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Original Article

Stability Indicating HPTLC Determination of Triamcinalone Acetonide in Bulk Drug and Sterile Injectable Suspension

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ABSTRACT

A sensitive, accurate, precise and stability indicating high-performance thin layer chromatographic method was developed and validated for analysis of triamcinalone acetonide (TRIA) in bulk drug and sterile injectable suspension. The method employed HPTLC aluminum precoated plates with silica gel 60F-254 as the stationary phase. The solvent system consisted of Toluene: ethyl acetate: ammonia solution (33:67:0.1 %v/v). This system was found to give compact bands for TRIA (R_f value 0.38 ± 0.02). TRIA was subjected to acid, alkali and neutral hydrolysis, oxidation, sun light and dry heat treatment. The degraded products were well separated from the pure drug with notably different R_f values. CAMAG semi-automatic HPTLC used for the analysis. Densitometric analysis of TRIA 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 0.9996 ±/ 0.001 in the concentration range of 100.0-2000.0 ng spot^{-1}. The values of slope and intercept were 9.7841 and -86.13 respectively. The method was validated for precision, accuracy, robustness, and recovery. The limits of detection and quantitation were 33.0 and 100.0 ng spot^{-1} respectively. As the method could effectively separate the drug from its degradation products, it can be employed as a routine stability indicating assay method.

Key words: Triamcinalone Acetonide (TRIA), HPTLC, Stability indicating assay method, Degradation, ICH, Validation.

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INTRODUCTION

Triamcinolone acetonide is a more potent form of triamcinolone, and is used in the post operative period of certain cosmetic surgery procedure. It can be occasionally used to treat severe cases of hay fever.

Chemically, TRIA is 9-Fluoro-11,16,17-trihydroxy-17-(2-hydroxyacetyl)-10,13-dimethyl-6,7,8,9,10,11,12,13,14,15,16,17 dodeca hydro cyclopental [a]phenanthren-3-one (Figure 1) with a molecular weight of 394.434 g/mol.

To our knowledge, no article related to the stability indicating HPTLC densitometry determination of TRIA in bulk drugs and pharmaceutical dosage form has been reported in literature.

The International Conference on Harmonization (ICH) guideline entitled "Stability testing of new drug substances and products" requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance.[1] An ideal stability-indicating method is one that quantifies the standard drug alone and also resolves its degradation products. Consequently, the implementation of an analytic methodology to determine TRIA in pharmaceutical dosage form in presence of its degradation products is a pending challenge of the pharmaceutical analysis. Therefore, it was thought necessary to study the stability of TRIA towards acidic, alkaline, oxidative, UV and sunlight degradation processes. The aim of this work was to develop stability-indicating chromatographic methods for determination of TRIA in presence of its degradation products for assessment of purity of bulk drug and stability of its dosage forms by HPTLC densitometry. Over the past decade, high performance thin layer chromatography (HPTLC) has been successfully used in the analysis of pharmaceuticals, plant constituents and bio macromolecules.[2-6] Forced degradation studies can be useful in the investigation of chemical and physical stability of the crystal form .[7]

The method is simple, accurate, specific, reproducible, stability indicating. It has added advantage of short duration of the analysis and suitable for routine determination of TRIA in bulk drug and in formulations.

The proposed method was validated in compliance with ICH guidelines,[8,9] and its updated international convention.[10]

Experimental

Materials

TRIA 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 100 µl Hamilton (USA) syringe, CAMAG twin trough glass chamber, CAMAG TLC scanner III densitometer, Cats 3 software.

HPTLC instrumentation

The samples were applied in the form of bands on the plate, width 6 mm, and 10 mm from the bottom of the edge using a Merck 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 100 nL/s was employed and the standard, sample volume was 10 µL, the space between two bands were 7 mm and slit dimension was kept 5x0.45 mm micro and 5 mm sec^{-1} scanning speed was employed. The eluting solvent was consisted of Toluene: ethyl acetate: ammonia solution (33:67:0.1 % 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 the 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 TRIA in 10 ml of methanol. From this 10.0, 20.0, 35.0, 80.0, 100.0, 120.0, 125.0 and 200.0 μg mL^{-1} solutions were prepared in methanol. 10.0 μL of each of this solution were spotted on the HPTLC plate to obtain concentrations of 100.0, 200.0, 350.0, 800.0, 1000.0, 1200.0, 1250.0 and 2000.0 ng $spot^{-1}$ of TRIA respectively.

