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Stability Indicating Assay Method for the Determination of Medroxy Progestrone Acetate in Bulk Drug and Formulation by HPTLC

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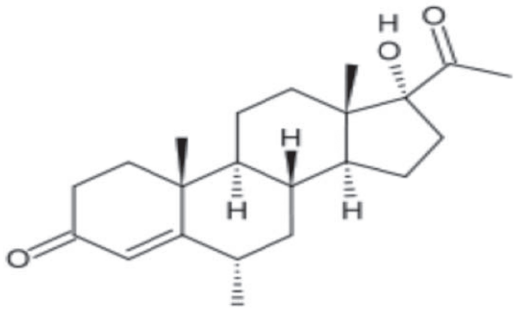
Abstract

A sensitive, accurate, precise and stability indicating high-performance thin layer chromatographic assay method for analysis of Medroxyprogestrone acetate (MPA) in both bulk drug and formulation was developed and validated. The method employed HTLC aluminium plates precoated with silica gel 60F-254 as the stationary phase. The solvent system consisted of Toluene: ethyl acetate: ammonia solution (80:20:0.1 %v/v). This system was found to give compact spots for MPA (*R*f value 0.32±0.02). MPA was subjected to acid, alkali and neutral hydrolysis, oxidation, sunlight and dry heat treatment. The degraded products were well separated from the pure drug with notably different R_f values. CAMAG HPTLC instrument was used for chromatographic separations. Densitometric analysis of MPA was carried out in the absorbance mode at 240 nm. The linear regression data for the calibration plots showed good linear relationship with correlation coefficient 0.9998 ±/0.001 in the concentration range of 50.0-1800.0ng spot^{-1}. The value of slope and intercept were 11.68 and 73.414 respectively. The method was validated for precision, accuracy, robustness, and recovery. The limits of detection and quantitation were 16.5 and 50.0 ng spot^{-1} respectively.

1. Introduction

MPA is a progestin, and is a common component of hormonal contraceptives. It can be used in the treatment of abnormal menstrual bleeding or amenorrhea. Chemically, MPA is 17-Acetyl-17-hydroxy-6,10,13-trimethyl-1,2,6,7,8,9,10,11,12,13,14,15,16,17 – tetradecahydrocyclopenta phenanthren-3-one (Fig.1) with a molecular weight of 344.488 g/mol.

The present study describes development and validation of a simple sensitive, specific, and precise stability indicating HPTLC method for the estimation of MPA in bulk drugs and pharmaceutical formulations. The International Conference on Harmonization [ICH] (1) guidelines emphasizes the stress testing of the drug substance which can help to identify the likely degradation products and validate the stability indicating power of the analytical procedures used.



Medroxyprogesterone

Fig. 1. Structure of Medroxyprogestrone

Chromatographic techniques are widely used for the stability studies as it can be efficient tools to separate and quantify the analyte in presence of decomposition products. The advantage of HPTLC is that several samples can be run simultaneously by using a small quantity of mobile phase unlike HPLC, thus lowering analysis time and cost. The aim of the present work was to develop an accurate, specific, and reproducible stability indicating HPTLC method for the determination of MPA in the presence of degradation products under stress testing in Bulk drugs and Pharmaceutical formulations as per ICH guidelines (1-2).

The parent drug stability test guidelines (Q1A R2) issued by International Conference on Harmonization (ICH) requires that analytical test procedures for stability samples should be fully validated and the assays should be stability indicating. Stress testing is a part of development strategy under ICH requirements and is carried out under more severe conditions than accelerated studies. Further, it is suggested that stress studies should be carried out to establish the inherent or intrinsic stability characteristics of the molecule by establishing the degradation pathways and help in validation of the analytical methods to be used in stability studies.

The primary goal of the present study was to develop and validate a HPTLC method as per ICH guidelines (3). A second objective was to develop a stability indicating technique, which could be employed for the routine quantification of low levels of medroxyprogestrone in the presence of degradation products for assessment of purity of the bulk drug and stability of its bulk formulations (4).

Structural information on drug degradants and impurities can serve to accelerate the drug discovery and development cycle (5). Forced degradation studies are used to facilitate the development of analytical methodology, to gain a better understanding of active pharmaceutical ingredient and drug product stability (6). The available regulatory guidance provides useful definitions and general comments about degradation studies. Forced degradation studies are carried for development and validation of stability indicating methodology, determination degradation pathways and structural elucidation of degraded products [7]. The objective of stability testing is to determine for what time period and under what condition the product is satisfactory (8). Pharmaceutical companies perform forced degradation studies during preformulation to help select compounds and excipients for further development, to facilitate salt selection or formulation optimization, and to produce samples for developing stability indicting analytical methods (9).

