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Research Article

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Development and validation of stability indicating HPTLC method for simultaneous estimation of paracetamol and mefenamic acid in liquid dosage form

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ABSTRACT

A simple, accurate, precise and selective stability- indicating high performance thin layer chromatographic method has been developed and validated for simultaneous estimation of Paracetamol and Mefenamic acid in suspension. Chromatographic separation of both the drugs was achieved by using Benzene: Methanol (8.5: 1.5, v/v) as mobile phase with UV detection at 226 nm. The retention factors for Paracetamol and Mefenamic acid were found to be 0.39 ± 0.006 and 0.69± 0.006, respectively. The dev eloped method was validated in terms of linearity, accuracy, precision and robustness as per ICH guidelines. The drugs were subjected to stress condition of hydrolysis (acid, base), oxidation. Linear response was observed in the concentration range of 250-1500 ng band^{-1} for Paracetamol and 100-600 ng band^{-1} for Mefenamic acid. The method has been applied for the analysis of drugs in suspension. The % assay (Mean ±S.D.) was found to be 99.00 ±1 .48 for Paracetamol and 99.84± 1.87 for Mefenamic a cid.

Keywords: Paracetamol, Mefenamic acid, HPTLC, Stress degradation, Validation

INTRODUCTION

Paracetamol (PARA), chemically, *N*-(4-hydroxyphenyl) ethanamide *N*- (hydroxyphenyl) acetamide is used to reduce pain and fever [1]. Mefenamic acid (MFA), 2-(2, 3-dimethylphenyl) amino benzoic acid is non-steroidal anti-inflammatory drug used to treat mild to moderate pain and is sometimes used to prevent migraines associated with menstruation [2].

Literature survey reveals high performance thin layer chromatographic [3] high performance liquid chromatographic [4, 5] and UV Spectrophotometric [6, 7] methods for estimation of PARA in human plasma and pharmaceutical dosage form in combination with other drugs. Analytical methods reported for MFA includes HPLC [8-10], HPTLC [11, 12] and UV [13] either as single or in combination with other drugs. Reports are also available for simultaneous determination of PARA and MFA by HPLC [14, 15] and HPTLC [16] method.

To best of our knowledge, no reports were found for stability-indicating high performance thin layer chromatographic method for simultaneous estimation of PARA and MFA in liquid dosage form. The present study describes development of stability indicating HPTLC method for simultaneous estimation of PARA and MFA as bulk drugs and in suspension and validation of developed method as per International Conference on Harmonisation Guidelines.

EXPERIMENTAL SECTION

Chemicals and reagents

Pharmaceutical grade working standards PARA and MFA were kindly supplied by Sun Pharma (Mumbai, India). The pharmaceutical dosage form used in this study was Metagesic-DS suspension (Blue Cross Laboratories Pvt. Ltd., India) labeled to contain 250 mg of PARA and 100 mg of MFA was procured from the local market. Benzene, Methanol (AR grade) was purchased from Merck specialties Pvt. Ltd. (Mumbai, India).

Instrumentation and chromatographic conditions

Chromatographic separation of drug was performed on precoated silica gel aluminium plate 60 F_{254} (10 ×10) with 250 µm thickness (E. MERCK, Darmstadt, Germany) using a CAMAG Linomat 5 sample applicator (Switzerland). Samples were applied on the plate as a band with 6 mm width using Camag 100 µL sample syringe (Hamilton, Switzerland).

Linear ascending development was carried out in 10 x 10 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland) by using Benzene: Methanol (8.5: 1.5, v/v) as mobile phase with chamber saturation time of 15 min. The length of chromatogram run was 9 cm and development time was approximately 15 min. After development, TLC plates were dried and densitometric detection was performed on CAMAG thin layer chromatography scanner at 226 nm operated by WINCATS software version 1.4.2. Deuterium lamp emitting a continuous UV spectrum between 200 to 400 nm was used as source of radiation.

