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Development and Validation of Stability Indicating Rp-Hplc Method for Simultaneous Estimation of Mefenamic Acid and Dicyclomine Hydrochloride in Bulk and in Pharmaceutical Solid Dosage Form

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Abstract:

A simple and sensitive stability indicating reversed phase High Performance Liquid Chromatography method was developed and validated for the simultaneous estimation of Mefenamic acid (MEF) and Dicyclomine hydrochloride (DCL) in bulk and in pharmaceutical solid dosage form. The column GLS-ODS C18 (250 x 4.6mm i.d. 5µm) having isocratic mode with mobile phase containing methanol was used. The flow rate was 1mL/min and the wavelength of detection was 263nm. The linearity of MEF and DCL were in the range of 25-125µg/ml and 100-500µg/ml respectively. The retention time of MEF and DCL were found to be 2.27min and 7.41min respectively. The percentage recoveries were found 99.999% and 99.993% of MEF and DCL respectively. The developed method was found to be accurate, precise and selective for simultaneous estimation of MEF and DCL in pharmaceutical solid dosage form (Tablet).

Keywords*:* Mefenamic acid, Dicyclomine hydrochloride, RP-HPLC, Stability studies

INTRODUCTION

Mefenamic acid (MEF) is chemically, 2-[(2,3-dimethylphenyl) amino] benzoic acid (Fig. 1). It is having non-steroidal anti-inflammatory properties (NSAID). It acts by binding with COX-1 and COX-2 receptors, inhibiting the action of prostaglandin synthetase. It is used in the treatment of osteoarthritis, rheumatoid arthritis, mild moderate pain, dysmenorrhea, inflammation and fever [1-2].

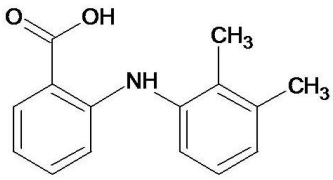

Fig. 1: Chemical Structure of Mefenamic acid

Dicyclomine hydrochloride (DCL) is chemically, 2-(diethyl amino) ethyl 1-cyclohexylcyclohexane -1-carboxylate (Fig 2). It is a muscarinic antagonist used as an antispasmodic and urinary incontinence. It does have antispasmodic properties and is used in biliary, gastrointestinal and urinary tract spasm [3].

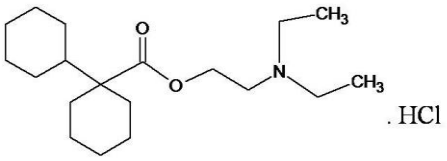

Fig. 2: Chemical Structure of Dicyclomine hydrochloride

Several analytical methods have been developed for simultaneous estimation of MEF and DCL in pharmaceutical formulations. Literature survey reveals that few analytical methods have been reported in combination like, spectrophotometry, HPLC and HPTLC. There is no stability indicating RP-HPLC method reported for the simultaneous estimation of MEF and DCL in pharmaceutical tablet dosage form. Hence attempts have been made to develop stability indicating RP-HPLC method for estimation of MEF and DCL in bulk and in tablet dosage form. The proposed method was validated according to ICH guidelines [4-14].

MATERIALS AND METHODS

HPLC instrument (Agilent Technology, Model series-1220), having variable wavelength UV detector, manual

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injector system, isocratic system, GLS, ODS-C18 column (250 x 4.6 mm, 5µ) was used. Chromatograms were obtained by EZ-Chrome software. Analytical balance (Anamed), and ultrasonic bath (HMG, India) were used for weighing and sonication respectively.

The samples of MEF and DCL were obtained from Alexo Chemicals (Thane) and Palam Pharma Pvt. Ltd. (Gujarat) respectively. The tablet dosage form containing MEF and DCL (MEFTAL-SPAS Manufactured by Blue Cross Ltd. Mumbai) purchased from local market. All chemicals and solvents were used HPLC grade and obtained from S.D. Fine Chemicals Ltd., Mumbai.

Chromatographic Conditions

Agilent Technology (1220) HPLC instrument equipped with variable wavelength UV detector, Rheodyne injector (20 µl capacity), GLS C18-ODS column (250 x 2.5mm, 5µ particle size) and EZ-Chrome software were used.

Preparation of Standard Solutions

Accurately weighed quantity of MEF (25 mg) and DCL (100 mg) were transferred each to a 100 ml clean dry volumetric flask and dissolved and diluted upto mark with methanol and resultant solutions were sonicated to obtain standard solutions having concentration 250µg/ml for MEF and 1000µg/ml for DCL.