In one of the literature stability indicating analytical method was reported for the determination of medroxyprogestrone in bulk drug and pharmaceutical dosage form by HPLC (10).

Till to day no article related to the stability indicating high-performance thin layer chromatographic (HPTLC) determination of MPA in bulk drug and pharmaceutical dosage forms was reported in literature or in Pharmacopoeias.

Experimental

Materials: MPA bulk drug and its injectable suspension were given by Star drugs and research laboratories, Bangalore as a gift sample. Ethyl acetate, Toluene, methanol and ammonia solution (Qualigens Fine Chemicals, Mumbai) used were of analytical grade, camag linomat IV sample applicator equipped with 100ml Hamilton (USA) syringe, Camag twin trough glass chamber, CAMAG HPTLC scanner III densitometer, Cats 3 software.

HPTLC instrumentation: The samples were applied in the form of bands on the plate, width 6 mm, and 10mm from the bottom of the edge using a Camag precoated silica gel aluminium plate 60F-254 (20 × 10 cm with 0.2 mm thickness, E.Merck, Germany) with Linomat IV (Switzerland) sample applicator equipped with a 100µL Hamilton (USA) syringe. A constant application rate of 100nL/s was employed and the standard, sample volume was 10µL, the space between two bands were 7

mm and slit dimension was kept 5 mm x 0.45 mm micro and 5 mm sec^{-1} scanning speed was employed. The eluting solvent was consisted of Tolune: ethyl acetate: ammonia solution (80:20:0.1 % v/v). Linear ascending development was carried out in twin trough glass chamber (Camag) saturated with mobile phase. Previously the glass chamber was saturated with help of filter paper and the optimized chamber saturation time was found to be 30 min at room temperature. The length of chromatogram run was approximately 80 mm. After the development the plates were dried in air with the help of an air-dryer. Densitometric scanning was performed on Camag TLC scanner III in the absorbance mode at 240 nm with CATS3 software. The source of radiation utilized was a deuterium lamp. The slit dimensions were 5mm x 0.45 mm micro and the scanning speed was 5 mm sec^{-1}.

Stock Preparation

Preparation of stock solution: Stock solution of 1mg/ml was prepared by dissolving 10 mg of MPA in 10 ml of methanol. From this 5.0, 10.0, 20.0, 40.0, 50.0, 100.0, 135.0 and 180.0µg mL^{-1} solutions were prepared in methanol. 10.0µl of each of this solution were spotted on the HPTLC plate to obtain concentrations of 50.0, 100.0, 200.0, 400.0, 500.0, 1000.0, 1350.0 and 1800.0ng $spot^{-1}$ of MPA respectively.

Method validation

***Calibration curve of* MPA:** The working standard solution (mentioned in section 2.3.1) each of 10 µl were applied on HPTLC plate to obtain concentration of 50.0, 100.0, 200.0, 400.0, 500.0, 1000.0, 1350.0 and 1800.0ng $spot^{-1}$ of MPA. The curve was plotted using peak area against the drug concentrations and the data was treated by the linear least square regression. The range was chosen based on the maximum concentration to be kept for the stress studies.

Accuracy and precision of the assay

Precision: Repeatability of sample application and measurement of peak area were carried out using six replicates of the same spot (500 ng $spot^{-1}$ of MPA). The intra-day and inter-day variation for the determination of MPA was carried out at a concentration of 500 ng $spot^{-1}$.

Accuracy: The analysed samples were spiked with extra 80, 100, 120 % of the standard MPA and the mixture were analysed by the proposed method. At each level of the above said amount, three determinations were performed. This was done to check the recovery of the drug at different levels in the formulations. Recovery was calculated using the standard formula

Recovery [%] = [(Total conc.

ÿ Formulation conc.)/ Standard conc.] × 100.

Repeatability

Repeatability of measurement of peak area: MPA (500 ng/spot) of 10 µl was spotted on a HPTLC plate, developed, dried and the spot was scanned seven times without changing the plate position and % RSD for measurement of peak areas were estimated.

Repeatability of measurement of sample applications : The drug solution, MPA, 50 µl (500 ng/spot) was spotted seven times on a HPTLC plate, developed and dried. The spots were scanned and RSD% for measurement of peak areas was estimated.

Robustness: By introducing small changes in the mobile phase composition, the effects on the results were examined. Mobile phases having different composition of Toluene: ethyl acetate: ammonia solution (78:22:0.1 and 78:22:0.1 v/v) were tried and samples were run. The mobile phase composition, chamber saturation time and temperature for activation of plate were tried in the varied range (25 and 30 min at 100°C and 120°C respectively). Robustness of the method was done at concentration level of 500 ng $spot^{-1}$.

