IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

DEVELOPMENT AND VALIDATION OF IN VITRO RELEASE METHOD OF TRIAMCINOLONE HEXACETONIDE IN ITS PHARMACEUTICAL DOSAGE FORM

B Jinal [1], P Jaymin[2],R Urvi[3], P Ronak[4], P Bhumi[5]
student[1], Assistant professor[2], Assistant professor[3], Assistant professor[4], Assistant professor[5]
Department of Quality Assurance
Address: Sharda School of pharmacy, Pethapur, Gandhinagar, Gujarat 382610

ABSTRACT: Simple, rapid, economical, precise and accurate method to identify actual % drug release of Triamcinolone Hexacetonide form its pharmaceutical dosage form has been developed. The reverse phase HPLC method was selected and developed for the identifying to its simplicity, suitability, ruggedness and its wider usage. The final chromatographic condition was achieved column Xselect CBH C18 (150 mm x 4.6 mm ID, 5 µ particle size) ,20 mM phosphate buffer: Methanol (20:80) as mobile phase, at a flow rate of 1.2 ml/min. Detection was carried out at 238 nm retention time of Triamcinolone Hexacetonide is 10 min. Dissolution medium that has a similar pH to the small intestine, dissolution testing can better simulate the in vivo conditions under which the drug will be absorbed. Therefore, based on pH dependent solubility testing, 6.8 pH phosphate buffer was used as dissolution medium for the trials by using USP type II apparatus. Linearity observed for Triamcinolone Hexacetonide is 0.99944. At 60 min time interval %drug release is 88.7 so we can conclude that developed method was found to be accurate, precise and rapid.

KEYWORDS: Triamcinolone Hexacetonide, dissolution media, RP- HPLC method, phosphate buffer, Dissolution study, type II apparatus, validation

I .INTRODUCTION:

Dissolution: [7-8] Dissolution tests, also known as 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 in an aqueous medium under specified conditions.

Dissolution Rate: It is the amount of drug substance that goes in solution per unit time under standardized conditions of temperature and solvent composition. Ideally, the dissolution method used for particular drug products in vitro, relates to the bioavailability of the drug in vivo. The USP-NF (United States Pharmacopoeia-National Formulary) sets standards for dissolution and drug release tests of most drug products. Identification, assay, uniformity of the dosage units, dissolution, impurities, microbial limits are some of the mandatory tests that are commonly performed to release a QC report of a dosage form after each batch production.

Dissolution testing measures how quickly the API is released from the dosage form and becomes available for absorption. It is essential in quality control (QC) as it enables QC personnel to evaluate the rate and extent of drug release from a solid dosage form under standardized conditions. By testing the dissolution of multiple batches of the same drug product, QC personnel can ensure batch-to-batch consistency and verify that the manufacturing process is consistent.This helps to ensure that the drug product will have similar dissolution profiles across different batches, leading to better outcomes for patients and improved product quality. For development of an appropriate dissolution tests, deterrent agitation rates, different media (volume and pH of dissolution medium), and different kinds of apparatus are taken into consideration. Size and shape of the dissolution vessel: It may affect the rate and extend of dissolution. Drugs that are poorly

water soluble may require use of a very large capacity vessels. United States Pharmacopeia (USP) and the National Formulary (NF) provide several official methods for carrying out dissolution tests of tablets, capsules and other drug products. **Apparatus-II - Paddle Apparatus.**

USP Apparatus type is widely used for evaluation of dissolution performance of solid dosage forms like IR Tablets, SR tablets, capsules, beads, etc. Advantages:- Standardized ,Robust ,Sink Conditions are maintained ,Change in pH can be possible ,Membrane effect is minimal ,Easy sampling method ,Broad Application

II .MATERIALS AND METHODS

Shimadzu HPLC, LC 2010 CHT model and LC Solution software was used. Acetonitrile, methanol, Diammonium hydrogen phosphate, Mili-Q water of AR grade from Merck Life Science Pvt. Ltd, was used. A commercial dosage form Zita-D was purchased from local market.

IR identification and wavelength selection

Identification of Triamcinolone hexacetonide was determined by FTIR and obtained IR spectrum was compared with the reference spectrum of Triamcinolone hexacetonide to confirm identity of the drug. Small quantities of drug were kept directly in the sample compartment of IR and they were scanned in the range of 400-4000 cm-1. An IR spectrum of Drug was interpreted.

