP-HPLC and spectrophotometric methods. The method was validated as per ICH-guidelines.

MATERIALS AND METHODS

Drugs and Chemicals

The Pharmaceutical grade pure samples of mefanamic acid(99.26%) and Drotaverine(99.65%) was supplied by DR.CEEL ANALYTICAL LAB, CHENNAI, INDIA. acetonitrile , methanol HPLC-grade solvents and all analytical grade solvents obtained from E-merk limited, Mumbai, India. Potassium dihydrogen orthopasphate analytical grade reagent was procured from Qualigens fine chemicals, Mumbai, India. The HPLC-grade water was obtained from a Milli-Q water purification system.

HPLC- apparatus and condition

The separation was performed by using inerestic C18 (250×4.6mm,5µm) column on a shimadzu LC 20 AT Isocratic solvent delivery system. Shimadzu SPD-10A dual wavelength absorbance detector and Rheodyne injector with 20mM phosphate buffer (adjusted to PH-4)and Acetonitrile: methanol in ratio of (50:15:35v/v)were used. The mobile phase was freshly prepared, filtered, sonicated before use and delivered at a flow rate of 1.5 ml/min and the detector wave length was set at 240 nm. The injection volume was 20µl(fixed loop).

Stock solution and standard

Standard stock solutions were prepared for 1000µg/ml by using mefanamic acid and Drotaverine separately by using mobile phase. From the standard stock solution different concentrations of working standard were prepared from the range of 200 to300µg/ml for Mefanamic acid and 64-96µg/ml for Drotaverine.

Calibration Curve

The calibration curve were constructed for the determination of the linearity and the curves were plotted with the concentration range verses area must obey the Beer's law. The linearity was evaluated by the analysis of serially diluted sample in the range of 64-96µg/ml for Drotaverine and 200-300µg/ml for Mefanamic acid. An aliquot was injected by using mixture of 20mM Phasphate buffer: Acetonitrile: Methanol (50:15:35v/v). The 20µl mixture was injected for the estimation under the optimized chromatographic conditions. The typical chromatogram was recorded for standard as shown in figure-1. The retention time of standard Mefanamic acid and Drotaverine found to be 2.9 min and 6.6 min respectively with a good resolution.

Analysis of Formulation

Twenty tablets were weighed and finely powdered. A quantity equivalent to 250 mg Mefanamic acid and 79.96 mg Drotaverine were transferred in to 100ml volumetric flask and dissolved on about 50ml mobile phase. The solution was ultra sonicated for 10 minutes and filtered through 0.45µ nylon membrane and degassed and the volume was made upto the mark with same system.Above solution was taken to prepare a dilution of 80µg/ml Drotaverine and 250µg/ml mefanamic acid. The amount of drug was determined and three replicate injections were done.

RESULT AND DISCUSSION

Method Development

Several tests were performed in order to get satisfactory separation and the resolution of mefanamic acid and Drotaverine in different mobile phases with various ratios of organic phase and buffers by using C18 column. The ideal buffer was 20mM phosphate buffer (PH-4): Acetonitrile: Methanol in ratio (50:15:35v/v) by Isocratic elution to obtain satisfactory and good resolution. The changes in PH of mobile phase by ±0.2 does not shows any significant change in retention time of each analyte. The retention time of Mefanamic acid and Drotaverine on analytical column was evaluated at the flow rate of 1.0 ml/min and the Injection volume was 20µl. The retentions time of standard and sample for Mefanamic acid and Drotaverine were satisfactory with good resolution.

Linearity

The linearity for HPLC method was determined at five concentration levels. The linearity of Mefanamic acid and Drotaverine were determined by calibration curves and the linearity based on the area observed in the range of 64-96µg/ml for Drotaverine and 200-300µg/ml for Mefanamic acid. The % relative standard deviation (%RSD) of peak area and the retention time was within the limit of ±0.2%. This indicates that, the method was system suitable. The regression co-efficient value (r^2) for Mefanamic acid and Drotaverine is 0.9999 and 0.9998 respectively . The reports are tabulated in Table-1.

