

A new approach for evolution and quantification of Triamcinolone acetonide in medication shots by using RP-HPLC

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ABSTRACT

Triamcinolone acetonide is a synthetic glucocorticosteroid has great immunosuppressive and anti-inflammatory activity. The most significant applications are to treat redness, itching, scaling, crusting like inflammatory skin disorders, and other conditions like dryness with psoriasis on the skin. The effective importance of Triamcinolone acetonide is the main objective of the study. A significant economic high responsive unique approach was developed for quantification of Triamcinolone acetonide in pharmaceutical dosage form by reverse phase high performance liquid chromatographic way. This drug samples were detached by passing through X-bridge phenyl column has a stationary phase and a mobile phase of 50:50 v/v of acetonitrile with octane sulphonic acid as a buffer solution to adjust the desirable pH-2.5 condition by employing ortho phosphoric acid. The Triamcinolone acetonide was eluted at 6.22 minutes with absorbance is 235 nano meters at input flow rate of 1ml/minutes with photodiode array detector. The linearity results of Triamcinolone acetonide gave regression equation $y = 43,591 \times + 14,344$ and correlation coefficient is (R^2) 0.9997. The percentage of relative standard deviation is 0.24 for intra-day precision and 0.65 for inter-day precision supports the ruggedness and the proposed approach is eco-friendly by AGREE-Analytical GREENness metric shows 0.72. This new method was validated in accordance with the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines consisting of several analytical parameters like method precision, accuracy, ruggedness, robustness, and forced degradation studies results are supportive to this method for quantification and validation for Triamcinolone acetonide in medication shots and industrial use.

INTRODUCTION

Triamcinolone acetonide is a synthetic glucocorticosteroid (Mohamadi *et al.*, 2017) has great immunosuppressive and anti-inflammatory activity. It is acetonide salt form of Triamcinolone. This medication works as intermediate to strong efficacy on various skin diseases like dry allergies, eczema, dermatitis, and rashes on the skin and rheumatic disorders (Decker, 1983). It is also used to prevent deterioration of asthma, Chronic obstructive pulmonary disease (COPD) (Vogelmeier *et al.*, 2017). The intake of this medication by mouth, injection into a muscle and inhalation and ointment on surface use only. The prolonged intake of this

results adverse effects like osteoporosis (Curry *et al.*, 2018), cataract (Mohammadpour *et al.*, 2019), most serious case leads to psychosis (Leucht *et al.*, 2009), thrush and muscle weakness. Usage during the pregnancy is generally safe. It works by decreasing inflammation (Chiba *et al.*, 2012) and the activity of the immune system (Kurosaki *et al.*, 2015).

When this ointment was applied on affected area reduces the strength of swelling, itching, and redness at affected body part except underarms, face, and groin it cannot applied. The efficacy of this medication is depending on the form which we use on affected skin area. The pharmacologic activity of Triamcinolone acetonide alters gene expression it binds particular receptors of cytosolic glucocorticoid and glucocorticoids response on DNA results for the synthesis of anti-inflammatory proteins and inflammatory medications. This synthesis gave reduction in chronic inflammation, autoimmune reactions, and vital medicinal importance are the main reasons for studying quantification and validation. The structure of Triamcinolone is shown in Figure 1.

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MATERIALS AND METHODS

Chemicals

Merk made analytical grade (A.R) of acetonitrile, ortho phosphoric acid (OPA), and octane sulphonic acid were procured from Merck India Pvt. Ltd, Worli, Mumbai, India. The active pharma ingredient (API) of Triamcinolone acetonide taken as a reference internal standard was procured from Dr. Reddy's laboratory, Hyderabad. Carbonate free triple distilled water was used throughout the experiment.

Instrument

High sensitive with accuracy of Waters alliance make high performance liquid chromatography (HPLC) of model e-2,695 consisting of quaternary pump with Photodiode-Array Detection (PDA) detector-2996 and inbuilt chromatographic software Empower-2.0 was used for all chromatograms.

