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Original Article

RP-HPLC Method Development and Validation for Estimation of Triamcinolone Acetonide in Injectable Suspension using USP-Type-IV Dissolution Apparatus

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ARTICLE INFO	ABSTRACT

A new simple, precise, accurate and selective RP-HPLC method has been developed and validated for stability indicating RP-HPLC method for estimation of Triamcinolone acetonide injectable suspension Pharmaceutical dosage form.In this method to optimize the mobile phase, various combinations of buffer, acetonitrile and water were studied on Inertsil ODS 3V, 150x4.6mm, 5 μm with a flow rate of 1.0 ml/min .The detection was carried out at 254nm.The retention time for Triamcinolone acetonide were found to 2.72 min respectively. The method was validated according to the ICH guidelines for specificity, precision, accuracy, linearity and robustness. The method showed good reproducibility and recovery with %RSD less than 2. So the proposed method was found to be simple, specific, precise, accurate and linear. Hence it can be applied for routine analysis of Triamcinolone acetonide in bulk and pharmaceutical preparations.

Keywords: Triamcinolone acetonide, acetonitrile, linearity and robustness.

1. INTRODUCTION

Dissolution and drug release tests are in-vitro tests that measure the rate and extent of dissolution or release of the drug substance from a drug product, usually aq.medium under specified conditions. It is an important QC procedure for the drug product and linked to product performance in-vivo. HPLC continues to be very biological samples. Detection of a drug or related substance in biological samples is usually complicated by the matrix. Therefore clean up procedures are employed to effectively separate drugs from endogenous materials. The ultimate sensitivity of an assay may be limited by efficiency of cleanup methods.

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Pharmaceutical formulation and in biological material is primarily by the LC techniques the interest of chiral separation has developed along with the increased axis of useful separation methods in particular owing to new chiral stationary phases. The new awareness combined with increased axis of tools for selective separation by HPLC has generated most rapidly growing application field in bio analytical chemistry. In the modern pharmaceutical industry, HPLC is the major and integral analytical tool applied in all stages of drug discovery and drug development and production. HPLC coupled with MS to support target selection (proteomics), biological screening and assay development, high throughput compound analysis and characterization, UV and mass directed fractionation for un attended, automated compound purification and high throughput in vitro ADME screening. The number of drugs introduced into the market is increasing every year. These Drugs may be either new entities or partial structural modification of the existing one. Very often there is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopeias. This happens due to the possible uncertainties in the continuous and wider usage of these drugs, reports of new toxicities (Resulting in their withdrawal from the market), development of Patient resistance and introduction of better drugs by competitors. Under these conditions, standards and analytical procedures for these drugs may not be available in the Pharmacopeia. Therefore it becomes necessary to develop newer analytical methods for such drugs.

2. MATERIAL AND METHODOLOGY

In the present investigation, we have developed a simple and sensitive RP-HPLC method for quantitative estimation of Triamcinolone acetonide inbulk drug Pharmaceutical injectable suspension dosage forms.These are trails performed for HPLC method development ofTriamcinolone acetonide injectable suspension.

Selection of wavelength:

The detection of wavelength was selected by dissolving triamcinolone acetonidein acetonitrile to get concentration of the 10 µg/ml. The resulting solution was scanned in U.V range from 200-400nmAfter thorough examination of the spectra, the wave length 254 nm was selected for further analysis.

Method development trails

TRIAL: 1

Mobile phase :(90:10% Water: Methanol

➤ Column **:** ZORBAX ECLIPSE XDB C8 150x4.6mm, 5 µm
➤ Flow rate **:** 1.4ml/min
➤ Column temp **:** 25C
➤ Sample temp **:** Ambient
➤ Injection volume **:** 20 µl
➤ Run time **:** 20 min
➤ Wavelength **:** 254 nm

➤ Mode of elution**:** Isocratic
➤ Rt : 11.2 min

Fig 1: chromatogram of triamcinolone acetonide for trail-1

Trial: 2: Mobile phase :(70:20:10% Water:Methanol: ACN)

Mix water, methanol and acetonitrile in the ratio of70:20:10 %v/v and sonicate to degas.