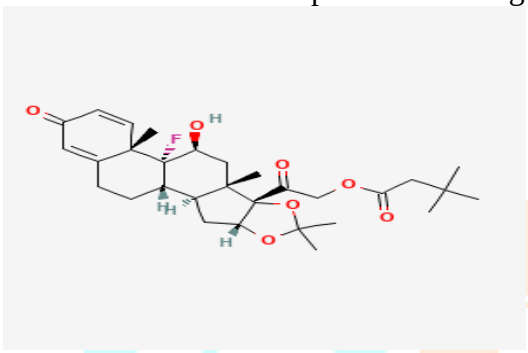

Figure 1. Structure of Triamcinolone Hexacetonide

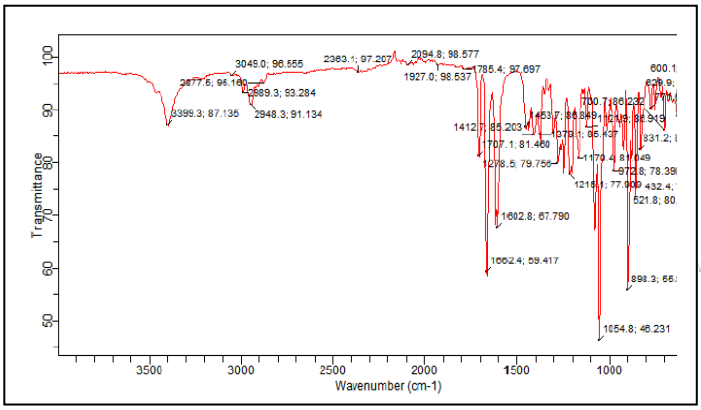

Figure 2: IR spectrum of (API)

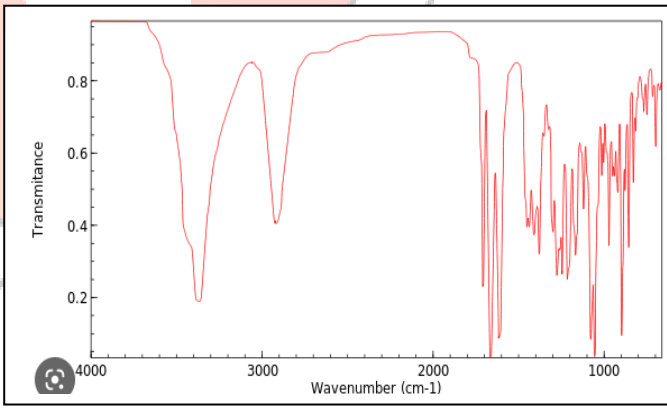

Figure 3: IR Spectrum of Triamcinolone Hexacetonide (Std.)

Table 1: IR spectrum of Triamcinolone Hexacetonide

Sr. No.	Functional group	Observed value	Standard value
1	O-H stretching	3399.3	3200-3600
2	C-H stretching	893.3	2850-2950
3	C-O stretching	1215.1	1000-1300
4	C-F stretching	1054.8	1000-1400
5	C=O stretching	1662.4	1650-1750

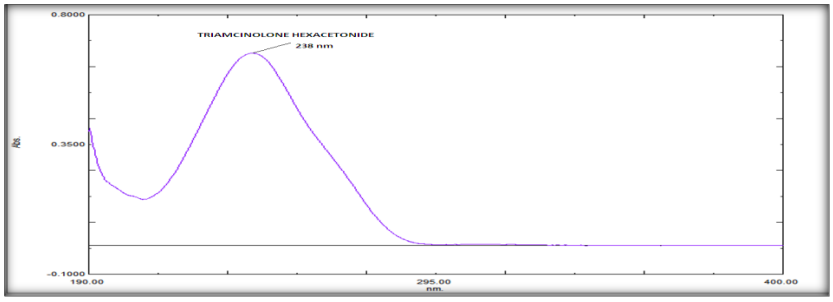

Figure 4: Determination of wavelength maximum (238 nm)

Selection of Mobile phase

For the analysis, methanol and phosphate buffer were used as mobile phases in varying compositions. To start with, 30 µL of a standard solution of Triamcinolone Hexacetonide was injected into the HPLC system and multiple runs were performed to optimize the method
parameters. Various parameters were considered in the optimization process, such as the composition of the mobile phase, the type of column used, and the column temperature. Chromatogram in optimized mobile phase is shown in Figure.

