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Simultaneous Determination of Paracetamol and Mefenamic acid in Tablet Dosage Form by High Performance Thin-Layer Chromatography

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ABSTRACT

A normal-phase simple, rapid and precise high-performance thin-layer chromatographic (HPTLC) method has been developed for simultaneous quantitative determination of Paracetamol and Mefenamic acid in a pharmaceutical formulation. The analysis was performed on silica gel 60F254 on aluminum plates with Acetonitrile-Toluene 7 : 3 (v/v), as mobile phase. Detection and quantitation were performed densitometrically at λ = 275 nm.. The developed method was validated for linearity, accuracy, precision and robustness parameters. Responses of Paracetamol standard and Mefenamic acid standard were linear functions of concentration in the ranges 36-180 µg/mL and 40-200 µg/mL, respectively.. The correlation coefficient of Paracetamol and Mefenamic acid were observed 0.9997 and 0.9992 respectively. Accuracy was checked by conducting recovery studies; average recovery from the pharmaceutical preparation was 99.88 ± 1.25% for Paracetamol and 99.92 ± 1.62% for Mefenamic acid. The Proposed HPTLC method has potential applications for simultaneous determination of Paracetamol and Mefenamic acid in combined tablet dosage form.

Keywords : Paracetamol, Mefenamic acid, HPTLC

I. INTRODUCTION

Paracetamol (PC) is chemically N - (4-hydroxyphenyl) acetamide and is used as analgesic and anti-pyretic agent. It has a narrow therapeutic index – the therapeutic dose is close to the toxic dose. Mefenamic acid (MA) is 2-[(2, 3-dimethylphenyl) amino] benzoic acid Mefenamic acid, an anthranilic acid derivative, is a member of the fenamate group of nonsteroidal anti-inflammatory drugs (NSAIDs).. Literature survey revealed that various methods reported for the analysis of Paracetamol and Mefenamic acid in pharmaceuticals viz, UV spectrophotometry, reverse phase HPLC stability indicating, visible spectrophotometry. Aim of present work was to develop simple, economical, rapid, precise and accurate method for simultaneous determination of Paracetamol and Mefenamic acid. The key advantage of developed HPTLC method is that several samples can run using a small quantity of mobile phase. The present study describes HPTLC method for the determination of paracetamol and mefenamic acid from tablet dosage form.

II. EXPERIMENTAL

Chemicals and reagents:
Reference standards of Paracetamol and Mefenamic acid were procured from Blue Cross India Limited Nashik. AR grade methanol and acetonitrile were purchased from Baker (Mumbai, India).

Instrumentation and Chromatographic condition:
The samples were spotted in the form of bands of width 5mm with a desaga 100 µL sample syringe on silica gel precoated aluminum plate 60 F_{254}, with 200 µm thickness. These bands were applied with the help of Desaga AS 30 - sample applicator at a distance of 10mm from X axis and 15mm from Y axis at the edge of the HPTLC plate with the speed of 150nl/sec for methanol. The plates were pre-washed by methanol and activated at 110 °C for 5 min prior to chromatography. The space between two bands was kept at 10 mm. The slit dimension was kept at 4 x 3 mm and 4.0 mm/s scanning speed was employed. The monochromator bandwidth was set at 10 nm, each track was scanned thrice and baseline correction was used. The mobile phase consisted of Acetonitrile : Toluene in the volume ratio 7 : 3 v/v.

Linear ascending development was carried out in a twin trough glass chamber saturated with the mobile phase.

The optimized chamber saturation time for mobile phase was 30 min at room temperature (25 °C±2) at relative humidity of 55% ± 5. Subsequent to the development; TLC plates were dried in current of air with the help of air dryer. Detection and quantification was performed in the absorbance mode using Degasa TLC scanner with Pro-Quant software. During the method development the spots on the TLC plate were visualized in a UV chamber equipped with a UV lamp (λ =254nm). The developed TLC plate was scanned between 200 and 400nm wavelength using CD-60 Densitometer/scanner. The wavelength chosen for further quantification was 275nm.

Preparation of standard solutions:
45mg of Paracetamol and 50 mg of Mefenamic acid was accurately weighed and transferred to a 50cm^3 volumetric flask. It was dissolved in a minimum quantity of methanol and then diluted up to the mark with methanol. The concentration of the solution obtained was 900 µg/mL for Paracetamol and 1000 µg/mL for Mefenamic acid **(Solution A).** 5cm^3 of this **solution A** was diluted to 50 cm^3 in a volumetric flask with mobile phase. The concentration of the solution obtained was 90 µg/mL & 100 µg/mL for Paracetamol and Mefenamic acid respectively **Preparation of**

Sample solutions:
Twenty tablets (MFTEL FORTY, BLUE CROSS LABORATORIES LTD) were weighed and their average weight was calculated. These tablets were powdered and weight equivalent to one tablet containing 450mg of Paracetamol and 500 mg of Mefenamic acid was taken in a 100mL dilution flask. Then about 50mL of diluent was added to it and sonicated for 20-25 mins at an ambient temperature with intermittent swirling, cooled to room temperature and diluted upto the mark with diluent. Then solution from the flask was filtered through syringe filter.