Column : ACE C18 (250×4.6×mm, 5µm)
Flow rate : 1.2ml/min
Column temp : 25ºC
Sample temp : Ambient
Injection volume : 10 µl
Run time : 20 min
Wavelength : 254 nm
Mode of elution : Isocratic
Rt : 4.24 min
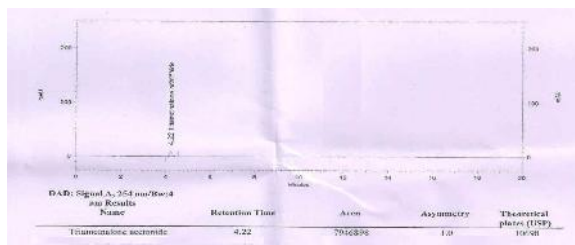
Fig 2: chromatogram of triamcinolone acetonide for trail-2

OPTIMIZED METHOD Mobile phase :(40:60% Water: ACN)

Mix water , acetonitrile in the ratio of40:90 %v/v and sonicate to degas.

Column : Inertsil ODS 3V, 150x4.6mm, 5 µm
Flow rate : 1.0 ml/min
Column temp : Ambient
Sample temp : Ambient
Injection volum : 10 µl
Run time : 5min
Wavelength : 254 nm
Rt : 2.72 min
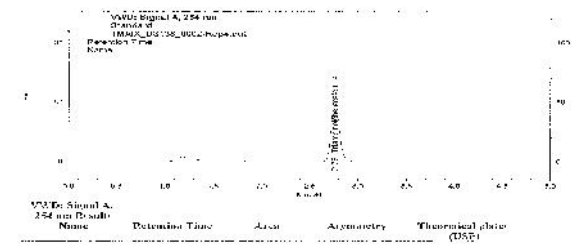
Fig 3: Chromatogram of Triamcinolone acetonide

METHOD VALIDATION

1. SYSTEM SUITABILITY: The HPLC system was stabilized for thirty min. by following the chromatographic conditions to get a stable base line. One blank followed by six replicates of a standard solution was injected to check the system suitability. The system suitability parameters were evaluated from standard Chromatograms obtained, by calculating the retention times, tailing factor, theoretical plates and %RSD peak areas from six replicate injections. The results are shown in the below table

Procedure: The blank and standard were prepared like as sample method and injected into HPLC system. The results of standard solution were summarized in below table.

Table 1: System suitability data Triamcinolone Acetonide

System suitability parameters	Results	Acceptance criteria
% RSD for analyte peak areas of five replicate standard injections	0.26	NMT 2.0
Theoretical plates	6123	NLT 2000
Tailing factor	1.24	NMT 2.0

Observation:

From the system suitability studies it is observed that all parameters are within limits. Hence it is concluded that the instrument, reagents and column are suitable to perform the assay.
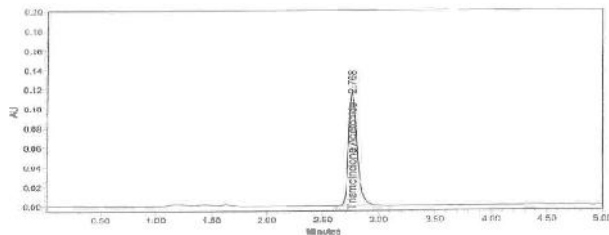
SPECIFICITY

The effect of wide range of excipients and other additives usually present in the Triamcinolone Acetonide the determinations under optimum conditions were investigated. Placebo solution, sample and standard solutions were analysed individually as per the method to examine interference. Chromatograms of placebo, standard and sample were shown in below Figures.

Blank interference:

Blank solution injected. It was observed that, no blank peaks were interfering with analyte peak.