Triamcinolone Hexacetonide Stock Solution:

About 22 mg of Triamcinolone working standard was accurately weighed and transferred into 100 ml volumetric flask. To this, 20 ml of acetonitrile was added and dissolved by sonication. The solution was diluted up to the mark with acetonitrile and used as a stock
solution.

Triamcinolone Standard Solution (22 mcg/ml):

Pipette out 2 ml of Triamcinolone stock solution into 20 ml volumetric flask. The solution was then diluted up to the mark with dissolution media.

METHOD DEVELOPMENT FOR HPLC

Trial-1

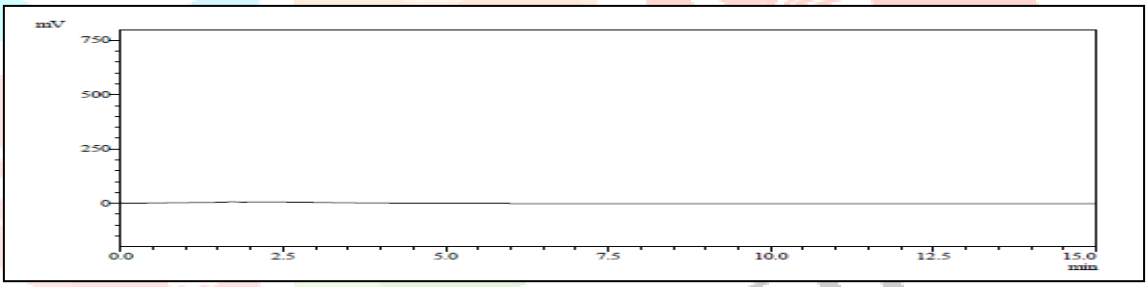

Figure 5: Chromatogram for Triamcinolone Hexacetonide Water:Methanol (50:50)

Trial-2

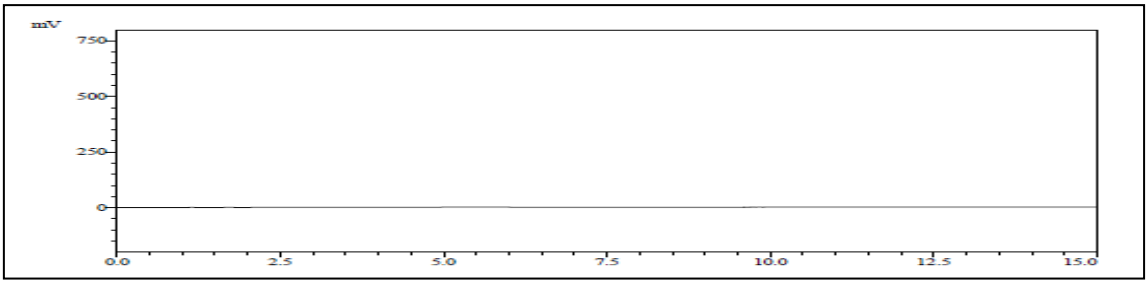

Figure 6: Chromatogram for Triamcinolone Hexacetonide Water:Methanol (40:60)

Trial-3

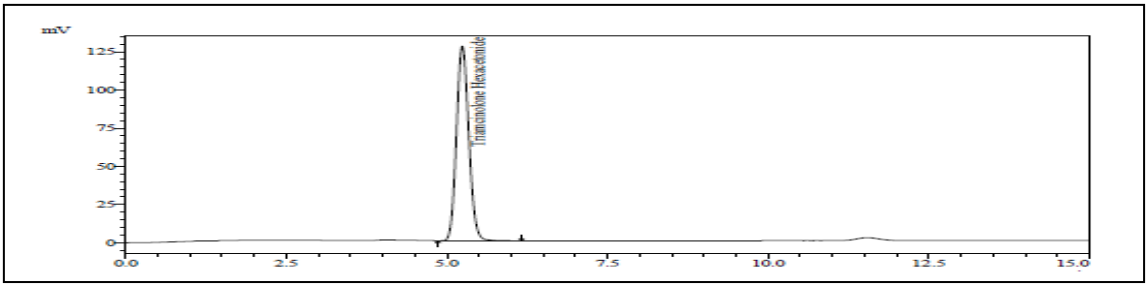

Figure 7: Chromatogram for Triamcinolone Hexacetonide Water:Methanol (25:75)

