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Research Article

A VALIDATED RP-HPLC METHOD DEVELOPMENT FOR THE ESTIMATION OF TRIAMCINOLONE ACETONIDE TABLET AND INJECTION

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ABSTRACT

In the present work is to established simple, accurate, rapid and validated (as per ICH) RP-HPLC method developed for the quantification of Triamcinolone acetonide tablet and injection. In RP-HPLC method the column used was Phenomenex Luna C18, Column (250 mm x 4.6 mm id; 5 µm particle size) and the mobile phase was composed of Acetonitrile: 0.05M Phosphate buffer pH adjusted to 6.8 using NaOH with 0.1% of Triethylamine (55:45 v/v) with flow rate 1ml/min. Eluents were monitored by UV detector at 238 nm. Calibration curve was linear in the concentration ranges 10-50 µg/ml (r2=0.9998) for TCA. The percentage purity of the TCA tablet and injection was found to be 99.48 ± 0.0203 and 99.48 ± 0.0076 respectively. The precision of the method was confirmed by the marketed formulation for six times. The % RSD was found to be 0.0204 and 0.0077 for TCA. The % RSD for tablet and injection accuracy was found to be 0.0127 and 0.0034 for TCA.

INTRODUCTION

To determine the composition of natural and manufactured chemicals, qualitative analysis was carried out. The purpose of these tests was to determine if the chemical or compound was present in the sample or not. Triamcinolone acetonide (TCA) is a corticosteroid with anti-inflammatory properties. These properties are used to treat inflammation in conditions that affect various organs and tissues. Triamcinolone acetonide should not be administered as an epidural injection [1-4]. The chemical structure of Triamcinolone acetonide shown in **Figure. 1.** Using HPLC, we can separate a bunch of compounds into their constituent parts, allowing us to determine what each one is and how much of it. In this present research work, new RP-HPLC method for quantifying the estimation of Triamcinolone acetonide drug product. As a result, the proposed method can find to be good and accurate for the quantitative determination of Triamcinolone acetonide tablet and injection [5].

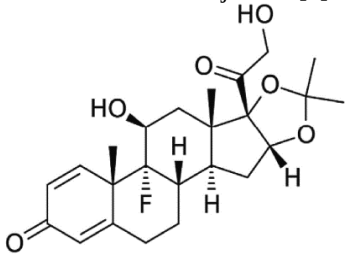

Figure 1. Structure of Triamcinolone Acetonide

MATERIALS AND METHODS

Triamcinolone acetonide was gifted by Strides pharma science ltd, Bangalore. Methanol (AR grade and HPLC grade), Acetonitrile (HPLC grade), Water (HPLC grade), Potassium dihydrogen orthophosphate, sodium hydroxide was purchased from Loba Chemie India Ltd, Mumbai and used as such.

RP-HPLC Method

The separation in the RP- HPLC is based on the non-polar nature of the drug and the polarity of the mobile phase. The columns used in RP- HPLC are usually a C8 or C18 column, which are less polar than the mobile phase. Hence the polar compounds are eluted first compared to the nonpolar compounds. Depending on the polarity of mobile phase the elution strength also varies. Lower the polarity of mobile phase, elution strength is higher. Isocratic elution and Gradient techniques are employed in RP-HPLC methods [6-8]. In the present study, an Isocratic elution method has been employed.

Selection of wavelength

10 µg/ml solution of Triamcinolone acetonide was prepared in the diluent and scanned in the range of 200 to 400 nm and the spectrum was recorded. The drug showed marked absorbance at 238 nm. Therefore, 238 nm was selected as the detection wavelength for the Isocratic elution of the drugs.

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Effect of mobile phase ratio

The ratios of the mobile phase were varied and the chromatograms were recorded. The system suitability of the chromatograms was compared and the ratio 55: 45 %v/v of Acetonitrile:0.05 M Potassium dihydrogen orthophosphate buffer pH adjust 6.8 using sodium hydroxide with 0.1 % triethylamine was finalized [9-10]. The optimized condition shown in Table 1.

