SIMULTANEOUS ESTIMATION OF MEFENAMIC ACID AND DICYCLOMINE HYDROCHLORIDE BY RP-HPLC METHOD

DIPAL PRAJAPATI* AND DR. HASUMATI RAJ1.

*Research Scholar, Department of Quality Assurance, Shree Dhanvantary Pharmacy College, Kim-Kudsad Road, Kim, Surat, Gujarat-394110, India.
1 Associate Professor, Department of Quality Assurance, Shree Dhanvantary Pharmacy College, Kim-Kudsad Road, Kim, Surat, Gujarat-394110, India.

ABSTRACT

RP-HPLC method was developed in mobile phase containing Acetonitrile:Monobasic potassium dihydrogen phosphate (60:40,v/v) using C8 Luna (150 mm × 4.6 mm id, 5µm) at wavelength 215nm. The method was linear in the concentration range of 2-6µg/ml for DICY and 50-150µg/ml for MEF. The method was validated for linearity, accuracy and precision as per ICH guidelines. The developed and validated method was successfully used for the quantitative analysis of commercially available dosage form.

KEYWORDS: Mefenamic acid, Dicyclomine hydrochloride, Simultaneous estimation, RP-HPLC.

DIPAL PRAJAPATI
Research Scholar, Department of Quality Assurance, Shree Dhanvantary Pharmacy College, Kim-Kudsad Road, Kim, Surat, Gujarat-394110, India.

*Corresponding author
INTRODUCTION

Mefenamic acid \[N-(2,3-xylyl)anthranilic acid\] is an Aminobenzoate, a subclass of analgesic with Non steroidal anti-Inflammatory properties\(^1\). It acts by binding the prostaglandin synthetase receptors COX-1 and COX-2, inhibiting the action of prostaglandin synthetase\(^2\, 3\). It is used for the treatment of rheumatoid arthritis, osteoarthritis, dysmenorrhea, and mild to moderate pain, inflammation, and fever\(^4\). Dicyclomine (bicyclohexyl-1-carboxylic acid) is an antispasmodic and anticholinergic agent\(^5\). Its action is achieved via a dual mechanism: a specific anticholinergic effect at the acetylcholine-receptor sites, a direct effect upon smooth muscle\(^5\). It is used to treat a certain type of intestinal problem called irritable bowel syndrome. It helps to reduce the symptoms of stomach and intestinal cramping. This medication works by slowing the natural movements of the gut and by relaxing the muscles in the stomach and intestines\(^6\, 7\). Combination of Mefenamic Acid and Dicyclomine HCl has a Synergistic effect. This Combination is highly effective and used in the treatment of spasmodic dysmenorrhoea, intestinal colic, biliary colic, ureteric colic\(^8\). Mefenamic Acid is a medication that helps in reducing the pain in the body. Antipyretic is a drug that helps in reducing the fever by reducing the temperature of the body to the normal temperature. Anti-inflammatory is a kind of medication that is mainly for reducing the inflammation by controlling the pain. Lastly Dicyclomine HCl helps in offering relief to the cramps of the stomach, bladder and intestine\(^8\). Both the drugs are official in Indian pharmacopoeia 2010\(^11\), United State Pharmacopoeia\(^12\) and British Pharmacopoeia\(^13\).

Literature survey reveals that RP-HPLC\(^14\), Liquid Chromatography\(^15\), Voltametry\(^16\), UV-Visible Spectrophotometry\(^20\, 21\), methods were reported for the estimation of Mefenamic Acid alone or in combination with other drugs except Dicyclomine HCl and Capillary Gas-Chromatography\(^30\), Stability indicating gas-liquid chromatography\(^30\), HPTLC\(^31\) methods were reported for the estimation of Dicyclomine HCl alone or in combination with other drugs except Mefenamic Acid. As per literature survey, no analytical method has been reported for simultaneous estimation of Mefenamic Acid and Dicyclomine HCl in pharmaceutical dosage forms. Therefore the present research work, our aim is to develop a novel, simple, accurate, sensitive, reproducible, economical analytical method to estimate Mefenamic Acid & Dicyclomine HCl in their combined dosage form in routine analysis.
MATERIALS AND METHODS

Reagents and Chemicals
The bulk drug of MEF and DICY were obtained as gift sample from Shree Dhanvantary pharmaceutical analysis & research centre, (Kim, India) and Mercury labs Ltd., (Vadodara, India), respectively. The commercial fixed dose combination product MEFTAL-SPAS (Mefenamic acid 250 mg, Dicyclomine HCl 10 mg) was procured from the local market. The solvent used was Methanol AR Grade, HPLC grade Methanol (Merck Ltd., Mumbai, India), HPLC grade Acetonitrile (Finar Chemicals Ltd., Mumbai, India). The water for RP-HPLC was prepared by triple glass distillation and filtered through a nylon 0.45µm–47 mm membrane filter (Gelman Laboratory, Mumbai, India).