Buffer solution

Under the present experimental conditions, the Chromatography was optimized at pH-2.5 was maintained by using buffer solution. It was prepared by dissolving 2.5 g of octane sulphonic acid in to HPLC grade of 1 l distilled water by using 0.1 % OPA and the buffer solution was filtered through 0.45 μ filter paper.

Mobile phase solution

Analytical grade acetonitrile and buffer solutions are mixed in equal ratio (50:50) to get homogeneous after that filtered from 0.45 μ filter paper to discard insoluble impurities.

Standard aqueous Triamcinolone acetonide solution

Triamcinolone acetonide stock solution was prepared in 100 ml volumetric flask by dissolving 20 mg of Triamcinolone acetonide with 70 ml of diluent was added with 30 minutes sonication to get homogeneous. From this stock solution, we prepared required concentrations (5 into 50 ml volumetric flask) makeup with diluent.

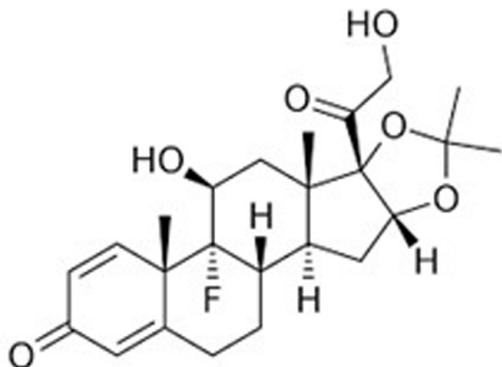


Figure 1. Structure of Triamcinolone.

Validation Methods

The evolution and validation of any chemical compound by the prescribed analytical methods, and it is a drug-specific methods (validation of analytical procedure, 1996; CIPAC, 2003; ISO, 1986). The validation process consisting of several assessments parameters like appropriateness, linearity, precision, accuracy, robustness, stability, and forced degradation studies results from the accuracy and suitability of analytical parameters of the system (European Commission, 2010; Horwitz, 1982; ISO, 1996; Thompson *et al.*, 2008).

RESULTS AND DISCUSSION

X-bridge phenyl column is an efficient separation column with significant dimensions like 150 \times 4.6 mm with a pore size is 3.5 μ was used for the separation of Triamcinolone acetonide. This column has a flow rate of 1 ml/minute with operational inflow strength of 10 μ l was cultivated at ambient temperature. The predicted drug was identified at wavelength region was fixed at 235 nm. The Photodiode Array Detector spectrum was operated under unique conditions shown in Table 1, and PDA spectrum of Triamcinolone acetonide was shown in Figure 2.

ACCURACY OF THE METHOD

In order to stabilize the HPLC system was balanced for about 1 hour to get standard baseline shown in Figure 3. There should be six trials were performed by injecting standard solutions into the chromatogram and the results are summarized in Table 2.

PRECISION PARAMETERS OF THE PROPOSED APPROACH

Linearity

This analytical parameter is used to study the variations of several ingredients with respect to their strengths of analytes within specific range. This can be performed by injecting standard analyte solution by using suitable solvent for six significantly different strengths between 10% and 150% working limit. The graph is drawn between the strengths against the peak areas of Triamcinolone acetonide shown in Figure 4. From this graph, the

Table 1. The improved chromatogram specifications.

