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# Development and Validation of High Performance Thin Layer Chromatography for Estimation of Mefenamic Acid, Paracetamol and Dicyclomine HCl in Tablet Dosage Form

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**ABSTRACT**

High performance thin layer chromatographic method was described for the analysis of mefenamic acid, paracetamol and dicyclomine HCl in tablet dosage form. The method employed HPTLC aluminium plates precoated with Silica gel $60F_{254}$ as the stationary phase and mixture of Toluene: Acetone: Formic acid (10:9.8:0.2) as mobile Phase. The densitometric evaluation of separated bands was carried out at 254 nm for mefenamic acid and paracetamol. In method development, Dicyclomine HCl was not detected in UV absorbance at 254 nm, so that same plate was poured in dragandroff reagent and measured at 510 nm wavelength. The linear response was observed in range of 100-700 ng/spot with correlation coefficient of, 0.998, 0.997 and 0.978. Precision was found to be within limits. The percentage recovery was found to be the limits of acceptance criteria between the range of 99.03% to 101.24%.

**Keywords:** High performance thin layer chromatography, Mefenamic acid, Paracetamol, Dicyclomine hydrochloride, Validation.

## INTRODUCTION

Mefenamic acid [N-(2,3-xylyl) anthranilic acid] is a white to off white crystalline solid with a bitter aftertaste. It will darken if exposed to light for long periods but is otherwise stable at room temperature. It is virtually water insoluble except at an alkaline pH. It exhibits anti inflammatory, analgesic, and antipyretic activities. It is used commonly to relive pain arising from rheumatic condition and dismenorrhea as it decreases inflammation (swelling) and uterine contractions.

Mefenamic acid is the only fenamic acid derivative which produces analgesia centrally and peripherally. It

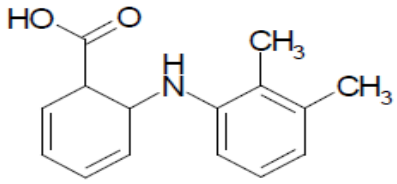


**Fig. 1: Chemical structure of Mefenamic acid**

binds to the prostaglandin synthetase receptors COX-1 and COX-2, inhibiting the action of prostaglandin synthetase; as a result the symptoms of pain are temporarily reduced. [1]

Paracetamol has analgesic and antipyretic properties and weak anti-inflammatory activity. The mechanism of analgesic action remains to be fully elucidated, but may be due to inhibition of prostaglandin synthesis both centrally and peripherally. Paracetamol is used for the relief of mild to moderate pain and minor febrile conditions. Paracetamol has a narrow therapeutic index. [2]

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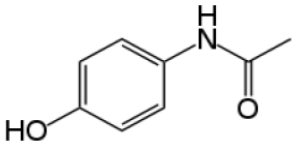

**Fig. 2: Chemical structure of Paracetamol**

Dicyclomine hydrochloride (DCL) is 2-(diethylamino) ethyl bicyclohexyl-1-carboxylate hydrochloride. It binds more firmly to M1 and M3 than to M2 and M4 receptors. It is used for its spasmolytic effect on various smooth muscle spams, particularly those associated with the gastrointestinal tract. It is also useful in dysmenorrheal, pylorospam and biliary dysfunction. 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. [3]

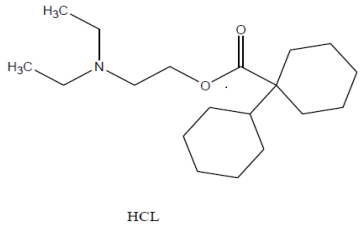

**Fig. 3: Chemical structure of Dicyclomine HCl**

Method has to be reported for analytical method development and validation for mefenamic acid, paracetamol and dicyclomine HCl for HPTLC. The aim of the work was develop a reliable, improved method that can be applied for the determination of mefenamic acid paracetamol and dicyclomine in tablet doasge form.

Nowadays, HPTLC is becoming a routine analytical technique in analytical laboratories due to its advantages. The major advantage of HPTLC is the simultaneous determination of several samples on single chromatogram using a small quantity of mobile phase .This reduces analysis time, cost per analysis and possibilities of pollution of the environment. The aim of the work is to develop a simple, accurate method that can be determination of mefenamic acid, Paracetamol and dicyclomine HCl in tablet dosage form. [4]

## MATERIALS AND METHODS

### Material

Mefenamic acid was supplied as gift sample from Vibhav Life science Pvt Ltd, Paracetamol was supplied as gift sample from Sun Pharma, Dicyclomine HCl was supplied as gift sample from Vista pharmaceutical. All reagents and solvents used were of analytical grade and obtained from Merck chemicals. All glass wares (Pipette, Beaker, volumetric flask, measuring cylinder) were of borosil.