Limit of detection and limit of quantification: In order to estimate the limit of detection (LOD) and limit of quantitation (LOQ), blank methanol was spotted six times following the same method as explained in Section 2.2. The signal-to-noise ratio was determined. LOD was considered as 3:1 and LOQ as 10:1.

Specificity: The specificity of the method was ascertained by analyzing standard drug with sample. The spot for MPA in sample was confirmed by comparing the R_f values and spectra of the spot with that of standard. The peak purity of MPA was assessed by comparing the spectra at three different levels, i.e., peak start (S), peak apex (M) and peak end (E) positions of the spot.

Analysis of MPA in prepared formulation: To determine the concentration of MPA in sterile injectable suspension (labeled claim: 150 mg mL^{-1}), 100 µL (15 mg) of MPA injectable suspension was pipetted out and transferred into three 10 mL volumetric flasks. 5.0 mL of methanol was added into each of the flasks, sonicated to dissolve the contents and then the volume was finally made up with methanol. Further this solution was diluted appropriately using methanol to obtain a concentration of 100.05 µg/mL (67 µL to 1000 µL), from this solution further diluted 100 µL to 1000 µL with methanol, from this solution 50µL (500 ng $spot^{-1}$) was applied on HPTLC plate followed by development and scanning as described in section 2.2. The analysis was repeated in triplicate. The possibility of excipients interferences in the analysis was studied.

Forced degradation of MPA: All stress degradation studies were performed at initial concentration of 100µg mL^{-1}. For these studies, 10 mg of MPA was accurately weighed and transferred to a 100 mL volumetric flask (step one).

Acid and base induced degradation: To the step one, 1mL of each 0.01N NaOH (for Alkaline degradation), 0.1N HCl (for Acid degradation),) were added to separate volumetric flasks and dissolved the content and diluted with methanol to make up the volume and prepared solution were placed at room temperature for 2 hrs for alkaline degradation and then refluxed at 80°C for 8 hrs.

The mixtures were allowed to cool and 1.0 mL of these solutions were then transferred to a 10 mL volumetric flasks neutralized with 1.0 mL of 1N NaOH for acid and neutralized with 1.0 mL of 1N HCl for alkaline degradation and the volumes were made up with methanol. From this solution 50µL and 2 x 50 µL samples were plotted in the form of bands by using Linomat IV, Switzerland applicator on precoated silica gel aluminium plate 60GF-254 to get 500ng/spot and 100ng $spot^{-1}$ respectively for MPA.

Hydrogen peroxide-induced degradation, Neutral Hydrolysis, Sunlight and Dry heat degradation.

To the step one, 1.0mL of 3 % Hydrogen peroxide (for Oxidative degradation) and 1.0 mL of water (for Hydrolytic degradation) were added in two separate volumetric flasks and dissolved the content and diluted with methanol to make up the volume and placed at room temperature for 8 hrs and refluxed at 80°C for 8 hrs respectively . Additionally, the drug powder in step one was exposed to dry heat at 80 ºC for 8 hrs and at sunlight for 8 hrs.

The mixtures were allowed to cool and made up to the mark with the diluent. 1.0 mL of this solution was then transferred to a 10 mL volumetric flask and the volume was made up with methanol. From this solution 50µL and 2 x 50µL samples were plotted in the form of bands by using linomat IV applicator on precoated silica gel aluminium plate 60GF-254 to get 500ng $spot^{-1}$ and 100ng $spot^{-1}$ respectively for MPA.

Results and Discussion

Method development

Development of mobile phase: HPTLC procedure was optimized with a view to developing a stability indicating assay method. Three solvent ratios were selected as to optimize the best among them. Initially, Tolune: ethyl acetate (80:20 v/v) gave good resolution with R_f value of 0.32 for MPA but typical peak nature was missing. Finally, the mobile phase consisting of toluene: ethyl acetate: ammonia solution (80:20:0.1 v/v %) gave a sharp and well defined peak at R_f value of 0.32 (Fig. 2). Well-defined spots were obtained when the chamber was saturated with the mobile phase for 30 min at room temperature.

Validation of Method

Calibration curves: The linear regression data for the calibration curves showed a good linear relationship over the concentration range 50-1800 ng spot^{-1} with respect to peak area. The regression-coefficient (r^2) was found to be 0.9998 which is within the acceptance criteria limit of ≥ 0.99.

