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DEVELOPMENT AND VALIDATION OF A METHOD FOR TRIAMCINOLONE HEXACETONIDE IN BULK AND DOSAGE FORM BY RP-HPLC

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Abstract:
In the present investigation, a simple, sensitive, precise and accurate Reverse phase high performance liquid Chromatography method developed and validated for estimation of Triamcinolone hexacetonide in injection formulation. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Triamcinolone hexacetonide was freely soluble in ethanol, methanol and sparingly soluble in water. ACN: Methanol: water (10:30:60) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed in tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Triamcinolone hexacetonide in injection formulation and in other Pharmaceutical dosage forms.

Key words: RP-HPLC, Triamcinolone hexacetonide.

INTRODUCTION:

Triamcinolone is a corticosteroid used to treat various inflammatory conditions in the body from allergic rhinitis to acute exacerbations of multiple sclerosis. Triamcinolone can be used as a one time adjunct treatment of osteoarthritic knee pain, or first line as a topical treatment of corticosteroid responsive dermatoses. Triamcinolone is more commonly seen in the forms triamcinolone hexacetonide, triamcinolone acetonide, and triamcinolone diacetate.[1-4] IUPAC name is 2-[(1S,2S,4R,8S,9S,11S,12R,13S)-12-fluoro-11-hydroxy-6,6,9,13-tetramethyl-16-oxo-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]icosa-14,17-dien-8-yl]-2-oxoethyl 3,3-dimethylbutanoate. Molecular formula $C_{30}H_{41}FO_7$. Molecular Weight is 532.6.

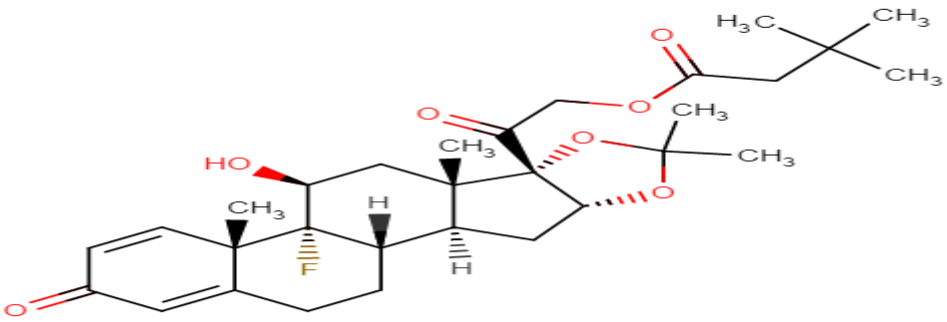

Figure 1: Structure of Triamcinolone hexacetonide

The literature survey revealed that There are very few methods reported in the literature for analysis of Triamcinolone hexacetonide alone or in combination with other drugs in the pure form and pharmaceuticals formulations.[5-15] In view of the need for a suitable, cost-effective RP-HPLC method for routine analysis of Triamcinolone hexacetonide estimation of in pharmaceutical dosage form. Attempts were made to develop simple, precise, accurate and cost-effective analytical method for the estimation of Triamcinolone hexacetonide. The proposed method will be validated as per ICH guidelines. The objective of the proposed work is to develop a new, simple, sensitive, accurate and economical analytical method and validation for the estimation of Triamcinolone hexacetonide in pharmaceutical dosage form by using RP-HPLC. To validate the developed method in accordance with ICH guidelines for the intended analytical application i.e., to apply the proposed method for analysis of the drug in its dosage form.

MATERIALS AND METHODS:

Chemicals and Reagents: Triamcinolone hexacetonide Gift samples obtained from Hetero Drugs Ltd, Hyderabad. NaH_2PO_4 was analytical grade supplied by Finerchem limited, Orthophosphoric acid (Merck), and Water and Methanol for HPLC (Lichrosolv (Merck).

Equipment: The chromatography was performed on a Waters 2695 HPLC system, equipped with an auto sampler, UV detector and Empower 2 software.

Preparation of solutions:

Preparation of standard solution:

Accurately weigh and transfer 10 mg of Triamcinolone hexacetonide working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.7ml of the above Triamcinolone hexacetonide stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure:

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization:
Initially the mobile phase tried was methanol: Water and Acetonitrile: Water with varying proportions. Finally, the mobile phase was optimized to ACN: Methanol :Water in proportion 10:30:60 v/v respectively.