Method validation

Calibration curve of TRIA

The working standard solution (mentioned in section 2.3.1) each of 10 μL were applied on HPTLC plate to obtain concentration of 100.0, 200.0, 350.0, 800.0, 1000.0, 1200.0, 1250.0 and 2000.0 ng $spot^{-1}$ of TRIA. 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 areas were carried out using six replicates of the same spot (1000 ng $spot^{-1}$ of TRIA). The intra-day and inter-day variation for the determination of TRIA was carried out at a concentration of 1000 ng $spot^{-1}$.

Accuracy

The analysed samples were spiked with extra 80, 100, 120% of the standard TRIA 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 following standard formula Recovery [%] = [(Total conc.－Formulation conc.)/ Standard conc.] × 100.

Repeatability

Repeatability of measurement of peak area

TRIA (1000 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, TRIA, 100 μL (1000 ng/spot) was spotted six 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 (31:69:0.1 and 35:65:0.1 v/v) were tried and chromatograms 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 a concentration level of 1000 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 TRIA in sample was confirmed by comparing the R_f values and spectra of the spot with that of standard. The peak purity of TRIA 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 TRIA in prepared formulation

To determine the concentration of TRIA in sterile injectable suspension (labeled claim: 40 mg mL^{-1}), 250 μL (10 mg) of TRIA 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 100.0 μg/mL. From this solution 10 μL (1000 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 interferences from excipients in the analysis was studied.

Forced degradation of TRIA

All stress degradation studies were performed at initial concentration of 100 μg mL^{-1}. For these studies, 10 mg of TRIA 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 solutions were placed at room temperature for 2 hrs for alkaline degradation and then refluxed at 80°C for 8 hrs for acid degradation.

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 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 1000 ng/spot for TRIA.

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

To the step one, 1.0 mL 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 solution so prepared were 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°C 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 2×50 μL samples were plotted in the form of bands by using linomat IV, applicator on precoated silica gel aluminium plate 60GF-254 to get 1000 ng $spot^{-1}$ for TRIA.

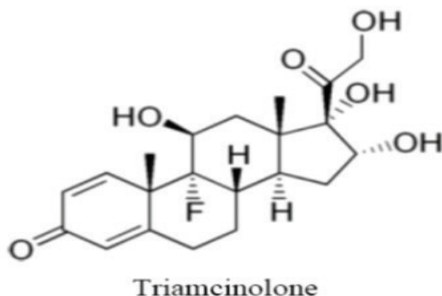

Figure 1: Structure of Triamcinalone.

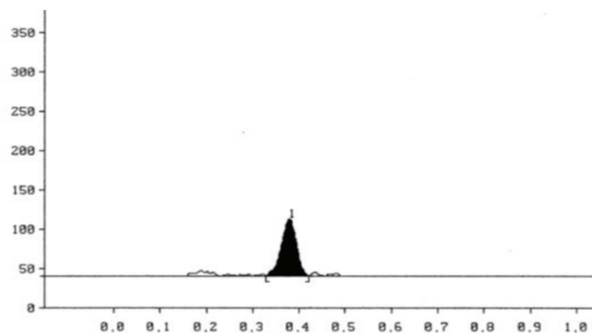

Figure 3: A HPTLC chromatogram of TRIA at the LOQ (100 ng spot^{-1}).

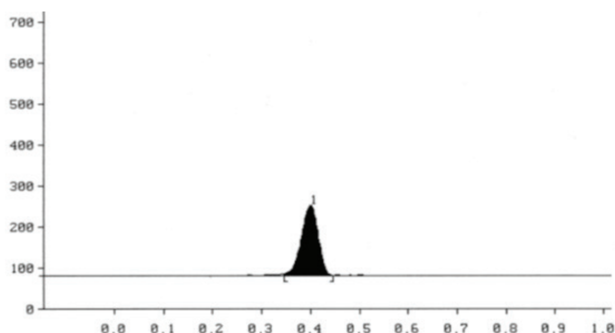

Figure 2: A typical HPTLC chromatogram ofTRIA (1000 ng spot -1) (Rf = 0.38 ± 0.02).