Preparation of Standard Stock Solution

Working standard solution of PARA was prepared by dissolving 25 mg of drug in 10 mL of methanol to get concentration of 2500 ng μL^{-1} from which 1 mL was further diluted to 10 mL to get final concentration of 250 ng μL^{-1}. Stock solution of MFA was prepared by dissolving 10 mg of drug in 10 mL of methanol to get concentration of 1000 ng μL^{-1} from which 1 mL was further diluted to 10 mL to get final concentration of 100 ng μL^{-1}.

Selection of Detection Wavelength

After chromatographic development bands were scanned over the range of 200-400 nm. It was observed that both the drugs showed considerable absorbance at 226 nm. So, 226 nm was selected as the wavelength for detection (Figure 1).

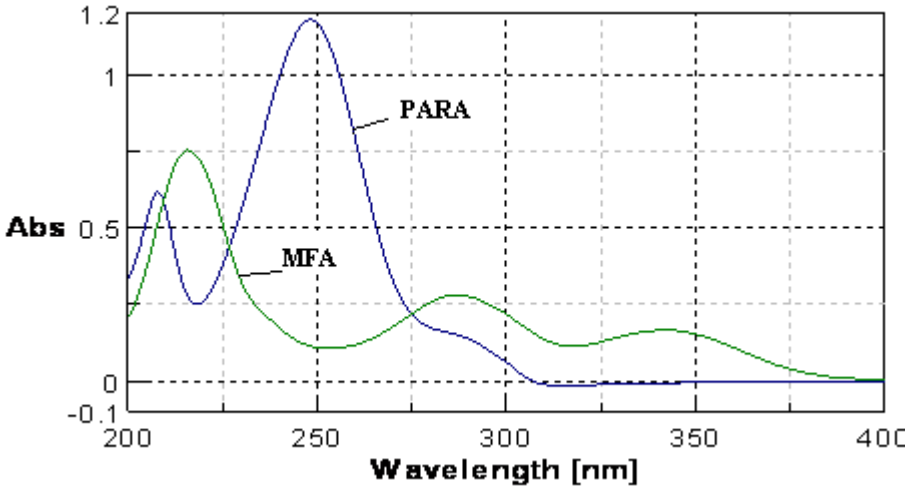


Fig. 1. In situ overlay spectrum of PARA and MFA

Analysis of Marketed Suspension

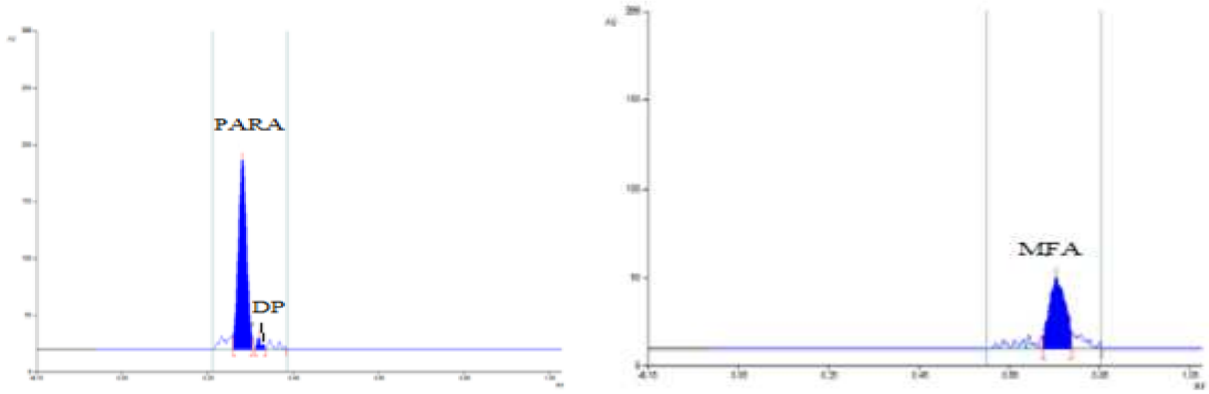
For the assay of marketed formulation, 1 mL of the marketed suspension labeled to contain 250 mg of PARA and 100 mg of MFA was pipetted out using a volumetric pipette and transferred to a 10 mL of volumetric flask and diluted with methanol to get the concentration of 2500 ng μL^{-1} for PARA and 1000 ng μL^{-1} for MFA. One mililitre of the above solution was diluted with methanol to 10 ml to get the concentration of 250 ng μL^{-1} for PARA and 100 ng μL^{-1} for MFA. Two microlitre volume of this solution was applied on the plate. After chromatographic development peak areas of the bands were measured at 226 nm and the amount of each drug present in each sample was estimated from the respective calibration curve. Procedure was repeated six times for the analysis of homogenous sample.