Precision: The repeatability of sample application and measurement of peak area were expressed in the terms of % RSD and the results are depicted in Table 1, which revealed intra-day and inter-day variation of MPA at concentration level of 500 ng spot^{-1}.

Robustness of the method: The standard deviation of peak areas was calculated for each parameter and %R.S.D. was found to be less than 2%. The low values of %RSD values as shown in Table 2 indicated robustness of the method.

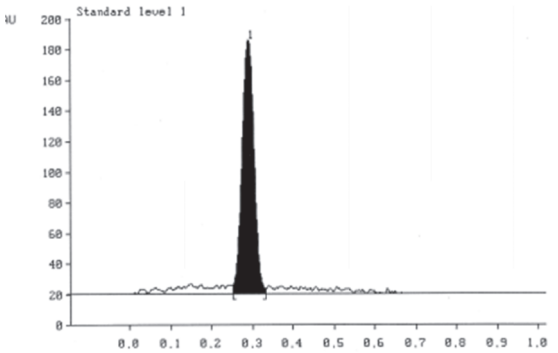


Fig. 2. A typical HPTLC chromatogram of MPA (500 ng spot -1) (Rf = 0.32).

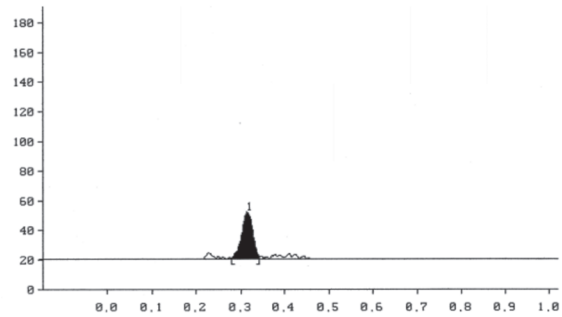


Fig. 3. HPTLC chromatogram of MPA at the LOQ (50 ng spot -1).

LOD and LOQ: The signal-to-noise ratio 3:1 and 10:1 were considered as LOD and LOQ, respectively. The LOD and LOQ were found to be 16.5 and 50 ng spot^{-1}, respectively. Chromatogram at LOQ of MPA is given in fig. 3.

Specificity: The developed method was used for the estimation of MPA in bulk drug and pharmaceutical dosage form. The excipients did not interfere in the estimation. Interferences from the formulation and degradants were absent. These results indicated the specificity of the method.

Recovery studies (Accuracy): The proposed method when used for extraction and subsequent estimation of MPA from pharmaceutical dosage forms after spiking with 80, 100 and 120% of additional drug afforded mean recovery of 100.09 ± 0.93 as listed in table- 3. The data of summary of validation parameters are listed in table-4.

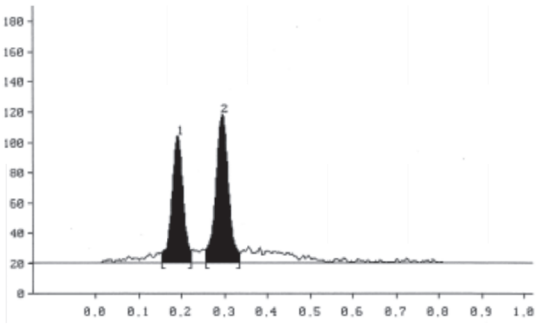


Fig. 4. HPTLC chromatogram of alkali degraded MPA.

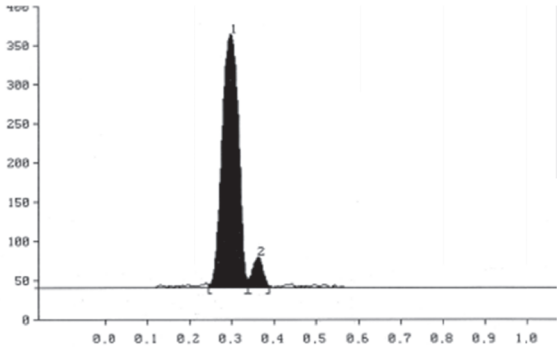


Fig. 5. HPTLC chromatogram of hydrogen peroxide degraded MPA.

Table 1. Intra- and inter-day precision of HPTLC method

Amount (ng/spot)	Intra-day precision [a]			Inter-day precision [a]		
	Mean area	S.D.	RSD%	Mean area	S.D.	RSD%
500	6106.9	52.36	0.86	6086.7	23.01	0.38

[a] n = 6.