Stationary phase	X-bridge phenyl column
Dimensions	150 \times 4.6 mm
Pore size of filter paper	3.5 μ
Mobile phase	Acetonitrile and octane sulphonic acid (50:50) ratio
Inflow speed	1 ml/minute
Injection volume	10 μ l
Column temperature	Ambient
Wavelength	235 nm
Run time	8 minutes
RT of Triamcinolone acetonide	6.229 minutes

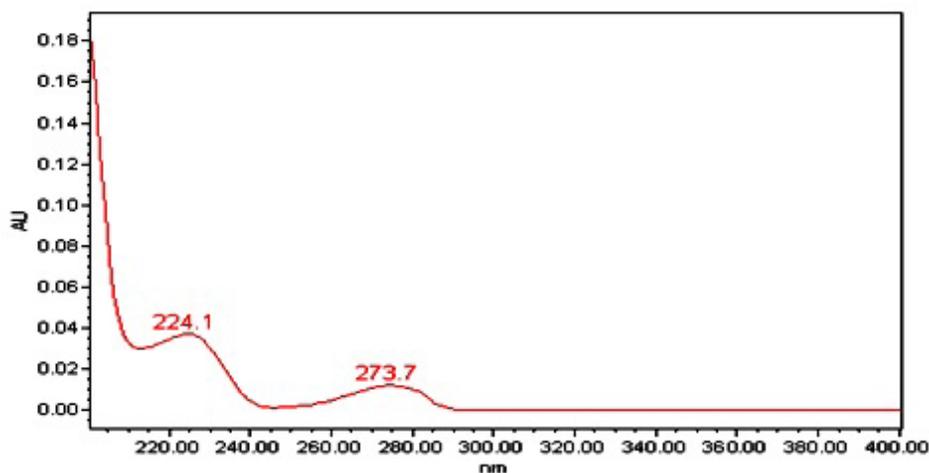


Figure 2. PDA spectrum of Triamcinolone acetonide.

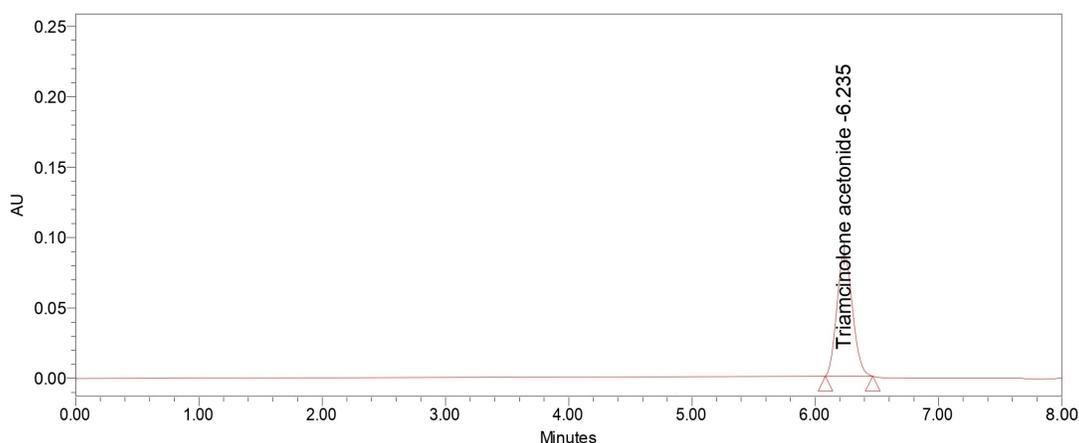


Figure 3. Chromatogram of system precision.

Table 2. Results of the system precision.

Parameter	Triamcinolone acetonide
Theoretical plate count	10,719
United States pharmacopeia (USP) tailing	1.12
USP resolution	4.57
Retention time	6.229

linear trend was observed where the strengths are in between 2 and 30 $\mu\text{g/ml}$ of Triamcinolone acetonide. The linearity results of Triamcinolone acetonide gave regression equation $y = 43,591x + 14,344$ and correlation coefficient (R^2) 0.9997 were calculated and the results are shown in Table 3.