Instruments
A HPLC instrument (LC-2010CHT, Shimadzu, Japan) equipped with a UVVisible detector and a photodiode array detector, manual injector with 20 µL loop, Phenomenex (Torrance, CA) C8 column (150 mm × 4.6 mm id, 5 µm particle size) and CLASS-VP software were used. Triple distillation unit consisting of borosilicate glass, Digital pH meter (LI 612 pH analyzer, Elico Ltd., Ahmedabad), Analytical balance (Sartorius CD2250, Germany), Ultra sonic cleaner (Frontline FS 4, Mumbai, India).

PREPARATION OF STANDARD SOLUTION

Preparation of standard stock solution of MEF
Accurately weighed quantity of MEF 100 mg was transferred into 100 ml volumetric flask, dissolved and diluted up to mark with Methanol. This will give a stock solution having strength of 1000 µg/ml.

Preparation of working standard solution of MEF
1000 µg/ml of MEF solution was prepared by diluting stock solution to 10ml with mobile phase. This solution was diluted in mobile phase further to get the concentration range of 50, 75, 100, 125, 150 µg/ml of MEF.

Preparation of standard stock solution of DICY
Accurately weighed quantity of DICY 10 mg was transferred into 100 ml volumetric flask, dissolved and diluted up to mark with Methanol. This will give a stock solution having strength of 100 µg/ml.

Preparation of working standard solution of DICY
100 µg/ml of DICY solution was prepared by diluting stock solution in 10 ml with Mobile phase. This solution was diluted in mobile phase further to get the concentration range of 2, 3, 4, 5, 6 µg/ml of DICY.

Sample preparation
Twenty tablets were weighed and finely powdered. The average weight of tablets is determined with the help of weight of 20 tablets. A portion of powder equivalent to the weight of 125 mg of MEF was accurately weighed into 50 ml A-grade volumetric flask and 25 ml Methanol was added. The volumetric flask was sonicated for 15 min to effect complete dissolution of the DICY and MEF, the solution was then made up to volume with Mobile phase. The solution was filtered through Whatman filter paper. The aliquot portion of the filtrate was further diluted to get final concentration of 2µg/ml of DICY and 50 µg/ml of MEF.

Mobile phase preparation
2.7218gm Potassium Dihydrogen Ortho Phosphate was added to 1000 mL triple distilled water and adjust the pH 7.5 with 10%NaOH. Mixture of 0.02M phosphate buffer : ACN (60:40v/v) was used as mobile phase.

HPLC METHOD DEVELOPMENTS AND OPTIMIZATION
The standard solution of MEF and DICY was used for method development trials to optimize the method for simultaneous determination of MEF and DICY.

Selection of detection wavelength
The standard solution of MEF and DICY were scanned over the range of 200 nm to 400 nm wavelengths.
Selection of Mobile phase
The mobile phase was selected on the basis of best separation, peak purity index, peak symmetry, theoretical plate etc. So a no.of trials was taken for the selection of mobile phase as shown in Table.1. MEF and DICY are freely soluble in methanol. Acetonitrile was used to increase resolution. Monobasic Potassium Dihydrogen Phosphate was used to optimize retention time and decrease tailing. So several combination of Phosphate Buffer : ACN were tried as shown in Table.1.

METHOD VALIDATION
Validation Approach
Validation of analytical method shall be done to establish by laboratory studies, that the performance of the method meet the requirement for the intended analytical application.

Check for interference from blank
Diluent was used as blank. Standard and sample were prepared as per test procedure.

Linearity and range
Accurately measured standard working solutions of MEF (2,3,4,5 & 6 ml and DICY (0.2, 0.3, 0.4, 0.5 & 0.6 mL) were transferred to a series of 10 mL of volumetric flasks and diluted to the mark with mobile phase. Calibration curves were plotted over a concentration range of 50-150µg/mL for MEF and 2-6 µg/mL for DICY.