Table 1. Optimized chromatographic conditions

Mode of operation	Isocratic Elution
Stationary phase	Phenomenex Luna C18 (250 mm × 4.6 mm, 5.0 µm particle size)
Mobile phase	Acetonitrile: 0.05 M Phosphate Buffer pH adjusted to 6.8 using sodium hydroxide 0.1% of Triethylamine
Ratio	55: 45 % v/v
Diluent	Acetonitrile: 0.05 M phosphate buffer (55:45)
Detection wavelength	238nm
Flow rate	1 ml/min
Temperature	Ambient
Sample load	20 µl
Method	External standard calibration method
Run time	10 min

Preparation of standard stock solution

Standard stock solution of Triamcinolone acetonide (1000 µg/ml) was prepared by dissolving 10 mg of Triamcinolone acetonide in 10 ml of diluent in 10 ml volumetric flask with vigorous shaking (stock – I), From this stock solution 1 ml was pipetted and diluted to 10 ml with diluent (stock -II), to set the concentration 100 µg/ml of Triamcinolone acetonide.

Linearity and Calibration

Different aliquots of 1 – 5 ml of TCA was transferred into series of 10 ml volumetric flasks separately, and the volume was made up to the mark with diluent to get concentration such as 10, 20, 30, 40 and 50 µg/ml solution was injected and chromatogram was recorded. The calibration curve was plotted between concentration Vs peak area (Figure 2D).

Quantification of the marketed tablet formulation

Ten Tablets were weighed, each containing 4 mg of TCA. Tablet powdered equivalent to 10 mg was transferred in to a 10 ml clean and dry volumetric flask containing 10 ml of diluent. The contents of flask were sonicated for 10 min and diluent was added to made up to mark to get a sample stock concentration of TCA. Filtered the solution. From the filtrate pipetted 1 ml of the above sample stock solution into a 10 ml clean volumetric flask and diluent was added and made up to the mark. Then, 2.5 ml was pipetted and diluted to 10 ml using diluent to get 25 µg/ml of TCA, the concentration of TCA was determined by measuring area of sample solution in 238 nm. The concentration of the drug was determined form the respective linear regression equations. The procedure was repeated for 6 times for the same concentration.

Quantification of the marketed injection formulation

One injection, each containing 40 mg of TCA. The content of the drug equivalent to 10 mg was transferred in to a 10 ml clean and dry volumetric flask containing 10 ml of diluent. The contents of flask were sonicated for 10 min and diluent was added to made up to mark to get a sample stock concentration of TCA. Filtered the solution. From the filtrate pipetted 1 ml of the above sample stock solution into a 10 ml clean volumetric flask and diluent was added and made up to the mark. Then, 2.5 ml was pipetted and diluted to 10 ml using diluent to get 25 µg/ml of TCA, the concentration of TCA was determined by measuring area of sample solution in 238 nm. The concentration of the drug was determined form the respective linear regression equations. The procedure was repeated for 6 times for the same concentration

Recovery studies

Recovery procedure of tablet formulation

The recovery studies were done by adding known concentrations of Triamcinolone acetonide raw material to pre-analyzed tablet formulation. The tablet powder equivalent to 10 mg of triamcinolone acetonide was weighed accurately and transferred into a series of three 10 ml standard flask. To that raw material Triamcinolone acetonide (50%, 75%, and 100%) were added, dissolved with minimum quantity of diluent and made up to the mark with the same solvent. The content was kept in a Sonicator for 15 minutes, after sonication the solutions was filtered through Whatman filter paper no.41 and from the clear solution further dilutions were made by diluting 1 ml to 10 ml volumetric flask with diluent and injected into RP-HPLC. The area values for 238 nm were used for the determination of Triamcinolone acetonide. The procedure was repeated for 3 times for each percentage recovery.