Trial-4

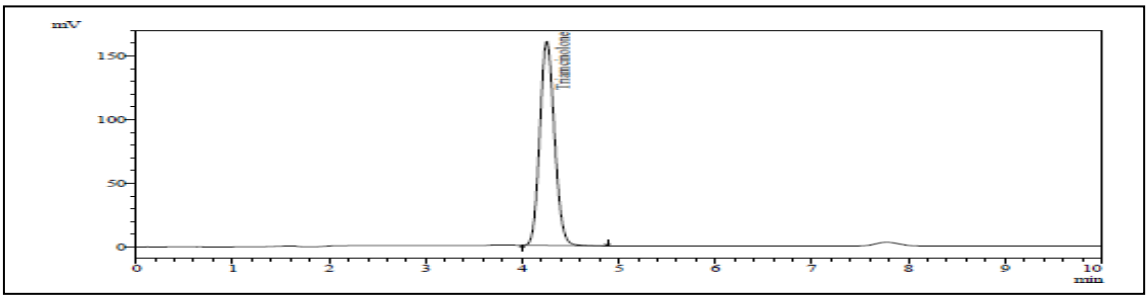

Figure 8: Chromatogram for Triamcinolone Hexacetonide Water:Methanol (20:80)

Trial-5

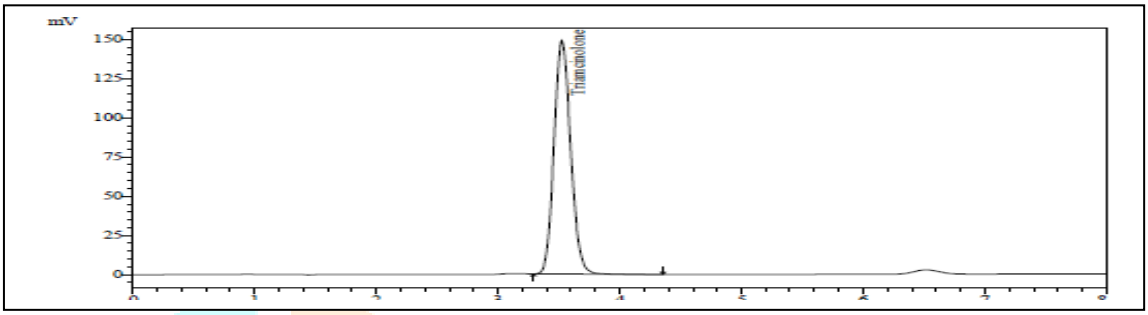

Figure 9: Chromatogram for Triamcinolone Hexacetonide Water:Methanol (20:80)

Trial-6 (Final)

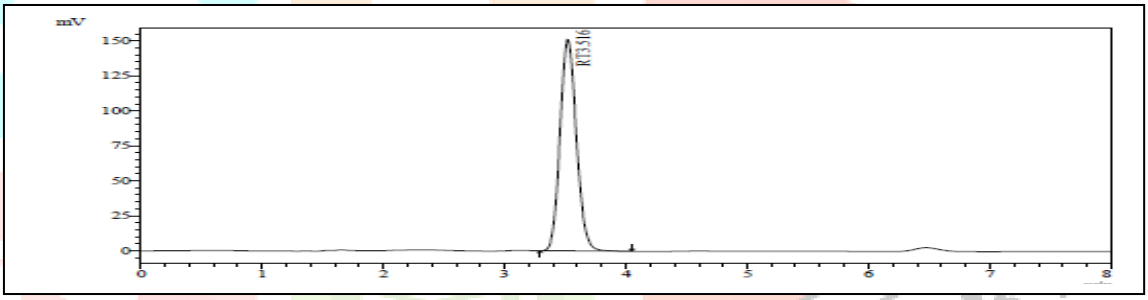

Figure 10: Chromatogram for Triamcinolone Hexacetonide 20 mM phosphate buffer:Methanol (20:80)