Accuracy (% recovery)
Accuracy of the method was calculated by recovery studies at three levels (80%,100% and 120%) by standard addition method. Twenty tablets were weighed and the average weight was calculated. The tablets were crushed to obtain fine powder. Tablet powder equivalent to 125 mg MEF was transferred to 50 ml volumetric flask. 25 ml Methanol was added. The volumetric flask was sonicated for 15 min to effect complete dissolution of the DICY and MEF, the solution was then made up to volume with Mobile phase. The solution was further diluted to get final concentration of 2µg/ml of DICY and 50 µg/ml of MEF. The % assay of the drugs was calculated

Limit of detection and Limit of quantification
The limit of detection (LOD) and limit of quantification (LOQ) of the drug were derived by visually method or calculating the signal-to-noise (i.e. 3.3 for LOD and 10 for LOQ) ratio using following equations designated by International Conference on Harmonization (ICH) guideline:
LOD = 3.3 X σ/S and LOQ = 10 X σ/S
Where, $\sigma$ = the standard deviation of the response, $S$ = slope of the calibration curve. In this method the limit of detection (LOD) and limit of quantification (LOQ) of the drug were derived by visual method.

**HPLC Parameters**
Stationary phase: Phenomenex C$_8$ column (150 mm x 4.6 mm i.d., 5 µm particle size) was used at ambient temperature
Mobile Phase: ACN : BUFFER(60:40v/v)
Flow rate: 0.7 mL/min
Injection volume: 20 µL
Detection: At 215 nm with PDA detector

**System suitability test**
Asymmetry of both the analytes’ peak in standard should not be more than 2.0,
Theoretical plates of both the analytes’ peak in standard should not be less than 2000,
Relative Standard Deviation for five replicates injections of both the standard preparation should not be more than 2.0%

**Procedure**
Injected diluent as a blank and recorded chromatogram.
Injected sample preparation for five replicates, recorded the chromatogram and % assay was calculated from regression equation.

**RESULTS AND DISCUSSION**

**HPLC method development and optimization:**

**Selection of detection wavelength**
Both drugs showed absorbance at 215 nm. So the wavelength selected for the determination of MEF and DICY was 215 nm.

**Selection of mobile phase**
The mobile phase was selected on the basis of I.P. best separation, peak purity index, peak symmetry, theoretical plate etc. So numbers of trials were taken for the selection of mobile phase.

**Table .1**

**Trials taken for selection of mobile phase**

<table>
<thead>
<tr>
<th>No.</th>
<th>Condition</th>
<th>Observation</th>
<th>Fig No</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Phosphate Buffer:ACN(30:70)</td>
<td>DICY drug eluted MEF drug not eluted</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>ACN:Monobasic Amm.Phosphate :THF (23:20:7)</td>
<td>DICY drug not eluted MEF drug eluted</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>ACN : Buffer (Disodium Hydrogen Ortho Phosphate) (50:50)</td>
<td>DICY drug not eluted MEF drug eluted</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>ACN : Buffer(60:40) Monobasic Potassium Dihydrogen Phosphate</td>
<td>MEFand DICY drug eluted</td>
<td>4</td>
</tr>
</tbody>
</table>
Figure: 1
**Phosphate Buffer: ACN (30:70)**

Figure: 2
**ACN: Monobasic Ammonium Phosphate: THF (23:20:7)**
**Specificity**

Acceptance Criteria: There should not be any interference from blank peaks with main peak.

The peak purity index for the main peaks in standard preparation and sample preparation should be equal to or more than 0.990.

**Method validation:**

**Chromatographic Condition**

The chromatographic estimation was performed using following conditions: Stationary phase: Phenomenex C8 column (150 mm x 4.6 mm i.d., 5 μm particle size) was used at ambient temperature Mobile Phase: ACN : BUFFER(60:40v/v) Flow rate: 0.7 mL/min Injection volume: 20 μL Detection: At 215 nm with PDA detector
Figure 5
Chromatograms of blank using optimized protocol

Figure 6
Chromatograms of standard using optimized protocol
Linearity
Linear correlation was obtained between peak areas versus concentrations of MEF in the ranges of 50-150 µg/mL and DICY in the ranges of 2-6 µg/ml. Regression parameters are mentioned in Table 2 and the calibration curves of MEF and DICY at 215 nm, respectively are shown in Fig.9 & Fig.10.