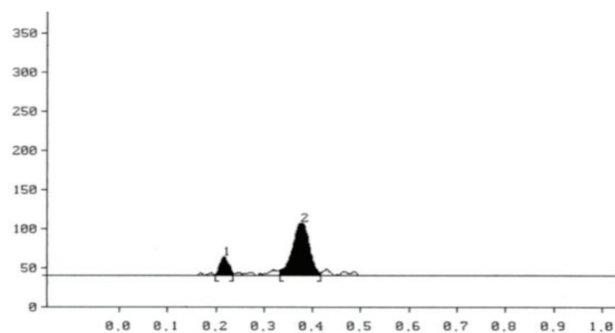

Figure 4: HPTLC chromatogram of alkali degraded TRIA.

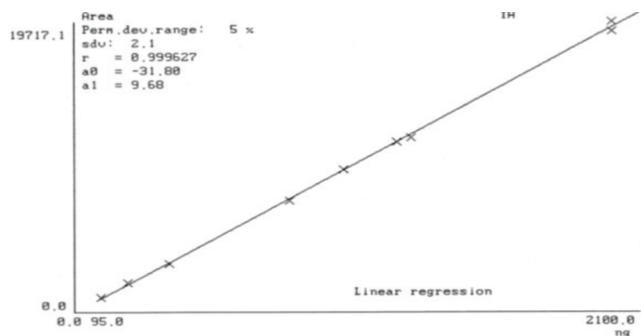

Figure 5: Calibration curve of TRIA.

Table 1: Concentration of standards and corresponding area of TRIA

Conc. (μg/mL)	Area
100	1017.4
200	2010.1
350	3290.4
800	7578.0
1000	9723.0
1200	11580.3
1250	11905.1
2000	19717.1

Table 2: 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
1000	9675.8	45.45	0.47	9844.5	30.73	0.31

a)n = 6.

RESULTS AND DISCUSSION

Method development

Selection of optimum 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, Toluene: ethyl acetate (33:67 v/v) gave good resolution with R_f value of 0.38 for TRIA but typical peak nature was missing. Finally, the mobile phase consisting of toluene : ethyl acetate : ammonia solution (33:67:0.1 v/v %) gave a sharp and well defined peak at R_f value of 0.38 (Figure 2). Well-defined spots were obtained when the chamber was saturated with the mobile phase for 30 min at room temperature.

Validation of the method

Calibration curves

The linear regression data for the calibration curves showed a good linear relationship over the concentration range 100-2000 ng spot^{-1} with

Table 3: Robustness of the method

Parameter	S.D.of peak area	%RSD
Mobile phase composition Toluene:Ethylacetate:Ammonia solution (31:69:0.1)	1.552	0.156
Mobile phase composition Toluene:Ethylacetate:Ammonia solution (35:65:0.1)	1.012	0.101
Chamber saturation time (25 min)	1.071	0.108
Chamber saturation time (35 min)	1.204	0.121
Temperature for activation of plates (100°C)	1.148	0.115
Temperature for activation of plates (120°C)	1.252	0.125

Table 4: Recovery studies a)

Recovery level (%)	Amt of drug added (ng/ spot)	Amount of drug found (ng/ spot)	Percentage recovery	Mean % recovery	%RSD
80	800.000	797.670	99.71	100.87	1.24
	800.000	805.512	100.69		
	800.000	817.606	102.20		
100	1000.000	990.851	99.09	98.92	0.46
	1000.000	992.806	99.28		
	1000.000	984.086	98.41		
120	1200.000	1184.615	98.72	98.59	0.36
	1200.000	1178.243	98.19		
	1200.000	1186.308	98.86		

Mean Recovery (Mean±S.D.)= 99.46 ± 1.23

a) (n=9)