Table: 2 Mobile phase selection

Sr. no	Mobile phase composition	Inference
1	Water:Methanol (50:50)	no peak observed
2	Water:Methanol (40:60)	no peak observed
3	Water:Methanol (25:75)	peak eluted at 5.24 minutes
4	Water:Methanol (20:80)	Need to decrease retention time
5	Water:Methanol (20:80)	Retention time was further decreased by increase in flow rate.
6	20 mM phosphate buffer:Methanol (20:80)	Peak observed with good sharp

METHOD DEVELOPMENT FOR DISSOLUTION STUDY

A sink condition is crucial in dissolution testing because it allows for accurate measurement of the dissolution rate of a substance without being affected by its solubility in the medium. To investigate sink condition of Triamcinolone Hexacetonide pH-dependent solubility study was carried out by gradually adding the API in 10 mg increments into 50 ml solvents. Three different pH buffers (1.2 pH, 4.5 pH and 6.8 pH) were selected for initial solubility studies. Solubility study experiment was carried out using saturation shake-flask method.

Table: 3 Different solvent for dissolution test

Sr No.	Solvent	Preparation of the solvent	Solubility(mg/ml)	Sink Condition
1.	0.1 N Hydrochloric Acid	8.3 ml of concentrated HCL in 1000 ml water	0.018931	No
2.	pH 4.5 Acsetate Buffer (USP)	Take 2.99 g of Sodium Acetate Trihydrate in water. Add 14 ml 2 N acetic acid into this. Dilute to 1000 ml with water.	0.019757	No
3.	pH 6.8 Phosphate Buffer (USP)	Place 250 ml of 0.2 M potassium dihydrogen phosphate into 1000 ml volumetric flask. Add 112 ml of 0.2 M sodium hydroxide. Dilute to 1000 ml with water.	0.036271	Yes

Using 6.8 pH phosphate buffer, at rotation speed of 75 rpm discriminating release profile was obtained. Finalized dissolution method parameters are as summerized in table

Table:4 Finalized dissolution method

Apparatus	USP Apparatus –II (Paddle)
Stirrer speed	75 rpm
Dissolution medium	6.8 pH phosphate buffer
Bath temperature	37 °C±0.5 °C
Media volume	900 Ml
Replenishment	Yes
Time point	5, 30,60 minutes
Filters	Whatman PVDF 0.45 µ syringe filter