### HPTLC instrumentation

Chromatographic separation was performed on aluminum plate precoated with silica gel $60F_{254}$, (Merck, Darmstadt, Germany). Densitometric scanning was performed on Camag TLC scanner 4 in the absorbance mode at 254 nm and 510 nm utilizing deuterium lamp as a source of radiation with WINCATS software (Camag, Muttenz, Switzerland).

### Chromatographic Condition

Aliquot amount of the working standard or sample solutions were spotted on the plate in the form of bands of width 3 mm with 1µl micro-syringe, using autosampler. A constant application rate of 1µl/s was employed and space between two bands was 3 cm. The bands were applied at 1 cm from the bottom edge of the plate. The plate was then allowed to dry on air for 5 min before its transfer to the mobile phase tank. The mobile phase consisted of Toluene: Acetone: formic acid (10:9.8:0.2 v/v/v). Linear ascending development was carried out in chromatographic chamber previously saturated with the mobile phase for 10 min at room temperature. The length of chromatogram run was 8 cm subsequent to the development. TLC plates were dried in a current of air with the help of an air dryer. Densitometric scanning was performed at 20 mm/s scanning speed and the slit dimension was kept at 6.0 × 0.30µm. The TLC chromatogram was manipulated by WINCATS software.

### Standard solution preparation

Standard stock solution of pure drug containing 10 mg of mefenamic acid, paracetamol and dicyclomine HCl prepared in methanol. The working standard solutions of the drug were obtained by dilution of the stock solution in the methanol (100µg/ml).

### Method Validation [5-10]

### Precision

Precision was performed by intraday and interday precision. 3 times on the same day and on 3 different days.

### Linearity

The amount of standard solution equivalent to of 1000-7000 ng/spot mefenamic acid, paracetamol and dicyclomine HCl was spotted on the prewashed TLC plates. The linearity of response was assessed in terms of slope, intercept and correlation coefficient values.

### Accuracy

Aliquot amounts of the dosage forms extract were spiked with extra 50, 100 and 120% of the standard mefenamic acid, paracetamol and dicyclomine HCl and the mixtures were reanalyzed in triplicate by the proposed method.

### LOD and LOQ

The limits of detection (LOD) and quantification (LOQ) were calculated from the slope (s) of the calibration plot and the standard deviation of the response (SD).

### Specificity

It is the ability of the method to remain unaffected in the presence of components which may be expected to be present; typically these might include impurities, degradation products and diluent.

## RESULT AND DISCUSSION

The result of validation studies on the simultaneous HPTLC determination method developed for mefenamic acid, paracetamol and dicyclomine HCl with toluene: acetone: formic acid (10:9.8:0.2 v/v/v) as

the mobile phase gave resolution with $R_f$ value of 0.6 for mefenamic acid, 0.4 for paracetamol and 0.14 for dicyclomine HCl.

**Linearity**

The drug response was linear over the concentration range between 100–700 ng/spot for mefenamic acid, paracetamol and dicyclomine HCl in Table 1.

**Table 1: Linear regression data for calibration plots**

| Parameter | Mefenamic acid | Paracetamol | Dicyclomine HCl |
|---|---|---|---|
| Linearity range ng/spot | 100-700 ng/spot | 100-700ng/spot | 100-700ng/spot |
| r2 | 0.998 | 0.997 | 0.978 |
| Slope | 6.59 | 7.75 | 7.00 |
| Intercept | 777.5 | 195.2 | 394.6 |

The proposed method was found to be linear at concentration of 100-700 ng/spot for mefenamic acid, paracetamol and dicyclomine HCl. The regression coefficient (r2) was found to be 0.998 and 0.997 which is well within the acceptance criteria limits.

**Precision**: The results of the repeatability and intermediate precision experiments are shown in table. The developed method was found to be precise, with RSD values for repeatability.

**Inter-day**

**Table 2 : Inter-day precision for MEF, PCM and DCL**

| Sample No. | Peak area ± % RSD | | |
|---|---|---|---|
| | MEF | PCM | DCM |
| 1 | 3767.7 ± 0.59 | 4225.5 ± 1.49 | 3881.1 ± 0.27 |
| 2 | 4045 ± 1.3 | 4203..4 ± 1.40 | 3970.2 ± 0.40 |
| 3 | 4261.6 ± 2.0 | 4146.4 ± 1.95 | 4090.2 ± 0.48 |

**Intra-day**

**Table 3: Intra-day precision for MEF, PCM, DCL**

| Sample No. | Peak area ± % RSD | | |
|---|---|---|---|
| | MEF | PCM | DCM |
| 1 | 1994.6 ± 0.9 | 4244.4 ± 1.6 | 3756.8± 0.20 |
| 2 | 3748.7 ± 1.9 | 4141.1 ± 1.68 | 3978.0 ± 0.49 |
| 3 | 4215.1 ± 2.0 | 4081.4 ± 1.45 | 4268.35 ± 0.048 |

The % RSD values were found to be < 2%, which indicates that the proposed method was precise.