Validation of the Method:
The method was validated for linearity, precision (inter-day, intra-day and intermediate precision), accuracy, specificity. Standard plots were constructed for both paracetamol and Mefenamic acid in the range of 36-180 µg/mL and 40-200 µg/mL, respectively L. The experiment was repeated thrice on the same day and additionally on two consecutive days to determine intra- and inter-day precision, respectively. The intermediate precision of the method was determined by repeating the experiment on two different instruments. Accuracy was determined by recovery studies. It was carried out by spiking 10%, 20% and 30% of the standard drugs to the pre-analysed marketed sample of Paracetamol and Aceclofenac. Three determinations were performed at each level. Further, specificity of the method was assessed by study of the resolution factor of the drug peaks from nearest resolving peaks. Robustness of the method was carried out by small changes in the mobile phase composition (± 0.1 mL for each component) were made and the effects on the results were examined. Time from spotting to chromatography and from chromatography to scanning was varied by ± 15 min.

Analysis of marketed formulation:
The developed method can be applied in determination of paracetamol and mefenamic acid in tablet MFTEL FORTY which is marketed oral solid dosage formulation. To determine the contents of paracetamol and mefenamic acid (label claim: 450mg paracetamol and 500 mg mefenamic acid per tablet), the contents of tablet were emptied and weighed. The drug from the powder was extracted with 10 ml methanol. To ensure complete extraction of the drug, it was sonicated for 30 min. The resulting solution was allowed to settle for about an hour and the supernatant was suitably diluted to give desired concentration. Ten microlitres of the solution was applied on TLC plate followed by development, visualization and scanned. The analysis was repeated in triplicate. The possibility of excipient interference in the analysis was studied.

III. RESULTS AND DISCUSSION

Optimization of the chromatographic conditions:
In order to develop an normal phase HPTLC method for the determination of paracetamol and mefenamic acid in combined dosage form the chromatographic conditions were optimized. For better separation and resolution the mixture of different solvents of varying polarity were tried. The different compositions of mobile phase were changed for getting better separation of analytes. Initially, chloroform-ethyl acetate 4: 6 (v/v) and acetonitrile, toluene 5:5 (v/v) were used. The best

results were obtained by the use of acetonitrile, toluene in the ratio of (7: 3 v/v). This mobile phase showed good resolution of paracetamol and mefenamic acid peak from other formulation components or excipients tested. Densitometric scanning of all the tracks showed compound with *Rf* value 0.51for mefenamic acid and 0.62 for paracetamol The present method uses acetonitrile –toluene (7:3 v/v) as the mobile phase for development. The present method is quicker as the time needed for development of plate is reduced considerably to less than half an hour for chamber saturation and development of plate as compared to the previously reported method.

Table 1. Optimized chromatographic conditions

Parameters	**Chromatographic conditions**
Development chamber	Twin trough chamber
Stationary phase	Silica gel
Mobile Phase	Acetonitrile : Toluene (7 : 3 v/v)
Chamber saturation	15 min
Sample applicator	AS 30 - SAMPLE APPLICATOR
Band	8mm
Space	12mm
Scanning speed	20mm/sec
Development distance	8 cm
Drying of plate	Room temperature
Densitometric scanner	CD 60 - DENSITOMETER / SCANNER
Lamp	Deuterium
Wavelength	275 nm
Volume	10µl

Method Validation:

Linearity and range

Linearity was observed over the concentration range of 36-180 µg/mL for paracetamol and 40-200 µg/mL, for mefenamic acid (see table 2). The linearity of the calibration plots was confirmed by the high value of the correlation coefficients (r^2 = 0.9997 for paracetamol and 0.9992 for aceclofeanc).

Table 2. Linear regression data

Drug	**Linearity range**	**Correlation coefficient (r^2)**	**Slope**	**Intercept**
Paracetamol	36-180 µg/mL	0.9997	3.430	-6.383
Mefenamic acid	40-200 µg/mL	0.9992	7.946	-13.73

Precision:

The developed method was validated for system precision and method precision.
The precision study of the proposed method gave the results in the prescribed limits of relative standard deviation. This is less than 2 % for both analytes. The low value of RSD showed that the proposed method was reliable and reproducible.