IV. METHOD VALIDATION

Specificity

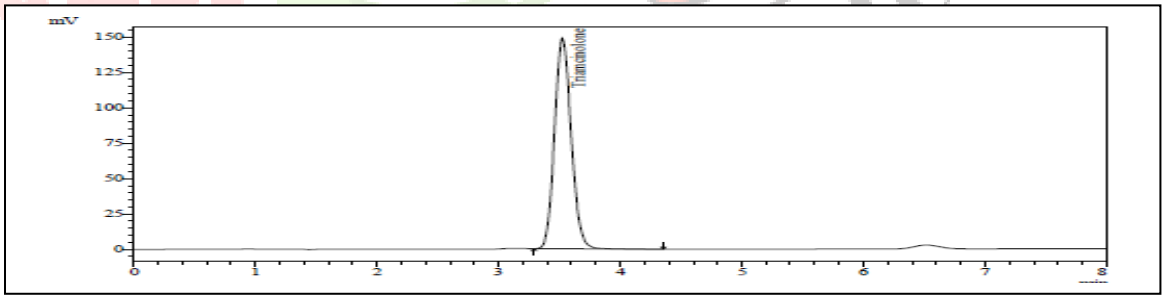

Figure 11: Chromatogram of Standard Triamcinolone Hexacetonide

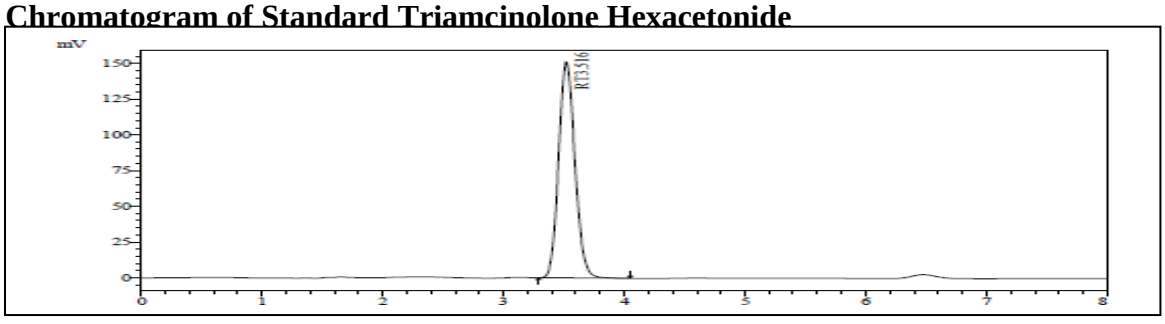

Figure 12: Chromatogram of Sample Triamcinolone Hexacetonide

Linearity

First stock solutions of Triamcinolone Hexacetonide were prepared as per test method parameters. Then serial of dilutions were performed from the stock solutions of Triamcinolone Hexacetonide. Linearity solution preparation is mentioned in table 4 solution was then injected into the HPLC system. Detector responses (area) were noted. Concentration versus area graph is plotted. Correlation coefficient, Y-intercept and slope were computed using Microsoft excel.

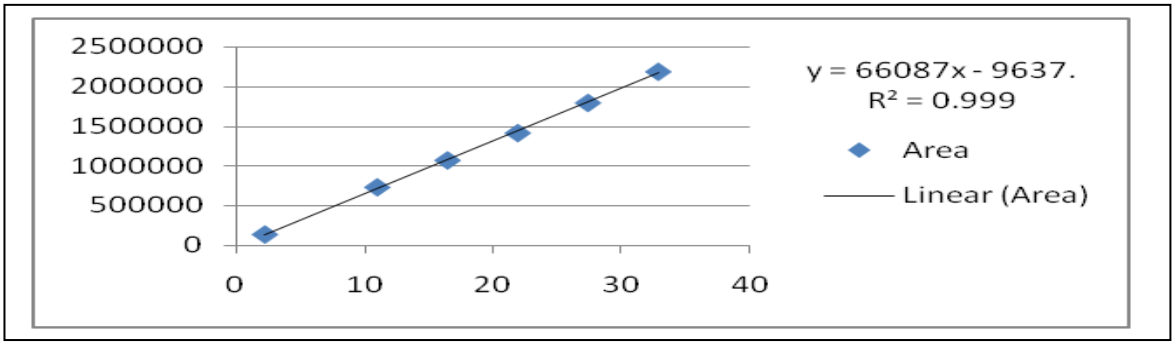

Figure 13: linearity graph of Triamcinolone Hexacetonide

Table 5: Linearity study of Triamcinolone Hexacetonide

Level	**Conc.(µg/mL) of Triamcinolone Hexacetonide**	**Area of Triamcinolone Hexacetonide**
10%	2.20	137774
50%	10.98	732948
75%	16.47	1071460
100%	21.96	1415842
125%	27.45	1795445
150%	32.93	2188881
Correlation (R)	**0.99972**	
Correlation co-efficient (R^2)	**0.99944**	
Y-intercept	**-9637.19**	
Slope	**66087.37230**	

Repeatability

Method precision was carried out for dissolution experiment using IM injection (Label: 20 Triamcinolone Hexacetonide) as per proposed dissolution method parameters. Sample aliquots were taken at 5, 10, 15, 30, 45 and 60 minutes. Quantitation was done using developed HPLC method parameters

Table 6: Results of Method Precision of Triamcinolone Hexacetonide

Time	**Cumulative (%) Drug release**						**Avg,**	**%RSD**
	Unit 1	Unit 2	Unit 3	Unit 4	Unit 5	Unit 6		
5 min	55.5	54.8	54.1	55.0	53.6	54.6	54.6	1.2
10 min	65.5	66.7	66.0	65.1	65.6	64.5	65.6	1.1
15 min	70.4	70.4	78.7	69.8	69.9	73.4	72.1	2.9
30 min	80.3	80.3	79.4	79.2	78.3	78.8	79.4	1.0
45 min	87.8	87.8	88.6	88.2	87.7	87.4	87.9	0.5
60 min	92.5	92.4	96.9	92.0	91.9	91.7	92.9	2.1

Intermediate Precision

On a separate day, by separate analyst, intermediate precision was evaluated for the dissolution experiment of IM injection (Label: 20 Triamcinolone Hexacetonide) as per proposed dissolution method conditions. Sample aliquots were taken at 5, 10, 15, 30, 45 and 60 minutes

time points, and each samples were filtered through 0.45 µm filter and injected into HPLC and run as per developed HPLC method parameters.