Table 2. Robustness of the method

Parameter	**S.D. of peak area**	**RSD%**
Mobile phase compositionToluene: Ethylacetate:Ammonia (78:22:0.1)	2.303	0.463
Mobile phase compositionToluene: Ethylacetate:Ammonia (82:18:0.1)	0.981	0.196
Chamber saturation time (25 min)	0.883	0.177
Chamber saturation time (35 min)	0.602	0.120
Temperature for activation of plates (100°C)	1.656	0.333
Temperature for activation of plates(120°C)	0.646	0.129

Table 3. Recovery studies[a]

Recovery level (%)	**Amt of drug added (µg/ mL)**	**Amount of drug found (µg/ mL)**	**Percentage recovery**	**Mean % recovery**	**RSD%.**
80	400.000	407.794	101.95	101.12	0.73
	400.000	403.571	100.89		
	400.000	402.059	100.51		
100	500.000	497.384	99.48	99.29	0.16
	500.000	495.903	99.18		
	500.000	496.051	99.21		
120	600.000	600.571	100.10	99.87	0.26
	600.000	597.520	99.59		
	600.000	407.794	101.95		

Mean Recovery (Mean±S.D.)= 100.09 ± 0.93
[a] (n=9)

Analysis of prepared formulation: A single spot of R_f 0.42 was observed in chromatogram of the MPA samples extracted from sterile injectable suspension. There was no interference from the excipients commonly present in the formulation. The average MPA content was found to be 99.33% with ± SD of 0.28 and listed in Table 5. Therefore it may be inferred that degradation of MPA had not occurred in the formulation which were analysed by this method. The low %RSD value indicated the suitability of this method for routine analysis of MPA in pharmaceutical dosage forms.

Table 4. Summary of validation parameters

Parameter	Data
Linearity range (ng spot^{-1})	50-1800
Correlation coefficient	0.9998
Limit of detection (ng spot^{-1})	16.5
Limit of quantitation (ng spot^{-1})	50
Percent recovery (n = 9)	100.09 ± 0.93
Precision (RSD%.)	
Repeatability of application (n = 6)	0.96
Repeatability of measurement (n = 6)	0.07
Inter-day (n = 6)	0.86
Intra-day (n = 6)	0.38
Robustness	Robust
Specificity	Specific

Stability- indicating property: The chromatogram of samples degraded with acid, base, hydrogen peroxide, neutral, sunlight and heat showed well separated spots of pure MPA as well as some additional peaks at different R_f values. The spots of degraded products were well resolved from the drug spot as shown in Figs. 2-6. Percentage degradation was calculated and listed in table-6.

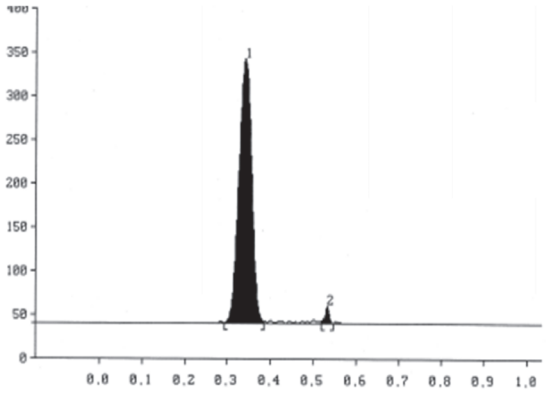


Fig. 6. HPTLC chromatogram of sun light degraded MPA

Table 5. Analysis of MPA sterile injectable suspension (500.00 ng spot^{-1})

Pharmaceutical formulation	Actual concentration of MPA (ng spot^{-1})	Amount of MPA calculated (ng spot^{-1})	% MPA (mean±SD) (n=3)
MPA Sterile injectable suspension	500.000	496.678	99.33±0.28

Table 6. Forced degradation of MPA

S.No.	Stress Condition	% Degradation ± S.D.
1	Base(0.01 N NaOH) @ RT for 2 Hour	24.91 ± 0.41
2	Acid(0.1 N HCl)@ 80°C for 8 Hour reflux	6.33 ± 0.11
3	Oxidative (3% H_2O_2) RT for 8 Hour	4.96 ± 0.26
4	Thermal (80°C) for 8 Hour	4.11 ± 0.02
5	Neutral (water)@ 80°C for 8 Hour	4.70 ± 0.03
6	Sunlight for 8 Hour	3.03 ± 0.13

Conclusion

The new HPTLC method is specific, and sensitive for the estimation of MPA. Statistical analysis proves the method is repeatable and selective for analysis of MPA. The proposed method is less time consuming and it can be used for routine Quality control test and stability studies of MPA in Bulk drug and in its pharmaceutical formulations. As the method separates the drug from its degradation products, it can be employed as a stability indicating one.

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