Table 3. Precision study for Paracetamol and Mefenamic acid

Obs No	Paracetamol		Mefenamic acid	
	Peak Area	% Assay	Peak Area	% Assay
1	2521	101.61	2665	101.89
2	2512	101.25	2689	99.82
3	2507	101.05	2710	98.24
4	2499	100.73	2708	98.34
5	2442	98.43	2650	97.65
6	2474	99.72	2743	99.53
	Mean	100.01	Mean	99.31
	S.D	0.899	S.D	1.307
	%R.S.D	0.8990	%R.S.D	1.310

Specificity:
An investigation specificity was conducted during the validation of identification tests, the determination of impurities and the assay. Demonstration of specificity requires that there should not be any interference of impurities and excipients. In practice this was done by taking the chromatogram of sample solution and the assay result was unaffected by the extraneous material. It has been found that there was no interference of the diluents , placebo at the Rf value of the analytes.

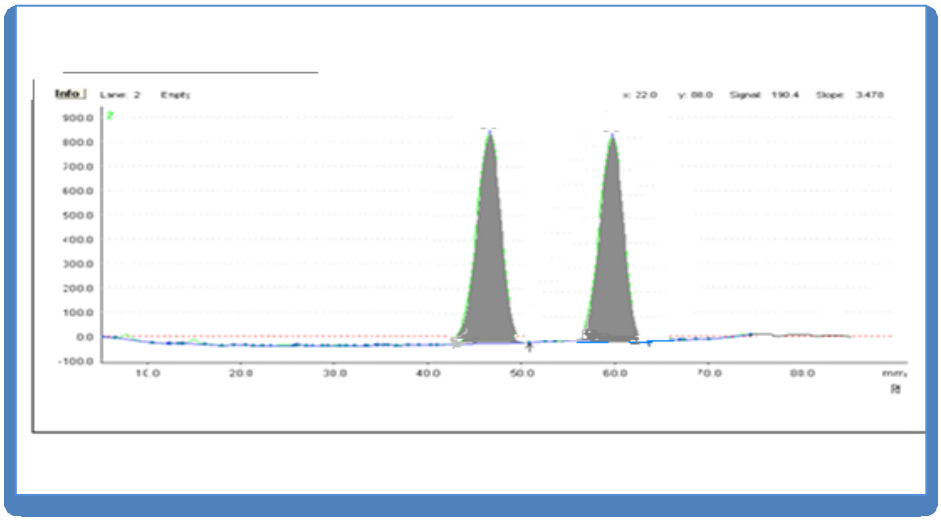

Figure 1. Typical HPTLC chromatogram 1) Mefenamic acid 2) Paracetamol

Accuracy (Recovery Experiment):

The accuracy of the method was determined by the standard addition method at three different levels. The sample solution of 100% level was considered as a zero level and 10% , 20% and 30% of the standard drug of paracetamol and mefenamic acid were added respectively. Each determination was performed in triplicates. The accuracy was then calculated as the percentage of the standard drug recovered by the recovery study. Mean recoveries for PC and

MA from the sample solution are shown in Table 4 and 5. The results are within the acceptance limit and hence the method is accurate.

Table 4. % Recovery of Paracetamol

Amount of Paracetamol in ppm								
Sr.No	**% Added**	**Original amount**	**Added amount**	**Total amount**	**Mean (n = 5)**	**% Recovery**	**S.D**	**% RSD**
1	10	450	45.40	495.40	495.45	99.97	0.3881	0.3786
2	20	450	90.87	540.87	540.02	99.50	0.4922	0.4896
3	30	450	135.66	685.66	685.66	99.10	0.645	0.681

Table 5. % Recovery of Mefenamic acid

Amount of Mefenamic acid in ppm								
Sr.No	**% Added**	**Original amount**	**Added amount**	**Total amount**	**Mean (n = 5)**	**% Recovery**	**S.D**	**% RSD**
1	10	500	50.36	550.36	550.12	100.15	0.9592	0.8582
2	20	500	100.38	600.38	600.33	99.49	0.4977	0.4902
3	30	500	150.16	650.16	650.87	99.82	0.8884	0.7953

IV. CONCLUSION

The HPTLC method for the determination of Paracetamol and Mefenamic acid from their tablet dosage form was found to be accurate , precise, specific and rapid. The results of the recovery studies show the high degree of accuracy of the proposed method. The advantage of the proposed method is that it require less time and cost effective method. Solvent consumption during the analysis is less. Therefore the proposed method can be applied successfully in routine analysis.

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