Table 7: Results of Intermediate Precision of Triamcinolone Hexacetonide

Time	Cumulative (%) Drug release						Avg,	%RSD
	Unit 1	Unit 2	Unit 3	Unit 4	Unit 5	Unit 6		
5 min	53.5	54.2	53.7	54.2	53.6	55.7	54.2	1.5
10 min	65.1	64.4	65.7	63.7	64.2	64.3	64.6	1.1
15 min	70.8	71.1	70.8	74.6	75.6	74.0	72.8	3.0
30 min	79.3	78.4	79.5	78.2	79.5	78.2	78.9	0.8
45 min	86.9	87.4	88.0	86.8	88.1	86.8	87.3	0.7
60 min	90.6	91.0	91.9	86.6	96.0	90.6	91.1	3.3

Accuracy:

Accuracy of analytical method was evaluated by spiking API stock and amount of sample stock solution equivalent to the target label claim into 1000 mL of dissolution medium at three levels of the range, i.e. 50%, 100% and 150% in triplicate. Recovery was calculated as percentage recovery.

Table 8: Recovery of Triamcinolone Hexacetonide

Level	Average Area	mg added	mg found	%Recovery	Mean %Recovery	%RSD
10%	135819.000	8.9	2.11	23.7	**24.0**	**1.0**
10%	134295.000	8.7	2.09	24.0		
10%	134683.000	8.7	2.09	24.2		
50%	727589.000	44.5	11.30	25.4	**25.2**	**0.8**
50%	726474.000	44.8	11.28	25.2		
50%	725394.000	45.0	11.26	25.0		
100%	1433091.000	90.0	22.25	24.7	**24.5**	**0.8**
100%	1417432.000	90.5	22.01	24.3		
100%	1417234.000	89.9	22.0	24.5		
150%	2132134.000	133.5	33.10	24.8	**24.7**	**0.4**
150%	2123487.000	134.0	32.97	24.6		
150%	2125967.000	133.80	33.01	24.7		

Robustness

Robustness of the HPLC method was carried out by making deliberate variations into method parameters such as flow rate (0.9 ml/min and 1.2 ml/min), column temperature (20 °C and 25 °C), mobile phase ratio (78:22 and 82:18 %v/v Methanol:Buffer). System suitability parameters were monitored to ensure robustness of method.

1. Flow rate of mobile phase was changed
2. Temp of column was changed
3. Ratio of Mobile phase was changed

Table:9 Robustness data for Triamcinolone Hexacetonide

Injection	As such	Temp **20° C**	Temp **25° C**	rate **0.9** mL/min	rate **1.2** mL/min	MeOH:Buffer **(78:22)**	MeOH:Buffr **(82:18)**
Mean	1414124	1378474	1374701	1529347	1264424	1400221	1387635
%RSD	0.1	0.1	0.1	0.1	0.1	0.2	0.4
Theoretical plates	3164	3052	2890	3294	2663	2619	2619
Tailing factor	1.2	1.1	1.2	1.2	1.2	1.2	1.2
Retention time	3.31	3.91	3.45	3.70	2.86	2.57	3.19

Stability of Solution

Standard solution as well sample solution (30 minute/unit – 1 withdrawal) were stored at room temperature and monitored in specific time intervals. Areas were compared with that of initial point and %difference in area was also noted.

Table: 10 Solution stability results

For Standard Triamcinolone Hexacetonide			**For Sample Triamcinolone Hexacetonide**		
Time point	Area	% Difference	Time point	Area	% Difference
Initial	1412803	-	Initial	794985	-
After 12 hr	1408967	-0.27	After 8 hr	792944	-0.26
After 24 hr	1393951	-1.35	After 24 hr	787400	-0.96

RESULT

Parameter	**Results of Triamcinolone Hexacetonide**
System Suitability Test (%RSD)	0.1
Specificity (%RSD)	No interference
Linearity (R^2 Value)	0.99944
Accuracy (n=9) (%RSD)	For 10% - 1.0
	For 50% - 0.8
	For 100% - 0.8
	For 150% - 0.4
Precision (%RSD)	
System Precision	0.1
Method Precision	For 5 min - 1.2
	For 10 min - 1.1
	For 15 min - 2.9
	For 30 min - 1.0
	For 45 min - 0.5