Figure.7
Chromatograms of tablet preparation using optimized protocol

Figure.8
Linearity Chromatograms of MEF and DICY
Figure 9
Calibration curve for Mefenamic acid

Figure 10
Calibration curve for Dicyclomine HCl

Table 2
Linearity of MEF and DICY by RP-HPLC method

<table>
<thead>
<tr>
<th>DICY Conc. (µg/mL)</th>
<th>MEF Conc. (µg/mL)</th>
<th>DICY Mean area (n=5)</th>
<th>MEF Mean area (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>50</td>
<td>414230</td>
<td>1417931</td>
</tr>
<tr>
<td>3</td>
<td>75</td>
<td>632425</td>
<td>2131076</td>
</tr>
<tr>
<td>4</td>
<td>100</td>
<td>850414</td>
<td>2834865</td>
</tr>
<tr>
<td>5</td>
<td>125</td>
<td>1074822</td>
<td>3555591</td>
</tr>
<tr>
<td>6</td>
<td>150</td>
<td>1290796</td>
<td>4259388</td>
</tr>
</tbody>
</table>

Correlation coefficient: 0.997
Slope of regression line: 19617
Y-intercept: 44205
**Accuracy (%recovery)**

Recovery for individual and mean value (n=3) at each level should be between 98.0% to 102.0% with RSD not more than 2.0%.

**Result:** % recovery, mean% recovery and %RSD were calculated at each level and recorded in Table 3. The % recovery is within limit (98.0 – 102.0 %) so the method is accurate.

### Table 3

**Recovery data of MEF and DICY**

<table>
<thead>
<tr>
<th>Formulation (MEFTALSPAS)</th>
<th>(%API) DICY+MEF (µg/ml)</th>
<th>Amt. recovered (DICY)* (Conc(µg/ml) ± SD)</th>
<th>%Recovery</th>
<th>Amt. recovered (MEF)* (Conc(µg/ml) ± SD)</th>
<th>%Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>01:25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(80) (1.8+45)</td>
<td>1.83±0.05</td>
<td>102.01</td>
<td></td>
<td>45.09±1.11</td>
<td>100.2</td>
</tr>
<tr>
<td>(100) (2+50)</td>
<td>2.03±0.13</td>
<td>101.4</td>
<td></td>
<td>49.73±0.21</td>
<td>99.47</td>
</tr>
<tr>
<td>(120) (2.2+55)</td>
<td>2.20±0.04</td>
<td>100.12</td>
<td></td>
<td>74.67±0.59</td>
<td>99.69</td>
</tr>
</tbody>
</table>

*Each value is the mean of three determinations

**Precision**

**Method precision (repeatability)**

The RSD of assay of six sample preparations should not be more than 2.0%.

### Table 4

**Precision data of MEF and DICY**

<table>
<thead>
<tr>
<th>Conc(µg/ml) (DICY:MEF)</th>
<th>Dicyclomine HCl</th>
<th>Mefenamic Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Conc(µg/ml) ± SD</td>
<td>%RSD</td>
</tr>
<tr>
<td>Repeatability</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.0 : 50</td>
<td>2.01±2803.39</td>
<td>0.67</td>
</tr>
<tr>
<td>4.0 : 100</td>
<td>4.10±242.677</td>
<td>0.03</td>
</tr>
<tr>
<td>6.0 : 150</td>
<td>6.07±14490.39</td>
<td>1.11</td>
</tr>
<tr>
<td>Intra Day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.0 : 50</td>
<td>2.03±4377.04</td>
<td>1.04</td>
</tr>
<tr>
<td>4.0 : 100</td>
<td>4.09±10318.49</td>
<td>1.18</td>
</tr>
<tr>
<td>6.0 : 150</td>
<td>6.08±15849.31</td>
<td>1.21</td>
</tr>
<tr>
<td>Inter Day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.0 : 50</td>
<td>2.01±1976.62</td>
<td>0.48</td>
</tr>
<tr>
<td>4.0 : 100</td>
<td>4.10±2491.25</td>
<td>0.29</td>
</tr>
<tr>
<td>6.0 : 150</td>
<td>6.06±12327.74</td>
<td>0.95</td>
</tr>
</tbody>
</table>

**System suitability**

**Acceptance criteria**

Asymmetry of both the analytes’ peak in standard should not be more than 2.0.