Table-1. System suitability parameters

PARAMETERS	MEFANAMIC ACID	DROTAVERINE
Calibration range(µg/ml)	200 - 300	64 - 96
Correlation Co-efficient(r2)	0.9999	0.9998
Retention time(min)	2.9±0.2	6.6±0.2
Resolution	6.2	6.2
Repeatability(%RSD)(n=5)	0.272%	1.169%
Theoretical plates	12,082.1306	8,361.6466
Tailing factor	1.00	1.00
Limit of quantification(µg/ml)	250µg/ml	80µg/ml

Precision

Precision was measured for both inter and intra-day and checked with repeatability and the %RSD for the repeatability was found to be 0.272% and 1.169% respectively for Mefanamic acid and Drotaverine. The % RSD was found within the limit and was tabulated in Table-1. The limit of quantification was determined by injecting minimum concentration of the drugs. The

limit of quantification was found to be 80µg/ml for Drotaverine and 250µg/ml for Mefanamic acid.

Recovery Studies

The assay procedure was repeated for standard and sample in five times and mean peak area ratio and concentration of drugs were calculated. The percentage of individual drugs found in formulation, mean and % RSD in formulation were calculated and shown in Table-2. Recovery studies carried out for both drugs. It is usually done by adding 80%, 100%, and 120% of the pure drug with the formulation taken for analysis. The average % recovery for Mefanamic acid and Drotaverine was found to be 99.80% and 99.95% respectively. The results were represented in Table-3.

Specificity and selectivity

Specificity was tested against standard compounds and potential interferences. To determine specificity with respect to sample compounds the response of standard and sample solution were compared. No interferences were detected at the retention time of either Mefanamic acid or Drotaverine in sample solution. The limit of detection (LOD) was determined at lowest concentration giving response and limit of quantification was determined at the lowest concentration. The limit of detection(LOD) for mefanamic acid and Drotaverine was found to be 200µg/ml and 64µg/ml respectively. The limit of quantification(LOQ) was 80µg/ml for Drotaverine and 250µg/ml for Mefanamic acid and was given in Table-1.

Stability

In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analysed over a period of 24 hours at room temperature. The results found for both solutions. The retention time and peak area of Mefanamic acid and Drotaverine remains almost similar (% RSD less then 3.0) and significant degradation within the indicated period, this indicates that both solutions were sufficient to complete the whole analytical process.

Table-2 Analysis of marketed formulations

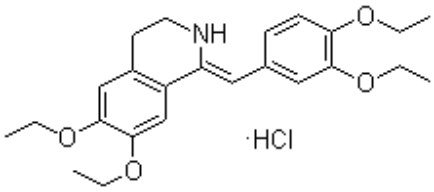
Formulation	Mefanamic Acid			Drotaverine Hydrochloride		
	Label Claim (Mg/Tab)	Amount Found * (mg/tab±RSD)	% Assay±RSD	Label Claim (mg/tab)	Amount Found* (mg/tab ± RSD)	% Assay ±RSD
ASMR	250	249.36±0.352	99.8±0.654	80	79.96±0.958	99.95±0.963

**Stands for the average reading taken in three reading*

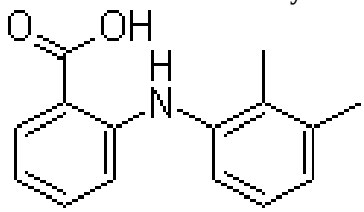
Table-3 Recovery studies of mefanamic acid and drotaverine hydrochloride in combined dosage form

Formulation	Mefanamic acid.			Drotaverine hydrochloride.		
	% added	%recovered*±RSD	%recovery ±RSD	%added	%recovered*±RSD	% recovery ±RSD
Brand. ASMR	80	0.0995	99.80±0.016	80	0.3213	99.90 ±0.380
	100	0.1892	99.80±0.012	100	0.1219	99.95±0.010
	120	0.09	99.83±0.064	120	0.1928	99.90±0.210

**Recovery experiment data for Mefanamic acid and Drotaverine hydrochloride showing the amount of drug recovered from sample solution at each level(n=3), percentage recovery and the avarage percentage recovery.*



1(a): Structure of Drotaverine Hydrochloride.