Fig 4: Typical chromatogram of standard
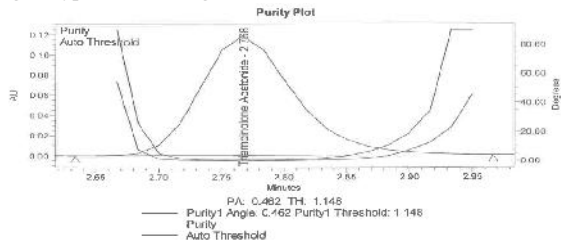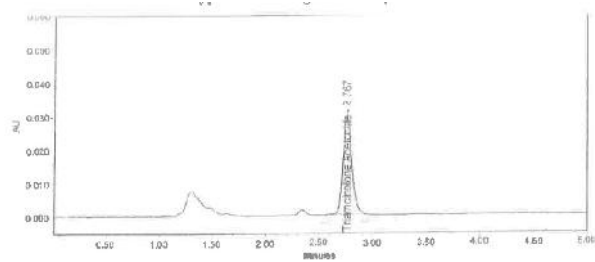

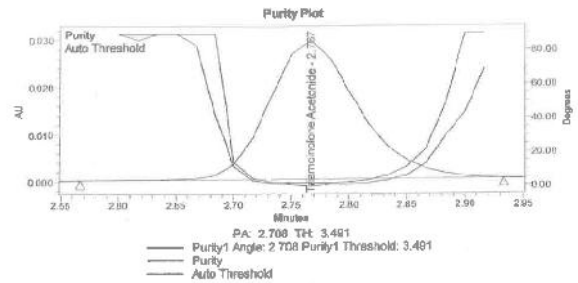Fig 5: Peak purity of standard

Observation:

There was no interference due to blank at the retention time of the analyte. Hence the method is specific.

Placebo interference:

Placebo of Triamcinolone Acetonide is 1 mL of placebo sample prepared and injected. It was observed that, no placebo peaks were interfering with analyte peak.

Fig 6: Typical chromatogram of sample

Fig 7: Peak purity of standard

Observation:

The chromatograms of the standard and sample were identical.

ACCURACY: The accuracy of the developed method was determined by assay and recovery studies. Recovery studies were carried out at three different levels. The pre-analysedsamples were spiked with 10%, 100%, and 120% of mixed standard solution. The mixtures were analysed by the proposed method.

Procedure

A series of sample solutions were prepared in triplicate by spiking the Triamcinolone Acetonide with placebo in the range of 10% to 120% and injected into HPLC system and analyzed .

Individual % recovery, mean % recovery and % RSD were calculated and the results were found to be within the acceptable limits.

Table 2: Accuracy results of Triamcinolone Acetonide

S. No.	% Spike level	Amount added(mg/ml)	Amount recovered(mg/ml)	% Recovery	Mean %recovery	%RSD
1.	10%	0.0045	0.00445	98.9	100.4	1.5
2.		0.0045	0.00453	100.7		
3.		0.0045	0.00458	101.8		
1.	100%	0.0453	0.0444	98.0	99.3	1.6
2.		0.0453	0.0458	101.1		
3.		0.0453	0.0448	98.9		

1.	120%	0.0542	0.0555	102.4	100.8	1.4
2.		0.0542	0.0543	100.2		
3.		0.0542	0.0541	99.8		

Observation:

The recovery results indicating that the test method has an acceptable level of accuracy. The results were found to be within the limits.

Linearity:

A series of solutions of Triamcinolone Acetonide, with concentrations of ranging from about 10% to 125% of specification level were prepared and injected into the HPLC system.

Linearity of detector response was established by plotting a graph of concentration and responses of Triamcinolone Acetonide. The detector response was found to be linear from about 10 to 150% of specification level. The squared correlation coefficient, slope and intercept were calculated by least square fit method for Triamcinolone Acetonide. The linearity results and graphs are summarized in below.
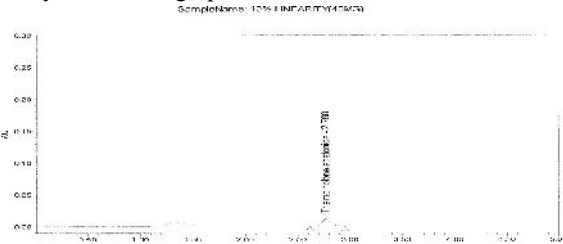

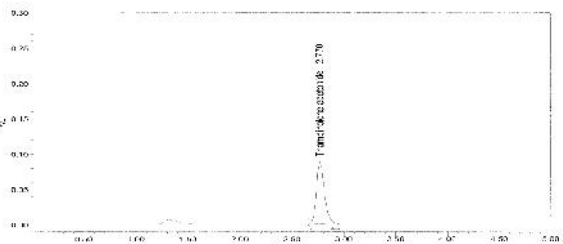**Fig 8: Typical Chromatogram of linearity of 10% Level**