Stress degradation studies of bulk drug

The forced degradation studies were carried out on bulk drug substance in order to prove the stability-indicating property and selectivity of the developed method. The degradation was carried out under hydrolytic, oxidative stress conditions.

Acid treatment

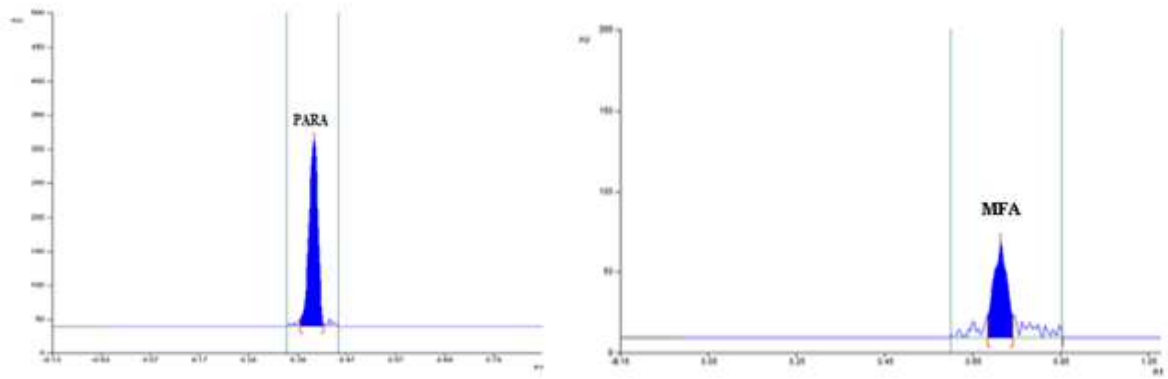
1 mL working standard solution of PARA (2500 ng μL^{-1}) was mixed with 1 mL of 0.1 N HCl and 8 mL of methanol. The solution was keep at room temperature for 4 h. 2 µL of resulting solution was applied on TLC plate to get concentration 500 ng $band^{-1}$. Same procedure was repeated for MFA (1000 ng μL^{-1}) to get final concentration 200 ng $band^{-1}$ applied on the TLC plate. After acid hydrolysis, PARA showed peak of degradation at Rf 0.42 and 13.11 % of degradation was observed for MFA with no degradation peak. The densitogram obtained after acid degradation is shown in Figure 2.



(a) **(b)**

Fig. 2. Representative densitogram of (a) PARA with degradation product DP (Rf =0.42) (b) MFA Alkali treatment

1 mL working standard solution of PARA (2500 ng μL^{-1}) was mixed with 1 mL of 0.1 N NaOH and 8 mL of methanol. The solution was keep at room temperature for 4 h. 2 µL of resulting solution was applied on TLC plate to get concentration 500 ng $band^{-1}$. Same procedure was repeated for MFA (1000 ng μL^{-1}) to get final concentration 200 ng $band^{-1}$ applied on the TLC plate. Both the drugs were found susceptible to alkali degradation with % degradation of 24.07 for PARA and 23.39 for MFA. The densitogram obtained after alkali degradation is shown in Figure 3.