Recovery procedure of injection formulation

The recovery studies were done by adding known concentrations of Triamcinolone acetonide raw material to pre-analyzed injection formulation. The content of the drug equivalent to 10 mg of triamcinolone acetonide was weighed accurately and transferred into a series of three 10 ml standard flask. To that raw material Triamcinolone acetonide (50%, 75%, and 100%) were added, dissolved with minimum quantity of diluent and made up to the mark with the same solvent. The content was kept in a Sonicator for 15 minutes, after sonication the solutions was filtered through Whatman filter paper no.41 and from the clear solution further dilutions were made by diluting 1 ml to 10 ml volumetric flask with diluent and injected into RP-HPLC. The area values for 238 nm were used for the determination of Triamcinolone acetonide. The procedure was repeated for 3 times for each percentage recovery (Table 3).

Precision

The reproducibility of this method was determined by analyzing formulation at different time intervals on same day in triplicated (intra-day assay precision) and on three different days (inter-day assay precision) in triplicated.

System suitability studies

The system suitability study was made (Table 2) based on USP. The various parameters such as capacity factor, tailing factor, asymmetric factor, number of theoretical plates and HETP were calculated.

Validation of developed method

Linearity

A calibration curve was plotted between concentration Vs peak area. Triamcinolone acetonide was linear with the

concentration range of 10 – 50 µg/ml at 238 nm by obeying Beer's law.

Accuracy

Accuracy of the method was confirmed (Table 5) by recovery studies. To the pre- analyzed formulation, a known quantity of raw material of Triamcinolone acetonide was added and the procedure was followed as per the analysis of formulation. The amount of each drug recovered was calculated. This procedure was repeated for three times for each concentration. The % RSD was calculated.

Precision

The repeatability of the method was confirmed (Table 4) by the analysis of formulation and repeated for 6 times with the same concentration. The amount of each drug present in the marketed formulation was calculated. The percentage RSD was calculated.

LOD and LOQ

The linearity study was carried out for six times. The LOD and LOQ were calculated (Table 2) based up on the calibration curve method. The LOD and LOQ were calculated using average value of slope and standard deviation of response (intercept).

RESULT AND DISCUSSION

The simple, precise and accurate RP-HPLC method was developed for the estimation of Triamcinolone acetonide in pharmaceutical dosage form. The mobile phase consists of Acetonitrile: 0.05 M phosphate buffer pH adjust 6.8 using sodium hydroxide (55: 45% v/v) with 0.1% of Triethylamine was selected for the analysis. From the spectral characteristics 238 nm was selected as the wavelength for the analysis.

The method developed has the advantages like the run time required for recording creators were less than 10 mins with greater sensitivity. The proposed method being precise and sensitive can be used for quantitative determination of Triamcinolone acetonide in raw material, tablet and injection formulation.

Statistical analysis proved that the method developed was accurate, precise and repeatable. The proposed method was found to be simple selective and sensitive for the analysis of Triamcinolone acetonide. The retention time was found to be 5.249 minutes for Triamcinolone acetonide. The drug was linear in the concentration of 10-50 µg/ml of Triamcinolone acetonide. The correlation coefficient of the Triamcinolone acetonide was found to be 0.9998. The optical characteristics were calculated. The percentage purity of drug in the tablet formulation was found to be 99.48 ± 0.0203 for Triamcinolone acetonide. The percentage purity of drug in the injection formulation was found to be 99.48 ± 0.0076 for Triamcinolone acetonide.