Method precision

Several Analytical parameters measures the degree of repeatability under optimum conditions, and it can be represented in % relative standard deviation (RSD) for specific samples which are studied according to the ICH guidelines. There should be three levels of precision, namely intermediate, repeatability, and reproducibility. Intermediate precision was performed by

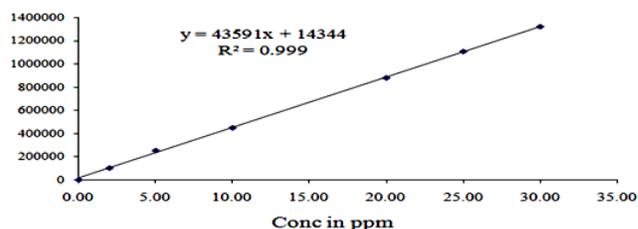


Figure 4. Linearity calibration plots of Triamcinolone acetonide.

chromatographic pattern shows no significant variations are observed for different HPLC systems operated at different analysts with different columns. The reports of intermediate precision are % RSD is 0.24 for intra-day precision and 0.65 for inter-day precision supports the ruggedness of the proposed approach.

Accuracy

Accuracy is the comparable parameter between expected reference value and actual measured value shows the correctness of the analytical value. To measure these three different strengths of 50%, 100%, and 150%, samples were injected for each cycle where the percentages of recovery should not less than 98% and not exceeded 99.6%. The results are shown in Table 4.

Table 3. Linearity results.

Linearity	Triamcinolone acetonide	
	Conc. ($\mu\text{g/ml}$)	Area
Linearity-10%	2	101,777
Linearity-25%	5	252,712
Linearity-50%	10	448,507
Linearity-100%	20	879,429
Linearity-125%	25	1,107,194
Linearity-150%	30	1,321,180
Slope	43,591.24	
Intercept	14,343.57	
CC	0.9997	

Table 4. Accuracy of the method.

Accuracy	Amount of Triamcinolone acetonide	% recovery
50%	10	99.6
100%	20	99.2
150%	30	98.6

Table 5. Robustness of method.

Parameter	% RSD of Triamcinolone acetonide
Flow (1.2 ml/minute)	0.30
Flow (0.8 ml/minute)	0.32
Org. phase (55:45)	0.13
Org. phase (45:55)	0.49

Table 6. Results of solution stability.

Stability	Triamcinolone acetonide	
	% Lable claim	% Deviation
Initial	99.9	0.1
6 Hrs	99.3	-0.12
12 Hrs	98.5	-0.22
18 Hrs	97.3	-0.27
24 Hrs	96.8	-0.34

Table 7. Results of forced degradation study.

Stress condition	% of degradation
	Triamcinolone acetonide
Acidic degradation (1 N HCl)	16.5
Basic degradation (1 N NaOH)	15.2
Peroxide degradation (30% H ₂ O ₂)	12.3
Hydrolysis degradation (1 ml of HPLC water)	11.6
Thermal degradation (Sample, 70°C, 3 hours)	14.7

Robustness

Robustness determines the appropriateness of the method by applied small variations like pH, ionic strength, % of solvent, and temperature on method results. The percentage of RSD indirectly assesses the robustness of the current new approach for neomycin, and it should be lower than 2%. The marginal variations are found in optimized parameters related to flow rate, it should be $\pm 20\%$, and another parameter like percentage of organic solvent in the movable phase is $\pm 10\%$. The results are shown in Table 5.

Solution stability test

To determine the standard solution stability by impurity barbed solution and control samples are studied for different intervals from 6 to 24 hours at relative wavelengths results from their percentage of RSD boundary is 2%. The reports are shown in Table 6. Under the present approach, there is no characteristic variations predicted for Triamcinolone acetonide in the standard solution stability test. From this study, the Triamcinolone acetonide solutions and sample solutions do not form any by-products during the experiment for about 24 hours.

Forced degradation study

This study, sometimes also known as stress testing, to know the stability of the API during this test drug was degraded by applying some external forces. Degradation study results from the

formation of any impurities established during the storage under existed environmental conditions. The study was carried under the ICH guidelines, including various degradative reagents like acidic, basic, hydrogen peroxide, hydrolyzed, and thermal degradations are studied. The graphical representation shows that there are no impurities are formed under these conditions. Results are shown in Table 7, and the drug Triamcinolone acetonide was stable.