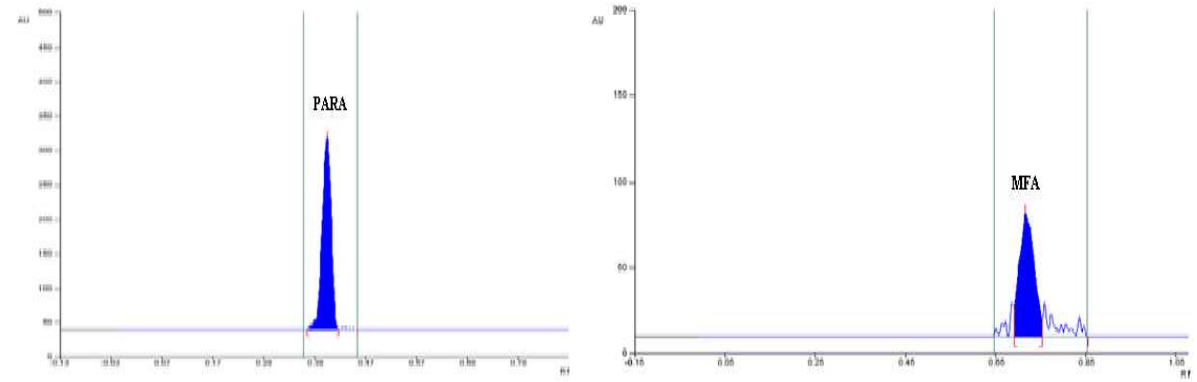


(a) **(b)**

Fig. 3. Representative densitogram of alkali induced degradation (a) PARA (b) MFA

Neutral Hydrolysis

1 mL working standard solution of PARA (2500 ng μL^{-1}) was mixed was mixed with 1 mL of water and 8 mL methanol. The solution was keep at room temperature for 4 h. 2 µL of resulting solution was applied on TLC plate to get concentration 500 ng $band^{-1}$. Same procedure was repeated for MFA (1000 ng μL^{-1}) to get final concentration 200 ng $band^{-1}$ applied on the TLC plate. About 15.95 % of PARA was degraded with reduction in peak area and 24.67 % degradation was observed for MFA in neutral condition. The representative densitogram after neutral degradation is shown in Figure 4.

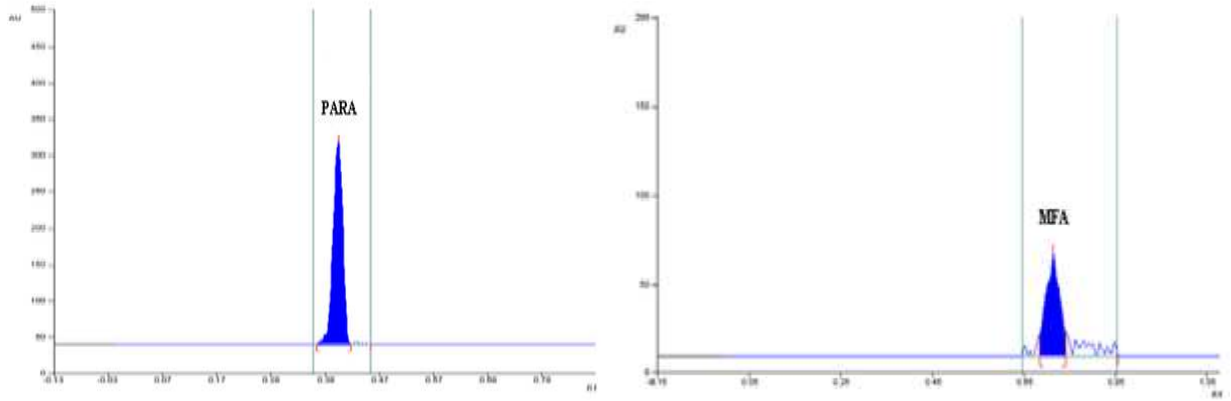


(a) **(b)**

Fig. 4. Representative densitogram obtained after neutral degradation (a) PARA (b) MFA