Preparation of Sample Solutions

Twenty tablets were weighed accurately and crushed to form fine powder. Powder weighed equivalent to 2.5mg of MEF and 0.1mg (and 9.9mg of standard) of DCL each were dissolved in volumetric flask with methanol. It was sonicated followed by filtration process. The final concentration was made to 25µg/ml of MEF and 100µg/ml of DCL.

Methodology

To optimize the RP-HPLC parameters, several mobile phase compositions were tried. The satisfactory separation and better peak symmetry for MEF and DCL were obtained with a mobile phase methanol, at a flow rate 1ml/min to get better reproducibility and repeatability. The analysis was carried out at 263 nm based on peak area as both drugs shows better absorbance at this wavelength (Fig 3).

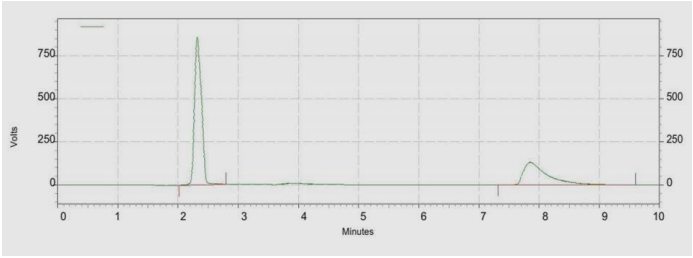

Fig. 3: Typical Chromatogram of MEF and DCL

VALIDATION OF PROPOSED METHOD

The proposed method was validated according to the ICH (international Conference on Harmonization) guidelines [13].

Linearity

Linearity was performed with five concentrations ranging from 25-125µg/ml and 100-500µg/ml for MEF and DCL respectively. The peak areas versus concentration of drug were plotted and a linear least square a regression analysis was conducted to determine the slope, intercept and correlation coefficient to demonstrate the linearity of method (Fig 4, 5 and Table 1).

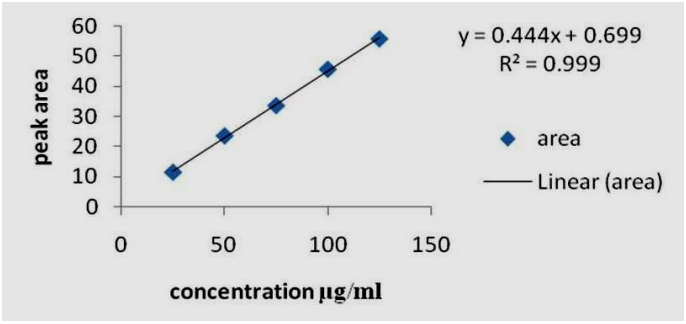

Fig. 4: Calibration Curve of MEF

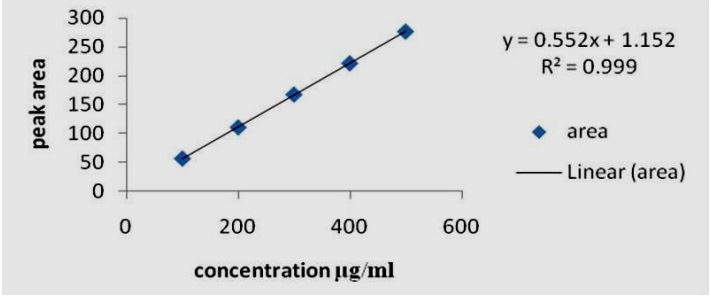

Fig. 5: Calibration Curve of DCL

Table 1: Linearity Study Data of MEF and DCL

Component	Linearity Range (µg/ml)	Slope	Intercept	Regression Coefficient
DCL	100-500	0.552	1.1526	0.9999
MEF	25-125	0.4443	0.6994	0.999

Accuracy (Recovery Study)

The accuracy of the method was determined by calculating recovery of MEF and DCL by the standard addition method. Known amounts of standard solutions of MEF and DCL (80, 100, 120 % level) were added to pre quantified sample solutions of MEF and DCL. The amounts of drugs were estimated by applying obtained values to the regression equation of the calibration value. The closeness of obtained results to the true results indicates that the proposed method is accurate (Table 2).

Precision (repeatability)

Intraday and interday precision was determined by injecting three standards solution of three different concentrations on the same day and interday precision was determined by injecting the same solutions for three conjugative days. The low % RSD (less than 2) value indicates that proposed method is precise.

Intermediate precision (reproducibility)

The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses three times on the same day and on three different days for same concentration of sample solutions that is 25µg/ml for MEF and 100µg/ml for DCL. The results were reported in terms of relative standard deviation (%RSD) (Table 3).