Optimization of Column:
The method was performed with various C18 columns like Gemini,ODS column, and X Bridge C18 column. XBridge C18 (4.6×250mm) 5µ was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Preparation of mobile phase:
Accurately measured 100 ml (10%) of HPLC grade ACN, 300ml Methanol (30%) and 600 ml of Water (60%) were mixed and degassed in a digital ultrasonicator for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:
The Mobile phase was used as the diluent.

METHOD:
The developed chromatographic method was validated for system suitability, linearity accuracy, precision, ruggedness and robustness as per ICH guidelines.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS:

Instrument used	:	Waters HPLC with auto sampler and RID detector.
Temperature	:	40ºC
Column	:	XBridge C18 (4.6×250mm) 5µ
Mobile phase	:	ACN: Methanol: Water (10:30:60% v/v)
Flow rate	:	0.8ml/min
Wavelength	:	260nm
Injection volume	:	10µl
Run time	:	6minutes

Retention time, tailing factor and USP theoretical plate count of the developed method are shown in table 1.

Assay of pharmaceutical formulation: The proposed validated method was successfully applied to determine Triamcinolone hexacetonide dosage form. The result obtained for was comparable with the corresponding labeled amounts and they were shown in Table-2.

Validation of Analytical method:

Linearity: The linearity study was performed for the concentration of 20 µg/ml to 100 µg/ml level. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient. Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient. The results are shown in table 3.

Accuracy studies: The accuracy was determined by help of recovery study. The recovery method carried out at three level 50%, 100%,150%. Inject the standard solutions into chromatographic system. Calculate the Amount found and Amount added for Triamcinolone hexacetonide and calculate the individual recovery and mean recovery values. The results are shown in table 4.

Precision Studies: precision was calculated from Coefficient of variance for five replicate injections of the standard. The standard solution was injected for five times and measured the area for all five Injections in HPLC. The %RSD for the area of five replicate injections was found. The results are shown in table 5.

Ruggedness: To evaluate the intermediate precision of the method, Precision was performed on different day. The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found. The results are shown in table 6.

Robustness: As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition was made to evaluate the impact on the method. The results are shown in table 7.

LOD and LOQ: The sensitivity of RP-HPLC was determined from LOD and LOQ. Which were calculated from the calibration curve using the following equations as per ICH guidelines. The results are shown in table 8.