Oxidative degradation

1 mL working standard solution of PARA (2500 ng μL^{-1}) was mixed 1 mL of 3 % solution of H_2O_2 and 8 mL of methanol. The solution was keep at room temperature for 4 h. 2 µL of resulting solution was applied on TLC plate to get concentration 500 ng $band^{-1}$. Same procedure was repeated for MFA (1000 ng μL^{-1}) to get final concentration 200 ng $band^{-1}$ applied on the TLC plate. About 73.91 % of PARA was recovered with reduction in peak area. On other hand, 72.14 % of MFA was recovered without appearance of additional product. The representative densitogram obtained after oxidative degradation is shown in Figure 5.



(a) **(b)**

Fig. 5. Densitogram of peroxide induced degradation (a) PARA (b) MFA

RESULTS AND DISCUSSION

Optimization of chromatographic method

The major objective in developing this stability indicating HPTLC method is to achieve the resolution of PARA and MFA and its degradation products. The separation was achieved by linear ascending development in 10 cm × 10 cm twin trough glass chamber using benzene: methanol (8.5: 1.5, v/v) as mobile phase and detection was carried out at 226 nm. The retention factors were found to be 0.39 ± 0.006 and 0.69± 0.006 for PARA and MFA, respectively. Representative densitogram of mixed standard solution of PARA and MFA is shown in Figure 6.

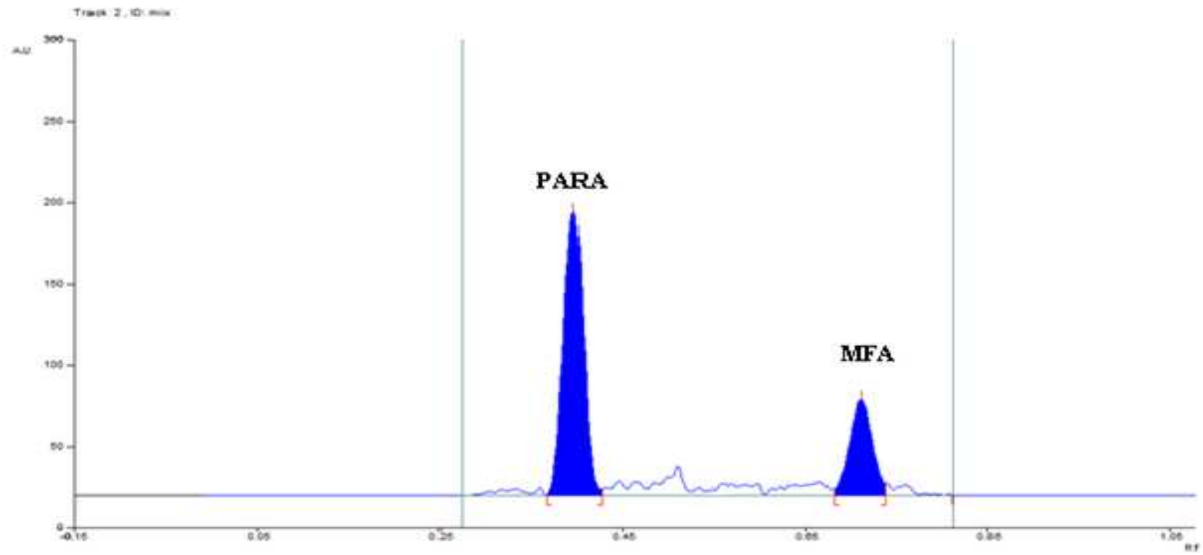


Fig. 6: Representative densitogram of mixed standard solution of PARA (500 ng band^{-1}, R_f = 0.39 ± 0.006) and MFA (200 ng band^{-1}, R_f = 0.69 ± 0.006)

Result of stress degradation studies

Stress degradation studies showed no interference of degradation products at retention time of drug. The degradation products were well resolved from both the drugs indicating specificity of the method. The results obtained after stress degradation studies are summarized in Table 1.