Advantages of the proposed method

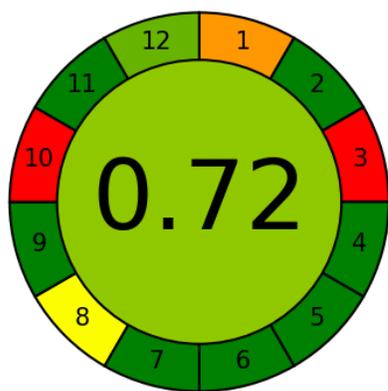
The proposed approach has not at reported and it has great advantages like commonly available column (X-bridge phenyl), mobile phase is 1:1 acetonitrile with octane sulphonic acid, good percentage of recovery (99.6%) was achieved at a wavelength of 235 nm. This approach is comparatively quick, rugged, economic with green chemical process was adopted. The literature results (Abasi *et al.*, 2020; Aquino *et al.*, 2011; Kedor-Hackmann *et al.*, 1997; Muralidharan *et al.*, 2016; Van Heugten *et al.*, 2018; Xiao *et al.*, 2016) are shown in Table 8.

AGREE-Analytical GREENness metric

AGREE is analytical tool (Pena-Pereira *et al.*, 2020) and others (Gamal *et al.*, 2021) for any analytical methods results show how best the proposed methods are environmental friendly and safety point of view toward humans. This tool was operated based on 12 parameters few of them are quantity of substances and reagents

Table 8. Comparison of the current approach with literature results.

Reference	Stationary phase	Mobile phase	Wavelength	Recovery %	Method	Run Time
Abasi et al., 2020	--	--	224 nm	100.13	Simultaneous equation	--
	--	--	274 nm	99.77	Ultraviolet spectrophotometry	--
Kedor-Hackmann et al., 1997	LiChrospher® 100 RP-18 (5 µm) in LiChroCART® (125-4) column	Acetonitrile-water (35:65 v/v) and methanol-water-96% acetic acid (55:44:1 v/v),	254 nm	99.84	HPLC	5–8 minutes
Aquino et al., 2011	--	--	242nm	--	UV-Vis spectrophotometric method	--
Van Heugten et al., 2018	Altima C18 RP18 HP column (250 × 4.6 mm ² , with 5 µm particles)	Acetonitrile and water buffered at pH 7 using 10 mM phosphate buffer	241 nm	99.5	HPLC	12 minutes
Xiao et al., 2016	Diamonsil C18	Methanol-0.1%Phosphoric acid solution(87:13,v/v),	254 nm	98.37–100.03	HPLC	5–20 minutes
Muralidharan et al., 2016	Thermo C18 column	0.5% triethylamine (TEA) (pH 3.48) and acetonitrile in the ratio of 50:50 v/v	--	79.5	RP-HPLC	4.9 minutes
Proposed method	X-bridge phenyl column	Acetonitrile with octane sulphonic acid (50:50 v/v)	235 nm	99.6	RP-HPLC	6.2 minutes

**Figure 5.** Analytical GREENess.

with toxicity, operational energy, number of run times, number of substances are identified in a single operation, etc. The proposed method was run under the AGREE-Analytical GREENess metric results 0.72 supports the approach is eco-friendly shown in Figure 5.

CONCLUSIONS

The developed new approach for validation and quantification of Triamcinolone acetonide in medication shots by RP-HPLC method was validated for both specific and unspecific impurities from ICH guidelines. This green chemical method is very economical, quick response with high reliability. The Forced Degradation study shows it is unique and more stable approach. The Photodiode Array Detector spectrum and calibrated curves are unique and linear with correlation coefficient >0.99. The quantification and appropriateness of the method was studied by the various testing parameters like recovery test, fidelity, accuracy, robustness, and stability of the solution, one can understand the correctness of the approach.

AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

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CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

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