LOD = 3.3σ/S and

LOQ = 10 σ/S, where

σ= Standard deviation of y intercept of regression line,

S = Slope of the calibration curve

RESULTS AND DISCUSSION

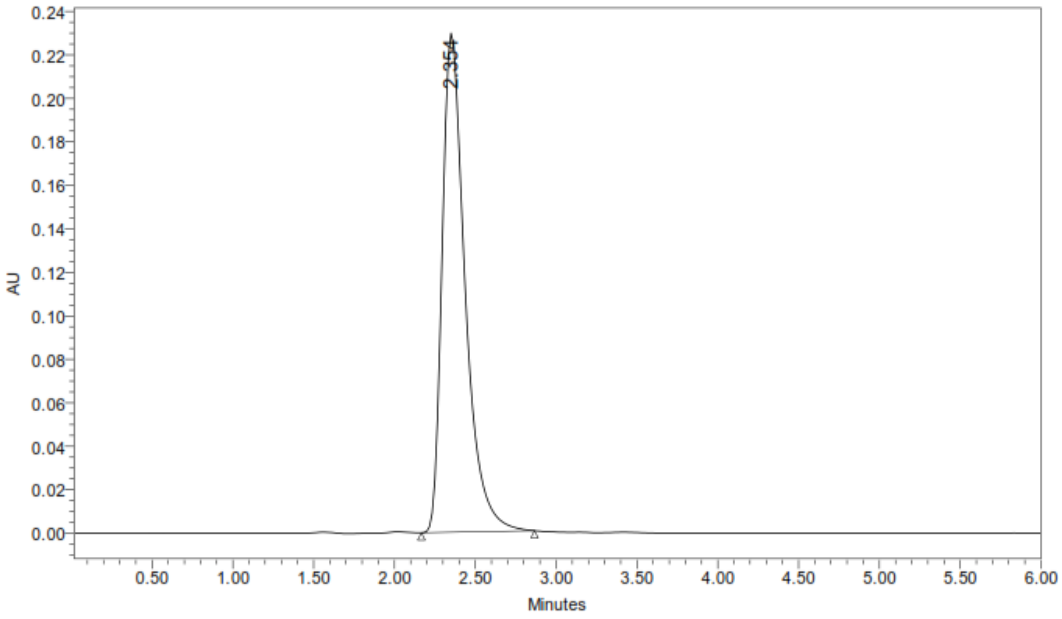

Figure 2: Standard chromatogram

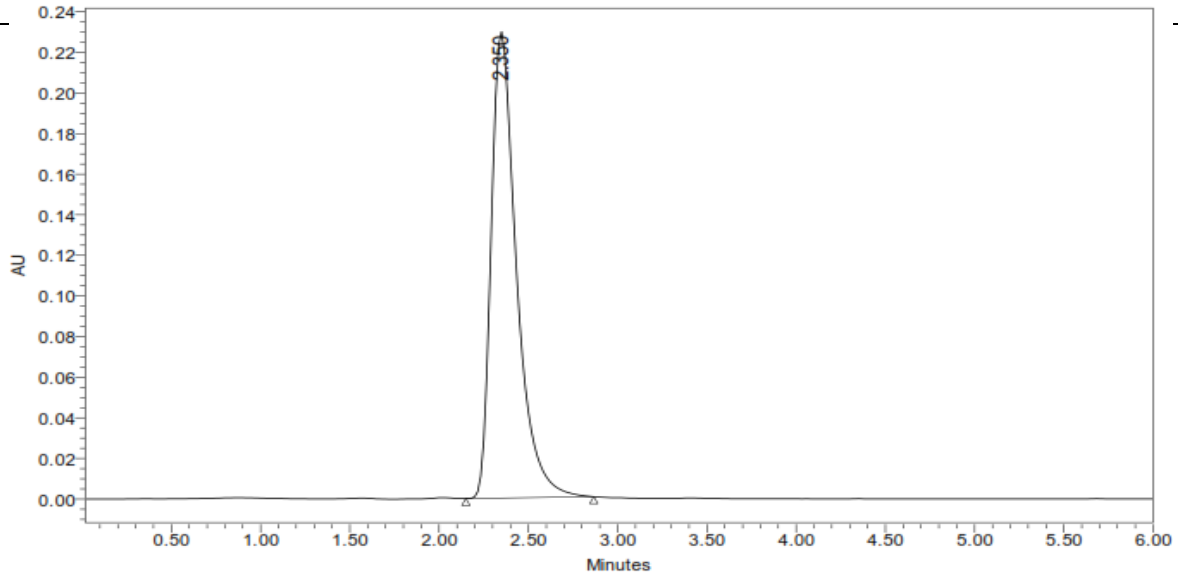

Figure 3: Sample chromatogram

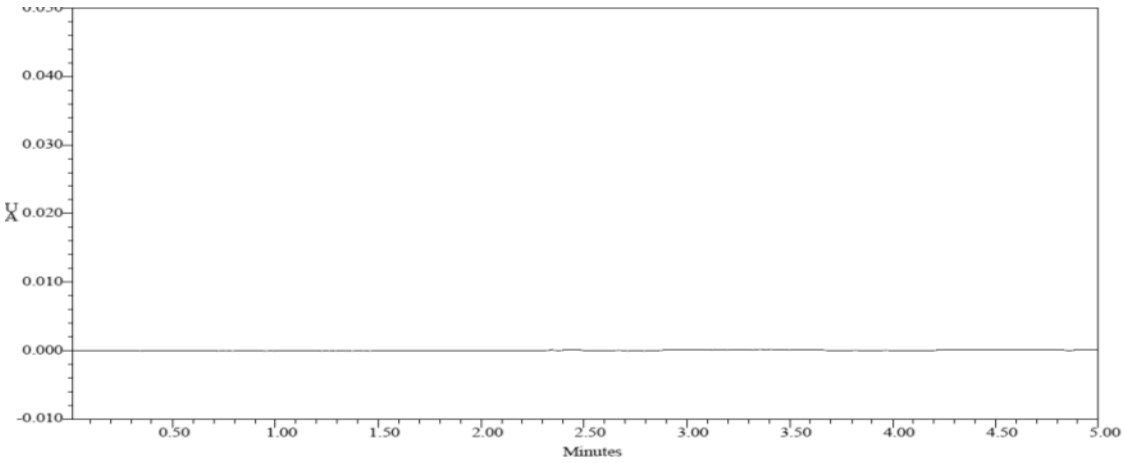

Figure 4: Blank chromatogram

Table 1: System suitability parameters

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Triamcinolone hexacetonide	2.321	2204850	239452	5281	1.2
2	Triamcinolone hexacetonide	2.302	2284721	239582	5093	1.2
3	Triamcinolone hexacetonide	2.323	2238127	236493	5391	1.2
4	Triamcinolone hexacetonide	2.343	2259349	249482	6139	1.2
5	Triamcinolone hexacetonide	2.317	2274631	239458	5728	1.2
Mean			2252336			
Std. Dev.			31827.08			