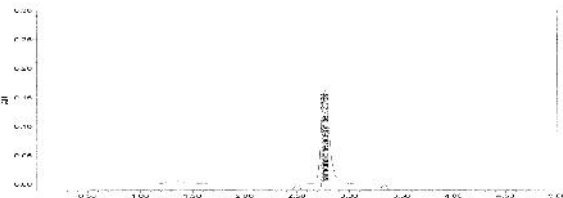**Fig 9: Typical Chromatogram of linearity of 75% Level**

Fig 10: Typical Chromatogram of linearity of 150% Level

PRECISION:

The precision of the analytical method was studied by analysis of multiple sampling of homogeneous sample. The precision expressed as standard deviation or relative standard deviation. According to the ICH, precision should be performed at three different levels:

➢ System precision (Repeatability)
➢ Method precision (Reproducibility)

➢ Intermediate precision

Method precision for 40 mg: USP-IV (Flow through cell):

To evaluate the method precision for dissolution method, six samples were prepared and analyzed.

% RSD of each time intervals were calculated and results summarized in below table.

Table 3: Method precision results of Triamcinolone Acetonide

Sample	% Drug dissolved											
	5 Min	10 Min	15 Min	20 Min	30 Min	40 Min	50 Min	60 Min	70 Min	80 Min	90 Min	120 Min
1	22	42	53	59	67	73	78	81	83	85	87	89
2	24	43	53	59	67	73	78	81	84	86	87	90
3	23	42	52	58	66	72	76	80	83	85	87	90
4	24	42	52	59	67	73	77	81	84	85	87	90
5	24	42	53	59	67	73	77	80	83	84	86	88
6	23	43	54	61	70	76	81	85	88	90	92	95
Mean	23	42	53	59	67	73	78	85	84	86	88	90
% RSD	3.5	1.2	1.4	1.7	2	1.9	2.2	2.3	2.3	2.5	2.5	2.7

Observation:

From the method precisions studies it is observed that all the parameters like %RSD of retention time and peak areas are within limits.

SOLUTION STABILITY:

The stability of solutions was evaluated by injecting the standard and samples from method precision at initial, 3rd day and 5th day at room temperature. The areas of standard and sample injections were compared.
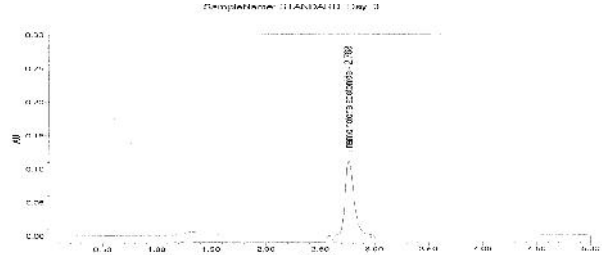

Fig 11: A Representative chromatogram of stabiliy day 3

Table 4: Stability data ofTriamcinolone Acetonide

	Area response	% Difference
Initial	3930435	N/A
Day-3	3936484	0.1
Day-5	3938072	0.1

Observation: All the samples were found to be stable up to 5days.

Robustness:

For demonstrating the robustness of the developed method, experimental conditions were purposely altered and evaluated. The method must be robust enough to withstand such slight changes and allow routine analysis of the sample. Following optimized conditions were slightly varied.

➢ To evaluate the Robustness for dissolution method, Standard solution was prepared and injected into HPLC as per test method by changing flow, and temperature

A study was conducted to determine the effect of variation in flow rate. Standard solution was prepared and injected into the HPLC system by keeping flow rates (± 0.2 ml/min) i.e., 0.8 ml/min and 1.2 ml/min. The effect of variation of flow rate was evaluated. The observations of variation of flow rate were given in below tables.