**Accuracy Studies**

Recovery studies were carried out by adding known amount of standard solution, to the sample solution. The %recovery was calculated and reported.

**Table 4: Recovery studies**

| Drugs | Recovery level | Amount of marketed sample (ng) | Amount spiked (ng) | Total amount of drug taken | Total amount recovery | % Recovery | % RSD |
|---|---|---|---|---|---|---|---|
| MEF | 80 % | 200 | 160 | 360 | 352.81 | 98.02 | 0.40 |
| | 100 % | 200 | 200 | 400 | 400.81 | 100.24 | 1.01 |
| | 120 % | 200 | 240 | 440 | 446.75 | 99.20 | 0.76 |
| PCM | 80 % | 200 | 160 | 360 | 365.41 | 99.01 | 0.66 |
| | 100 % | 200 | 200 | 400 | 418.55 | 101.8 | 1.31 |
| | 120 % | 200 | 240 | 440 | 439.01 | 99.7 | 0.45 |
| DCL | 80 % | 200 | 160 | 360 | 362.96 | 98..5 | 0.69 |
| | 100 % | 200 | 200 | 400 | 412.92 | 99.02 | 0.78 |
| | 120 % | 200 | 240 | 440 | 446.85 | 99.12 | 0.85 |

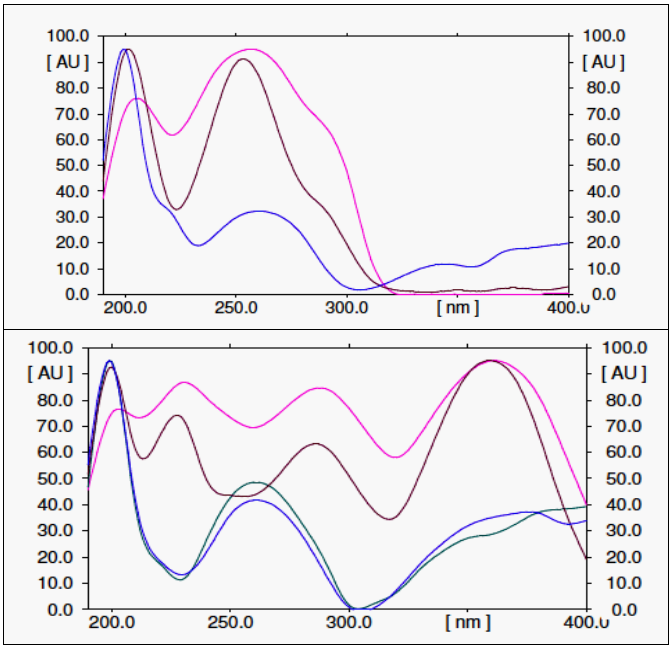


**Fig. 4: Specificity for Paracetamol and Mefenamic acid**

The percentage recovery was found to be 99.03-101.24%, which are well within the limit and hence the method was found to be accurate.

**Table 5: LOD and LOQ**

| | MEF | PCM | DCM |
|---|---|---|---|
| LOD | 13.08 ng/spot | 16.30 ng/spot | 12.74 ng/spot |
| LOQ | 39.65 ng/spot | 49.39 ng/spot | 38.63 ng/spot |

The proposed method can detect and quantify small amount of drugs with precisely.

**Specificity**

The spot for in sample was confirmed by comparing the $R_f$ and spectra of the spot with that of standard (Figure 4). The peak purity of was assessed by comparing the spectra at three different levels, i.e. peak start, peak apex and peak end position of the spot. In specificity, Dicyclomine HCl was derivatized. So, it was not take

**Analysis of marketed formulation by developed method**

**Table 6: Analysis of marketed formulation**

| Drug | Amount taken (ng) | Area ± SD (n=3) | Amount found (ng) ± SD | % Label claim ± SD |
|---|---|---|---|---|
| MEF | 250 | 2288 ± 0. 86 | 256.75 ± 0.072 | 101.20 ± 0.81 |
| PCM | 500 | 4450 ± 0.73 | 497.01 ± 0.045 | 99.60 ± 0.62 |
| DCL | 20 + 80 (Standard addition | 520.0 ± 0.69 | 18.90 ± 0.067 | 98.67 ± 0.58 |

The assay results were comparable to labelled value of each drug in tablet. These results indicate that the developed method is precise and accurate.