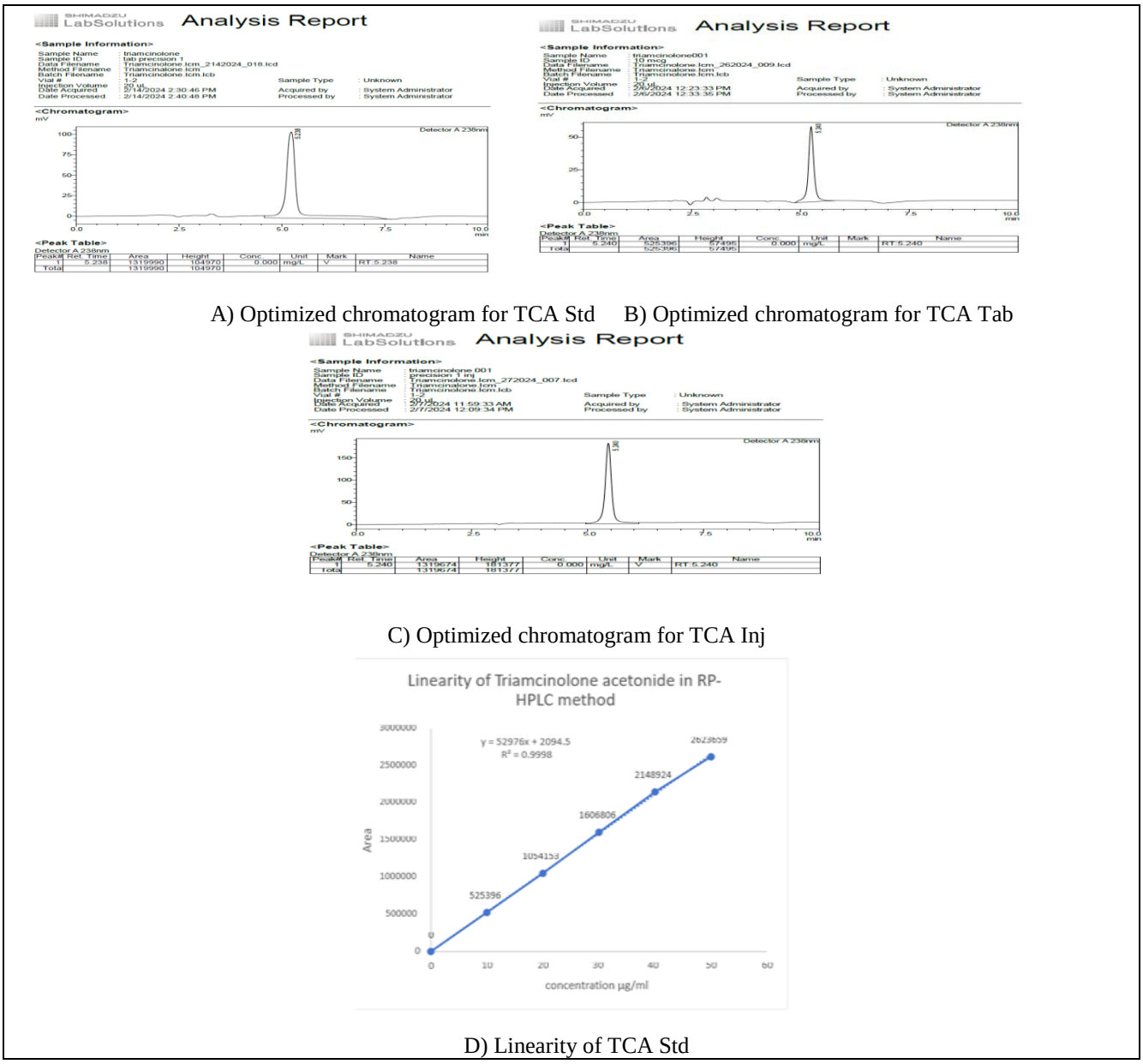
Table 2. System suitability and optical characteristics for TCA

Parameters	TCA @ 238 nm
Retention time	5.240
Peak area	1319990
Tailing factor	0.8
Theoretical plate	2745.76
Beer's law limit	10-50 µg/ml
Correlation coefficient	0.9998
Regression equation	Y = 52976x + 2094.5
Slope	52976.806
Intercept	2094.5
LOD	1.341 µg/ml
LOQ	4.064 µg/ml

Table 3. Quantification and Recovery of Triamcinolone acetonide (TCA) tablet and injection