Table 5: System suitability data for Column oven temperature variation

System suitability parameters	Results			
	As such (25°C)	-Column oven temperature (20°C)	+Column oven temperature (30°C)	Acceptance criteria
% RSD for analyte peak areas of five replicate standard injections	0.09	0.15	0.44	NMT 2.0
Theoretical plates	5035	4766	4776	NLT 2000
Tailing factor	1.1	1.1	1.1	NMT 2.0

3. RESULTS AND OBSERVATIONS

Out In the present work reports a new RP-HPLC method for estimation of Triamcinolone acetonide injectable suspension Pharmaceutical dosage form.In this method to optimize the mobile phase, various combinations of buffer, acetonitrile and water were studied on Inertsil ODS 3V, 150x4.6mm, 5 µm Initially. Then combination of the solvents were modified by using of the compounds were not satisfactory. Then the mobile phase containing a mixture of water and acetonitrile was carried out and found that the ratio 60:40 technique resulted in peaks with good shape and resolution. A flow rate of 1.0 ml/min was found to be optimum in the range of 0.8-1.2 ml/min resulting in short retention time, baseline stability and minimum noise and the retention times of Triamcinolone acetonide were found to be, 2.72min.

The developed method is validated in accordance with the ICH guidelines with all of the results within the limits. Quantitative linearity was obeyed in the concentration range of Triamcinolone acetonide 40 µg/ml of the regression equations of concentration over their peak areas were found to be Y = (R^2=0.9999) for Triamcinolone acetonide where Y is the peak area and x is concentration of drugs (µg/ml).

Precision of the method was studied by making the replicate injections of the standard solutions and standard deviation was determined. The % RSD value (below 2) indicates that the method was Précised.

The high percentage recovery indicates that the proposed method is highly accurate. The % Recovery of were found to be 100.4 for 10% concentration; 99.3, for 100% concentration and 100.8 for 150% concentration.Robustness of the method was performed by varying the flow rate of mobile phase and column temperature. Ruggedness of the method was determined by performing the experiment by different analysts.

Finally the developed HPLC method was applied for estimation of Triamcinolone acetonide in Pharmaceutical dosage forms. No interfering peaks were found in the chromatogram indicating that excipients used in tablet formulations didn't interfere with the estimation of the drugs by the proposed HPLC method.

Thus, a simple, sensitive, precise and accurate RP-HPLC method was developed and validated for the estimation of Triamcinolone acetonide injectable suspension pharmaceutical dosage form.

Table 6: Validation parametersTriamcinolone acetonide by HPLC

PARAMETER	ACCEPTANCE CRITERIA	Triamcinolone acetonide
Linearity Range Correlation Coefficient	Correlation coefficient $r^2 > 0.999$ or	$r^2 = 0.9999$
System Suitability	RSD < 2% Theoretical plates Tailing factor	%RSD = 0.26 6123 1.24
Intermediate Precision	RSD < 2%	%RSD = 0.72
Method precision	RSD < 2%	%RSD = 0.57
Accuracy	Recovery 98- 102% (individual)	% recovery=100.1
Solution Stability	> 12 hour % area difference < 2%	Stable up to 5days % area difference = 0.1
Robustness	RSD NMT 2% in modified condition Flow minus Flow plus Column temperature minus Column temperature plus	Complies %RSD= 0.14 %RSD= 0.17 %RSD=0.15 %RSD=0.44
Filter validation	% area difference < 2% Centrifugation Nylon filteration PVDF fiteration	-0.1 0 1

4. CONCLUSION

Development of new analytical methods for the determination of drugs in Pharmaceutical dosage forms is of great importance in quality control, pharmacokinetic, toxicological and biological studies. The commonly used tests of Pharmaceutical analysis generally entail compendia testing method development, setting specifications and limits, and method validation. New methods are now being developed, so that, new products can be assured to have comparable quality and can be brought to international markets faster.

Pharmaceutical analysis plays a pivotal role in statuary certification of drugs and their formulations either by the industry or by the regulatory authorities. The current good manufacturing practices (CGMP) and the Food Drug Administration (FDA) guidelines insist for adoption of

sound analytical methods with greater sensitivity and reproducibility. Therefore, the complexity of problems encountered in pharmaceutical analysis with the importance of achieving is the selectivity, speed, low cost, simplicity, sensitivity, specificity, precision and accuracy in estimation of drugs.

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