	For 60 min - 2.1
Intermediate Precision	For 5 min - 1.5
	For 10 min - 1.1
	For 15 min - 3.0
	For 30 min -0.8
	For 45 min - 0.7
	For 60 min - 3.3
Robustness	
Column oven temperature	Complies
Mobile phase ratio	Complies
Flow rate	Complies
Solution Stability	Stable up to 24 hrs

CONCLUSION

Furthermore, a new, precise, rapid, and cost-effective HPLC method was developed and validated for the invitro release method of Triamcinolone Hexacetonide in its pharmaceutical dosage form. The developed method was successfully applied to quantitative in-vitro dissolution samples of synthetic mixture of Triamcinolone Hexacetonide. Hence, this HPLC method can be used for the routine quality control analysis of these drugs. Based on the experimental results and statistical analysis, it was determined that the developed in-vitro dissolution method is discriminative to study dissolution of Triamcinolone Hexacetonide Injectable suspension. This method can be used as a reliable and routine quality control tool for the analysis of these drugs.

REFERENCES

1. Chatwal G.R., Anand S. (2002) Instrumental Methods of Analysis, 5 th Ed. Himalaya Publishing House, New Delhi. P.624-631.
2. Willard H.H., Mertift L.L., Dean J.A., & Senle F.A. (1986) Instrumental methods of analysis, Page 42 7 th Ed. CBS publishers and distributors, New Delhi
3. Beckett A.H., Stenlake J.B. (1997) Practical Pharmaceutical Chemistry, 4 th Ed. CBS Publishers and Distributors.
4. Journal of chromatography," **1984**, 309, 385-390
5. Synder L.R., Kirkland J.J., Glajch J.L. Practical HPLC method development, 2 nd Ed., Wiley Interscience Publication.
6. George L. HPLC methods for pharmaceutical analysis 3 rd Ed. (2005) p.357- 1094.
7. The Dissolution Procedure: Development and Validation, USP 36 page 735.
8. Martin Siewert, Jennifer Dressman,Cynthia Brown, Vinod Shah, "FIP/AAPS Guidelines for Dissolution/In Vitro Release Testing of Novel/Special Dosage Forms" dissolution technologies, **2003**, 6
9. Wang, Qingxi, "HPLC in dissolution Testing," Handbook of Pharmaceutical Analysis by HPLC, 2005, 6 (15), 379-400
10. FDA guidance for industry, analytical procedures and methods validation , 2014
11. ICH Harmonized Tripartite Guideline, ICH Q2A, Text on validation of analytical procedures, March 1995
12. ICH Harmonized Tripartite Guideline, ICH Q2B, Validation of analytical procedures: Methodology, May 1997
13. Drug bank Online; Triamcinolone Hexacetonide https://go.drugbank.com/salts/DBSALT000861
14. National library of medicine; Triamcinolone Hexacetonide https://pubchem.ncbi.nlm.nih.gov/compound/Triamcinolone-hexacetonide
15. United States Pharmacopeia 34/National Formulary 29, 2011
16. Approved Product Monograph 1.docx; Triamcinolone Hexacetonide . https://pdf.hres.ca/dpd_pm/00042568.PDF
17. Rx List ; Triamcinolone Hexacetonide https://www.rxlist.com/aristospan-injection-20-drug.html

18. Chung Chow Chan, Herman Lam, Y. C. Lee, Xue-Ming Zhang, Analytical method validation and instrument performance verification, John Wiley & Sons Publication, New York, 2004, pp 27
19. Albert R. (1997), Validation of analytical procedure, Ana. Chem. 789.
20. Volume 1, Published by The Indian Pharmacopoeia Commission , Ghazibad, 2010
21. Ketan Savjani, A.G., J.S. , Drug Solubility: Importance and Enhancement Techniques, National library of medicine;**2012**
22. National Institute of Standards and Technology ; Reference IR spectra Triamcinolone Hexacetonide
23. USP 35/solutions/buffer solutions pp 1068g
24. USP General Chapter <1225> Validation of Compendial method
25. USP Chapter <711> Dissolution Testing
26. Center for drug evaluation and research, Application No. 210045Orig1s000s