Quantification of TCA tablet and injection							
Drug	**Sample No.**	**Labelled amount (mg)**	**Amount found (mg)**	**% Obtained**	**% Average**	**SD**	**% RSD**
TCA Tab	1	4	3.98	99.50	99.48	0.0203	0.0204
	2	4	3.97	99.49			
	3	4	3.97	99.49			
	4	4	3.97	99.49			
	5	4	3.97	99.49			
	6	4	3.97	99.49			
TCA Inj	1	40	39.79	99.49	99.48	0.0076	0.0077
	2	40	39.79	99.49			
	3	40	39.79	99.49			
	4	40	39.79	99.49			
	5	40	39.79	99.49			
	6	40	39.78	99.47			
Recovery of TCA tablet and injection							
Drug	**Recovery %**	**Amount present (µg/ml)**	**Amount added (µg/ml)**	**Amount recovered (µg/ml)**	**Amount recovered %**	**SD**	**RSD**
TCA Tab	50	25	12.5	12.46	99.95	0.0111	0.0111
	75	25	18.75	18.62	99.43	0.0108	0.0109
	100	25	25	24.50	98.03	0.0125	0.0127
TCA Inj	50	25	12.5	12.43	99.92	0.0218	0.0218
	75	25	18.75	18.64	99.45	0.0155	0.0154
	100	25	25	24.59	98.14	0.0033	0.0034

Figure 2. Optimized chromatograms and linearity



A) Optimized chromatogram for TCA Std B) Optimized chromatogram for TCA Tab

C) Optimized chromatogram for TCA Inj

D) Linearity of TCA Std

Table 4. Precision for TCA tablet and injection

Sample No.	TCA Tab		TCA Inj	
	Peak area response	%	Peak area response	%
1	1319990	99.50	1319825	99.49
2	1319821	99.49	1319674	99.48
3	1319826	99.49	1319764	99.49
4	1319628	99.48	1319647	99.48
5	1319812	99.49	1319742	99.49
6	1319218	99.45	1319536	99.47
Average	99.48		99.48	
% RSD	0.0204		0.0077	

The precision of the method was confirmed by repeatability of tablet formulation for 6 times. The percentage RSD was found to be 0.0204 for Triamcinolone acetonide. The precision of the method was confirmed by repeatability of injection formulation for 6 times. The percentage RSD was found to be 0.0077 for Triamcinolone acetonide. The accuracy of the method was performed by recovery studies. To the pre-analyzed tablet formulation, a known quantity of standard API material solution was added at three different concentrations (50%, 75% and 100%) and the solutions were injected. The percentage recovery was found to be in the range of 99.95%, 99.45% and 98.03 % respectively. The percentage RSD values were found to be 0.0111, 0.0109 and 0.0127 for Triamcinolone acetonide. The low percentage RSD values indicated that there are no interferences due to the excipients used in tablet formulation. The accuracy of the method was performed by recovery studies. To the pre-analyzed injection formulation, a known quantity of standard API material solution was added at three different concentrations (50%, 75% and 100%) and the solutions were injected. The percentage recovery was found to be in the range of 99.92 %, 99.43 % and 98.03 % respectively. The percentage RSD values were found to be 0.0218, 0.0155 and 0.0034 for Triamcinolone acetonide. The low percentage RSD values indicated that there are no interferences due to the excipients used in injection formulation.

Table 5. Accuracy of TCA tablet and injection

Sample No.	TCA Tab			TCA Inj		
	% Recovery	SD	% RSD	% Recovery	SD	% RSD
1	99.50	0.0185	0.0186	99.47	0.0140	0.0141
2	99.47			99.50		
3	99.50			99.49		

CONCLUSION

By application of newer analytical techniques, the method was found to be simple, rapid and accurate and an isocratic RP-HPLC method has excellent sensitivity and reproducibility. The results obtained in recovery studies were indicating that there is no interference from the excipients undo in the formulation. Hence it is suggested that the proposed an isocratic RP-HPLC methods can be effectively applied for the routine analysis of Triamcinolone acetonide in marketed formulation and the obtained results will be presented elsewhere. RP-HPLC method reduces overall assay development time and provides essential information regarding the sensitivity of various chromatographic variables on separation attributes.

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