% RSD			1.41307			

Table 2: Assay results for Triamcinolone hexacetonide

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Triamcinolone hexacetonide	2.354	2255919	248281	1.2	6582	1
2	Triamcinolone hexacetonide	2.350	2255538	249382	1.2	5928	2
3	Triamcinolone hexacetonide	2.354	2253363	241533	1.2	5291	3

Table 3: Linearity results of Triamcinolone hexacetonide

Concentration µg/ml	Average Peak Area
20	791354
40	1657073
60	2293804
80	3158339
100	3939630

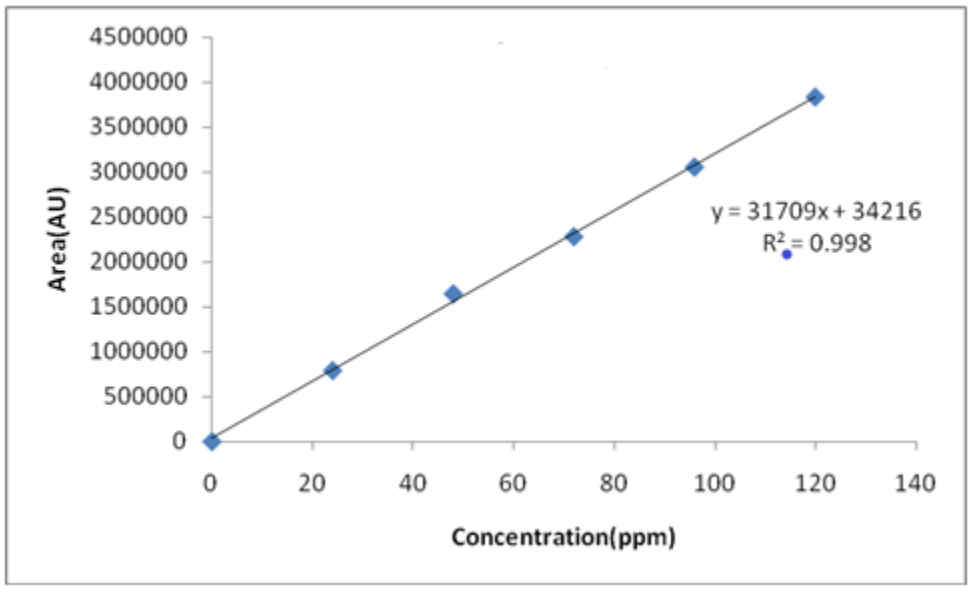

Figure 5: Linearity graph for Triamcinolone hexacetonide

Table 4: Showing accuracy results for Triamcinolone hexacetonide

Table 5: Precision results for Triamcinolone hexacetonide

S. No	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Triamcinolone hexacetonide	2.356	2279464	245362	5938	1.2
2	Triamcinolone hexacetonide	2.356	2285915	248293	5827	1.2
3	Triamcinolone hexacetonide	2.357	2282117	240795	5032	1.2
4	Triamcinolone hexacetonide	2.358	2288675	230139	5978	1.2
5	Triamcinolone hexacetonide	2.359	2272448	249605	6183	1.2
Mean			2275724			
Std.dev			9476.485			
%RSD			0.416416			

Table 6. Ruggedness results of Triamcinolone hexacetonide

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USPTailing
1	Triamcinolone hexacetonide	2.380	2236184	202188	5472	1.2
2	Triamcinolone hexacetonide	2.383	2238020	201837	6193	1.2
3	Triamcinolone hexacetonide	2.385	2239352	201273	5980	1.2
4	Triamcinolone hexacetonide	2.385	2242466	203923	7163	1.2
5	Triamcinolone hexacetonide	2.389	2244692	202938	6182	1.2
6	Triamcinolone hexacetonide	2.389	2247654	201982	7684	1.2
Mean			2241395			
Std. Dev.			4333.851			

%			0.193355			

Table 7: Robustness results for Triamcinolone hexacetonide

Parameter used for sample analysis	**Peak Area**	**Retention Time**	**Theoretical plates**	**Tailing factor**
Less Flow rate of 0.7mL/min	2650811	2.765	5551	1.2
More Flow rate of 0.9mL/min	2740254	2.234	5421	1.2
Less organic phase	2740658	2.763	4803	1.5
More organic phase	2740325	2.236	4691	1.5

Table 8: LOD, LOQ of Triamcinolone hexacetonide

Drug	**LOD**	**LOQ**
Triamcinolone hexacetonide	5.5	16.7

CONCLUSION:

The Developed HPLC method was validated and it was found to be simple, precise, accurate and sensitive for the estimation of Triamcinolone hexacetonide in injection formulation and in its pharmaceutical dosage forms. Hence, this method can easily and conveniently adopt for routine quality control analysis of Triamcinolone hexacetonide in injection formulation and in other pharmaceutical dosage forms.