Table 2: Recovery study Data of MEF and DCL

Level of % Recovery	Mean*		Standard Deviation*		% R.S.D.*	
	DCL	MEF	DCL	MEF	DCL	MEF
80%	99.9999	99.9999	0.0061	0.1167	0.0061	0.1167
100%	100.0001	99.9999	0.0073	0.0955	0.0073	0.0955
120%	99.9999	99.9999	0.0294	0.0199	0.0294	0.0199

**Denotes average of 3 Determinations at each level*

Table 3: Inter-day and Intra-day Precision data of MEF and DCL

Statistical Parameters	MEF		DCL	
	Intra-day	Inter-day	Intra-day	Inter-day
	99.98	99.99	100.003	99.99
	99.99	99.99	99.99	99.99
	99.98	99.98	99.99	100.33
Avg.	99.98	99.98	100.001	100.11
SD	0.0057	0.0057	0.0021	0.1924
% RSD	0.0057	0.0057	0.0020	0.1921

Table 4: Forced Degradation Study data of MEF and DCL

Degradation Condition	% Degradation		% Assay	
	MEF	DCL	MEF	DCL
Acid (0.1 N Hcl)	11.32	3.58	88.67	96.41
Base (0.1 N NaOH)	2.84	4.30	97.15	95.69
Neutral (H2O)	1.68	3.56	98.31	96.43
Oxidative (3% H2O2)	1.07	3.62	98.92	96.37
Sunlight	2.75	3.89	97.24	96.10
Thermal	2.66	4.32	97.33	95.67

Table 5: Summery of Proposed Method

Parameters	Observations	
	MEF	DCL
Linearity Range (µg/ml)	25-125	100-500
Regression Equation	y=0.4443x + 0.6994	y=0.552x + 1.1526
Correlation Coefficient (r^2)	0.999	0.9999
LOD (µg/ml)	1.0832	0.0033
LOQ (µg/ml)	2.3498	0.0100
Retention Time (min)	2.273	7.410
Tailing Factor	0.8750	1.3333
% Recovery	99.99	99.99
Precision (% RSD)		
Intra-day (n=3)	0.0057	0.0020
Inter-day (n=3)	0.0057	0.1921
Repeatability (n=9)	1.4024	1.0024

Quantification Limit

The LOQ (Limit of Quantification) is the lowest concentration that can be quantified and the LOD (Limit of Detection) is the lowest concentration of the analyte that can be detected. The LOQ and LOD of MEF were found to be 2.34µg/ml and 1.08µg/ml and for DCL was 0.0100 µg/ml and 0.0033µg/ml respectively.

STABILITY STUDY (FORCE DEGRADATION STUDY)

Stability testing is an important part of the process of drug product development. The purpose of stability testing is to provide evidence of how the quality of a drug substance or drug product varies with time under a variety of environmental conditions (ICH Q1A-R2 Guidelines). This study involves acid degradation, alkali degradation, neutral degradation, thermal degradation, oxidative degradation and sunlight degradation [14].

Acid/Alkali/neutral degradation

To perform Acid/alkali/neutral degradations take 10ml stock solution of MEF and DCL separately in 100 ml volumetric flask then add 10ml of 0.1N Hcl/0.1N NaOH/Water and 50ml of methanol and refluxed for 4 hrs at 60°C , then cooled at room temperature and completed upto the mark with methanol.

Oxidative Degradation

To study effect of oxidizing condition, 5 ml of stock solution of MEF and DCL was added in 10 ml of 3% hydrogen peroxide solution and solution was refluxed for 4 hrs at 60°C. Then cooled at room temperature and final volume mark with methanol.

Thermal and Sunlight Degradation

Thermal degradation was carried out by exposing pure drugs to dry heat at 60°C for 4 hrs and samples were withdrawn at interval of 4 hrs. The samples after exposure to heat were prepared and diluted with methanol to get 100 µg/ml DCL and 25 µg/ml of MEF.

Similarly, for sunlight degradation pure drugs were exposed to sunlight for 6 hrs and samples were withdrawn at interval of 6 hrs. The samples after exposure to sunlight were diluted with methanol to get 100µg/ml DCL and 25µg/ml of MEF.

CONCLUSION

The proposed stability indicating RP-HPLC method for simultaneous estimation of Mefenamic acid and Dicyclomine hydrochloride in pharmaceutical solid dosage form was, simple, accurate, precise and economic. The method has several advantages including simple mobile phase, simple sample preparation and rapid analysis. The method can be used for routine analysis of marketed products of Mefenamic acid and Dicyclomine hydrochloride in combined pharmaceutical tablet dosage form.

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