Theoretical plates of both the analytes’ peak in standard should not be less than 2000.

**Result**

Relative Standard Deviation for five replicates injections of both the standard preparation should not be more than 2.0%.

The mean values of system suitability parameters are shown in following Table 5.
Table 5

Mean values of system suitability parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>MEF (n = 6)</th>
<th>DICY (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retention time (min)</td>
<td>2.025</td>
<td>18.508</td>
</tr>
<tr>
<td>Tailing factor (limit &lt;2)</td>
<td>1.342</td>
<td>1.387</td>
</tr>
<tr>
<td>Theoretical plates (limit &gt;2000)</td>
<td>2500</td>
<td>3109</td>
</tr>
<tr>
<td>Resolution (limit &gt;2)</td>
<td></td>
<td>18.56</td>
</tr>
</tbody>
</table>

Application of developed method to pharmaceutical formulation
The proposed validated method was successfully applied to the simultaneous determination of MEF and DICY in tablet dosage form.

Table 6
Analysis of Marketed Formulation of MEF and DICY by Proposed Method

<table>
<thead>
<tr>
<th>Formulation (MEFTALSPAS) (DICY:MEF)</th>
<th>Mefenamic acid</th>
<th>Dicyclomine HCl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Conc(µg/ml) ± SD</td>
<td>%Assay (limit:92-107)</td>
</tr>
<tr>
<td>02:50</td>
<td>2.02±0.00921</td>
<td>100.6</td>
</tr>
</tbody>
</table>

Table 7
Summary of validation parameters for optimized RP-HPLC method

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Parameters</th>
<th>Dicyclomine HCl</th>
<th>Mefenamic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Range(µg/ml)</td>
<td>2 – 6</td>
<td>50 – 150</td>
</tr>
<tr>
<td>2</td>
<td>Linearity</td>
<td>R² = 0.999</td>
<td>R² = 0.997</td>
</tr>
<tr>
<td>3</td>
<td>Precision( %RSD)</td>
<td>1.04-1.21</td>
<td>0.75-1.89</td>
</tr>
<tr>
<td></td>
<td>(a) Intraday</td>
<td>0.29-0.95</td>
<td>0.15-0.48</td>
</tr>
<tr>
<td></td>
<td>(b) Interday</td>
<td>0.03-1.11</td>
<td>0.03-0.15</td>
</tr>
<tr>
<td></td>
<td>(c) Reproducibility</td>
<td>100.12-102.01</td>
<td>99.47-100.2</td>
</tr>
<tr>
<td>4</td>
<td>Accuracy</td>
<td>100.6 %</td>
<td>99.91 %</td>
</tr>
<tr>
<td>5</td>
<td>Assay</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In this proposed method the linearity is observed in the concentration range of 50-150µg/ml and 2-6µg/ml with coefficient of correlation, (r²) = 0.997 and (r²) = 0.999 for MEF and DICY, respectively.

The result of the analysis of pharmaceutical formulation by the proposed method is highly reproducible and reliable and it is in good agreement with the label claim of the drug. The method
can be used for the routine analysis of MEF and DICY in combined dosage form without any interference of the excipients.

**CONCLUSION**

The developed method was novel, simple, accurate, precise reproducible, economical, which would be used to estimate MEF & DICY in their combined dosage form in routine analysis.

**ACKNOWLEDGEMENTS**

Authors are highly thankful to Shree Dhanvantary Pharmaceutical Analysis & Research Centre & Mercury labs Ltd., (Vadodara, India) for providing gift sample of MEF and DICY. We acknowledge the Shree Dhanvantary Pharmacy College,Kim for providing the facilities to complete the work.

**REFERENCES**

8. The Indian practitioner: Mefenamic acid with Dicyclomine is a highly effective and well tolerated treatment for spasmodic dysmenorrheal, Medical advisor, Blue cross laboratories limited, Mumbai, nov;52(11): 767, (1999)
10. Aventis pharmaceuticals: Bentyl (Dicyclomine HCl, USP) prescribing information, bridgewater, nj. apr (2003).


33. ICH guidelines, Validation Of Analytical Procedure: Methodology Q2B; I.C.H.