REFERENCES:

1. FLORINI JR, SMITH LL, BUYSKE DA: Metabolic fate of a synthetic corticosteroid (triamcinolone) in the dog. J Biol Chem. 1961 Apr;236:1038-42.
2. Argenti D, Shah B, Heald D: A pharmacokinetic study to evaluate the absolute bioavailability of triamcinolone acetonide following inhalation administration. J Clin Pharmacol. 1999 Jul;39(7):695-702. doi: 10.1177/00912709922008335.
3. Derendorf H, Hochhaus G, Rohatagi S, Mollmann H, Barth J, Sourgens H, Erdmann M: Pharmacokinetics of triamcinolone acetonide after intravenous, oral, and inhaled administration. J Clin Pharmacol. 1995 Mar;35(3):302-5. doi: 10.1002/j.1552-4604.1995.tb04064.x.
4. Hochhaus G, Portner M, Barth J, Mollmann H, Rohdewald P: Oral bioavailability of triamcinolone tablets and a triamcinolone diacetate suspension. Pharm Res. 1990 May;7(5):558-60. doi: 10.1023/a:1015889305157. [Article]
5. G.W. Peng, G.G. Jackson, and W.L. Chiou. High-pressure liquid chromato-graphic assay of Triamcinolone hexacetonide in plasma. Antimicrob. Agents Chemother. 12: 707–709 (1977).
6. S.E. Back, I. Nilsson-Ehle, and P. Nilsson-Ehle. Chemical assay, involving liquidchromatography, for aminoglycoside antibiotics in serum. Clin. Chem. 25: 1222–1225 (1979).

7. J. Marples and M.D.G. Oates. Serum gentamicin, Triamcinolone hexacetonide and tobramycinassays by high performance liquid chromatography. J. Antimicrob. Chemother. 10: 311–318 (1982).
8. L. Essers. An automated high-performance liquid chromatographic method forthe determination of aminoglycosides in serum using pre-column sample clean-up and derivatization. J.Chromatogr. 305: 345–352 (1984).
9. M. Santos, E. Garcia, F.G. Lopez, J.M. Lanao, and A. Dominguez-Gil.Determination of Triamcinolone hexacetonide in plasma by HPLC. J. Pharm. Biomed. Anal. 13: 1059–1062 (1995).
10. S. Dionisotti, F. Bamonte, M. Gamba, and E. Ongini. High-performance liquidchromatographic determination of Triamcinolone hexacetonide in guinea-pig and human serum byfluorodinitrobenzene derivatization with spectrophotometric detection. J.Chromatogr. 434:169–176 (1988).
11. H. Kubo, T. Kinoshita, Y. Kobayashi, and K. Tokunaga. Micro-Scale Method forDetermination of Tobramycin in Serum Using High-Performance LiquidChromatography. J.Liq.Chromatogr. 7: 2219–2228 (1984).
12. H. Fabre, M. Sekkat, M.D. Blanchin, and B. Mandrou. Determination of amino-glycosides in pharmaceutical formulations—II. High-performance liquid chro-matography. J.Pharm.Biomed.Anal. 7: 1711–1718 (1989).
13. D.C. Rigge and M. F. Jones. Shelf lives of aseptically prepared medicines—sta-bility of Triamcinolone hexacetonide injection in polypropylene syringes. J. Pharm. Biomed. Anal. 35:1251–1256 (2004).
14. B. Li, A.V. Schepdael, J. Hoogmartens, and E. Adams. Characterization of impu-rities in sisomicin and Triamcinolone hexacetonide by liquid chromatography/mass spectrometry. Rapid Commun.Mass Spectrom. 22: 3455–3471 (2008).
15. J.J. Wright, P.J.L. Daniels, A.K. Mallams, and T.L. Nagabhushan, U.S. Patent4002742 (1977).N. Nagae, T. Enami, and S. Doshi. The retention behavior of reversed-phaseHPLC columns with 100% aqueous mobile phase. LC/GC 20: 964–972 (2002-2003)