Table 5: Summary of validation parameters

Parameter	Data
Linearity range (ng spot-1)	100-2000
Correlation coefficient	0.9996
Limit of detection (ng spot-1)	33.0
Limit of quantitation (ng spot-1)	100
Percent recovery (n = 9)	99.46 ± 1.23
Precision (RSD%.)	
Repeatability of application (n = 6)	0.35
Repeatability of measurement (n = 6)	0.06
Inter-day (n = 6)	0.41
Intra-day (n =6)	0.61
Robustness	Robust
Specificity	Specific

Table 6: Analysis of TRIA sterile injectable suspension (1000.00 ng spot^{-1})

Pharmaceutical formulation	Actual concentration of TRIA (ng spot^{-1})	Amount of TRIA calculated (ng spot^{-1})	% TRIA (mean±SD) (n=3)
TRIA Sterile injectable suspension	1000.000	991.453	99.15 ± 0.41

Table 7: Forced degradation of TRIA

Stress Condition	% Degradation ±S.D
Base(0.01 N NaOH) @ RT for 2 Hour	22.67 ± 0.16
Acid(0.1 N HCl)@ 80°C for 8 Hour reflux	6.98 ± 0.15
Oxidative (3% H_2O_2) RT for 8 Hour	4.49 ± 0.13
Thermal (80°C) for 8 Hour	4.90 ± 0.10
Neutral (water)@ 80°C for 8 Hour	4.27 ± 0.04
Sunlight for 8 Hour	9.23 ± 0.11

respect to peak area. Results were tabulated in Table 1. The regression-coefficient (r) was found to be 0.9996 (Figure 5) 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 2, which revealed intra-day and inter-day variation of TRIA at a concentration level of 1000 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 3 indicated robustness of the method.

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 33.0 and 100.0 ng spot^{1}, respectively. Chromatogram at LOQ of TRIA is given in Figure 3.

Specificity

The developed method was used for the estimation of TRIA 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 TRIA from pharmaceutical dosage forms after spiking with 80, 100 and 120% of additional drug afforded mean recovery of 99.46 ± 1.23 as listed in Table 4. The data of summary of validation parameters are listed in Table 5.

Analysis of prepared formulation

A single spot of R_f 0.38 was observed in chromatogram of the TRIA samples extracted from sterile injectable suspension. There was no interference from the excipients commonly present in the formulation. The average TRIA content was found to be 99.15% ± SD of 0.41 and listed in Table 6. Therefore it may be inferred that degradation of TRIA had not occurred in the formulation which were analysed by this method. The low SD value indicated the suitability of this method for routine analysis of TRIA in pharmaceutical dosage forms.

Stability- indicating property

The chromatogram of samples degraded with acid, base, hydrogen peroxide, neutral, sunlight and heat showed well separated spots of pure TRIA 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 Figure 4. Percentage degradation was calculated and listed in Table 7.

CONCLUSION

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

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CONFLICT OF INTEREST

None.

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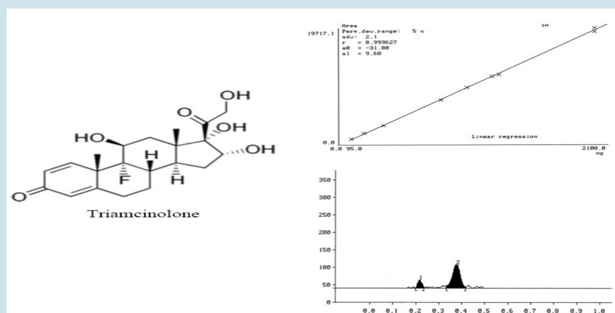
PICTORIAL ABSTRACT



SUMMARY

- A sensitive, accurate, precise and stability indicating HPTLC method was developed and validated for analysis of triamcinalone acetonide (TRIA) in bulk drug and sterile injectable suspension
- The solvent system consisted of Toluene: ethyl acetate: ammonia solution (33:67:0.1 %v/v). This system was found to give compact bands for TRIA (Rf value 0.38 ± 0.02).
- The linear regression date for the calibration plots showed good linear relationship with correlation coefficient 0.9996 ± /0.001 in the concentration range of 100.0-2000.0 ng spot-1.
- As the method could effectively separate the drug from its degradation products, it can be employed as a routine stability indicating assay method.

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