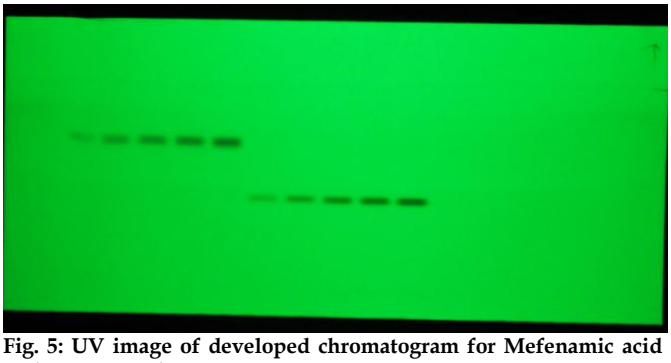

**Fig. 5: UV image of developed chromatogram for Mefenamic acid and paracetamol**

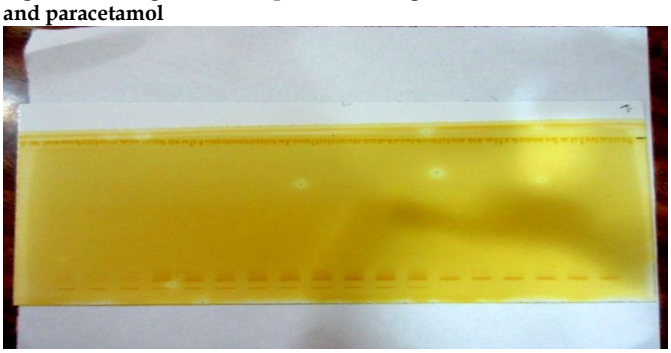

**Fig. 6: After Derivatization UV image of developed chromatogram for Dicyclomine HCl**

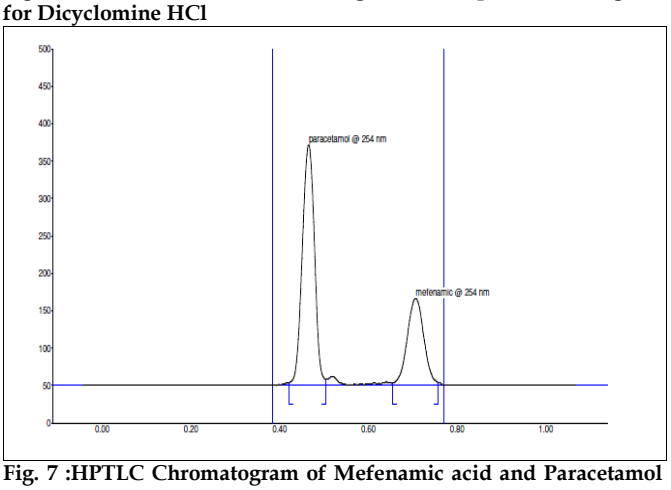


**Fig. 7 :HPTLC Chromatogram of Mefenamic acid and Paracetamol scan at 254 nm**

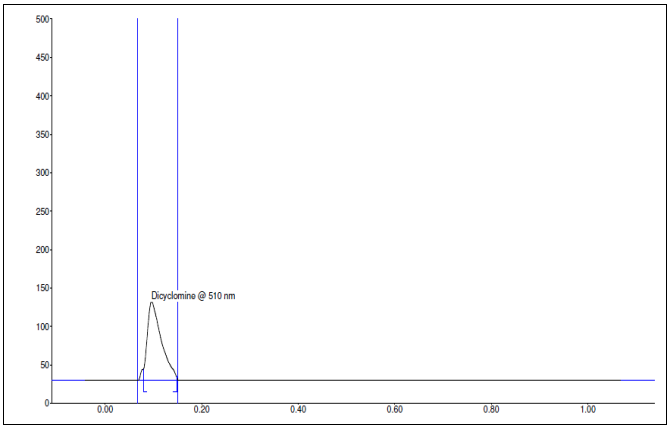


**Fig. 8: HPTLC Chromatogram of Dicyclomine HCl**

Today, HPTLC is rapidly becoming a routine analytical technique due to its advantages of low operating costs, high sample throughput, and the need for minimum sample preparation. The major advantage of HPTLC is that several samples can be run simultaneously using a small quantity of mobile phase-unlike HPLC; thus reducing the analysis time and cost per analysis. The developed HPTLC technique is precise, specific, and accurate. Statistical analysis proves that the method is suitable for the analysis of mefenamic acid, paracetamol and dicyclomine HCl in tablet dosage form.

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