Table 1: Data of forced degradation studies of PARA and MFA

Stress conditions/ duration	PARA		MFA	
	% Assay of active substance	% degradation	% Assay of active substance	% degradation
Acidic/ 0.1 N HCl/ Kept at RT for 4 h	93.00	7.00	86.89	13.11
Alkaline/1 N NaOH/ Kept at RT for 4 h	75.93	24.07	78.61	23.39
Oxidative/3 % H_2O_2/ Kept at RT for 4 h	73.91	26.09	72.14	27.86
Neutral/H_2O/ Kept at RT for 4 h	84.05	15.95	75.33	24.67

Method Validation

The method was validated for linearity, accuracy, intra-day and inter-day precision and robustness as per ICH guidelines [17, 18].

Linearity

The standard stock solutions of PARA (250 µL^{-1}) and MFA (100 ng µL^{-1}) were applied by over spotting on TLC plate in range of 1, 2, 3, 4, 5 and 6 µL. Linear results were observed in the concentration range of 250-1500 ng band^{-1} for PARA and 100-600 ng band^{-1} for MFA with high correlation coefficient > 0.99.

Precision

Set of three different concentrations in three replicates of standard solutions were prepared. All the solutions were analyzed on the same day in order to record any intra day variations in the results. Intra-day variation, as RSD (%), was found to be in the range of 0.34 to 1.96 for PARA and 0.14-1.59 for MFA. For Inter day variation study, three different concentrations of the standard solutions in linearity range were analyzed on three consecutive days. Interday variation, as RSD (%) was found to be in the range of 0.32 -1.34 for PARA and 0.62-1.06 for MFA.The lower values of % R.S.D. (< 2) indicated that method was found to be precise.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

LOD and LOQ were calculated as 3.3 σ/S and 10 σ/S, respectively; where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot. The LOD was found to be 49.27 ng band^{-1} for PARA and 15.68 ng band^{-1}for MFA. The LOQ was found to be 100 ng band^{-1} for PARA and 48 ng band^{-1} for MFA.

Recovery Studies

To check accuracy of the method, recovery studies were carried out by adding standard drug to sample at three different levels 80, 100 and 120 %. Basic concentration of sample chosen was 500 ng band^{-1} for PARA and 200 ng

band^{-1} for MFA. The drug concentrations were calculated from respective linearity equation. The results of the recovery studies indicated that the method is accurate for estimation of drugs in liquid dosage form. The results obtained are shown in Table 2.

Table 2: Recovery Studies of PARA and MFA

Drug	Amount taken (ng band^{-1})	Amount added (ng band^{-1})	Total amount found (ng band^{-1})	% Recovery	% RSD
PARA	500	400	897.12	99.68	0.24
	500	500	992.30	99.23	0.25
	500	600	1088.89	98.99	0.71
MFA	200	160	356.50	99.03	0.81
	200	200	397.60	99.40	0.11
	200	240	435.55	98.99	0.71

**Average of three determinations*

Robustness Studies

Robustness of the method was determined by carrying out the analysis under conditions during which mobile phase composition, chamber saturation time was altered and the effect on the area of drug was noted. The deliberate alterations of the analytical parameters showed that areas of peaks of interest remained unaffected by small changes of the operational parameters (% R.S.D. < 2) indicating robustness of the method. The results are given in Table 3.

Table 3. Robustness Data in Terms of Peak Area (% RSD)

Sr. No.	Parameter	(% RSD)	
		PARA	MFA
1	Mobile phase composition (± 2 % methanol)	0.34	0.33
2	Chamber saturation time (± 10 %)	0.41	0.43

**Average of three determinations*

CONCLUSION

The developed HPTLC method is simple, precise, accurate and stability-indicating without interference from the excipients or from degradation products. The developed method can be used for quantitative analysis of PARA and MFA in pharmaceutical dosage form.

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