1(b): Structure of Mefanamic acid

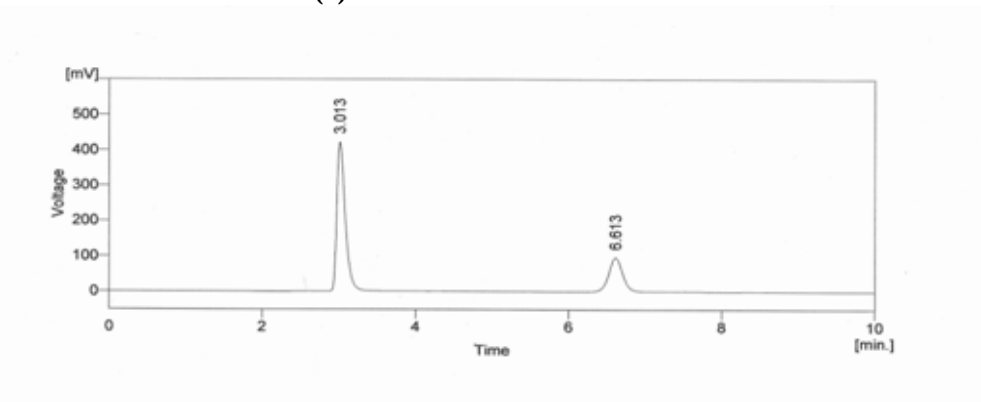


Fig 1: A Typical Chromatogram for Drotaverine hydrochloride and Mefanamic acid.

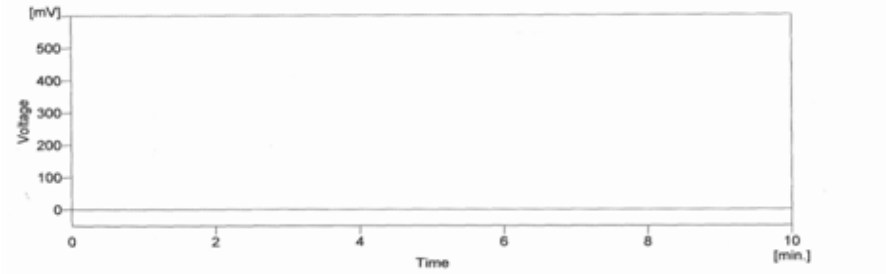


Fig 2(a): Blank

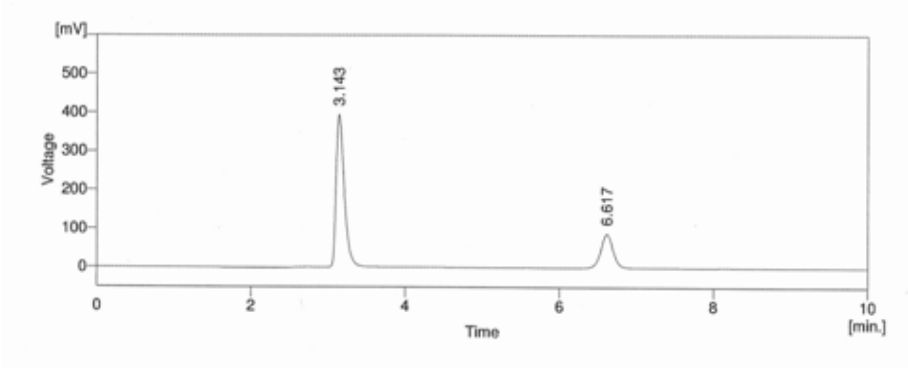


Fig 2(b): Linearity at 110% level.

Ruggedness and Robustness

Ruggedness test was determined by different analyst in different days using similar operational environmental conditions. Robustness of the method was determined by changing the wavelength and flow rate. The content of the drug was not adversely affected by these changes as evident from the low value of relative standard deviation indicating that the method was rugged and robust.

CONCLUSION

Mefanamic acid and Drotaverine combined tablet dosage form was analysed by UV-Spectrophotometric simultaneous equation and reverse phase high performance liquid chromatography. On comparing these methods, RP-HPLC method was found to be more precise, accurate, rugged, robust, simple and rapid then UV-Spectrophotometric method and it is was suitable for the quality control of the raw meterials, formulations, dissolution studies and also for bioequivalence studies of the same formulations.

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