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Development of a Validated High-performance Liquid Chromatographic Method in Reverse Phase for Simultaneously Determining Triamcinolone Acetonide and Benzyl Alcohol in Injectable Suspension

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ABSTRACT

A high-performance liquid chromatographic stability-indicating assay method in reverse-phase has been developed and validated for the simultaneous qualitative and quantitative measurement of triamcinolone acetonide and benzyl alcohol content in injectable suspension. The reverse-phase method was developed by using Inertsil ODS-2 column (100 x 4.6mm), 5µm with a mobile phase ratio comprised of water and acetonitrile mixed in 70 : 30 v/v ratio and pumped at a flow rate of 1.5 mL/min where the temperature of the column oven controlled at 40°C and sample compartment at 5°C with 10 µL injection volume. Benzyl alcohol and Triamcinolone acetonide had retention times of 1.80 and 5.60 min respectively with UV detection for benzyl alcohol at 215 nm and triamcinolone acetonide at 254 nm. The guidelines of the international conference on harmonization were used for the validation of this novel method. During validation, the developed approach was shown to be exact, accurate, linear, robust, rugged, and stable. The detector was showing linear response in a range of 10 μg/mL to 120 μg/mL and 2.5 μg/mL to 30 μg/mL for triamcinolone acetonide and benzoyl alcohol respectively.

Keywords: Validation, Development, Stability indicating, ICH, Triamcinolone acetonide, Benzyl alcohol.

INTRODUCTION

Triamcinolone acetonide Fig. 1 is a synthesized glucocorticosteroid available in salt form having immunosuppressive and anti-inflammatory action[1]. Triamcinolone acetonide interacts with the cytosolic glucocorticoid receptor response element on DNA and affects gene expression. The process of which produces anti-inflammatory proteins and inhibits inflammatory mediators. Therefore, there is a decrease in chronic inflammation and immunological responses[1]. Triamcinolone acetonide injection is used in treatment for gout, rheumatoid arthritis, synovitis, or osteoarthritis as short term treatment[1], and also used to treat atopic dermatitis, contact dermatitis, eczema, psoriasis, lichen planus, lichen

sclerosis, subacute cutaneous lupus erythematous with histopathology, and dermatomyositis.[2]

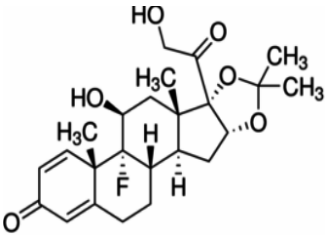

Fig. 1. Triamcinolone Acetonide Structure

Triamcinolone acetonide IUPAC name:

(1S,2S,4R,8S,9S,11S,12R,13S)-12-fluoro-11-hydroxy-8-(2-hydroxyacetyl)-6,6,9,13-tetramethyl-5,7-dioxapentacyclo[10.8.0.02,9.04,8.013,18]icosa-14,17-dien-16-one.

Molecular formula: $C_{24}H_{31}FO_6$
Molecular weight: 434.50 g/mols

Whereas, benzyl alcohol Fig. 2 is a compound that consists of a benzene ring having a single hydroxymethyl group attached as the substituent which is ortho para directing. It functions as a solvent, metabolite, antioxidant and perfume.[3] In intravenous pharmaceutical formulations, cosmetics, and topical treatments, benzyl alcohol is employed as a preservative at low doses. The USFDA has authorized benzyl alcohol in a 5% solution as safe.[3] It's often utilized in injectable pharmaceutical compositions as a preservative in the concentration range of 0.5–2.0%. The 0.9% saline solution (USP) which is used to flush deposited blood, tissues, or medications during the treatment of patients from dialysis machines, mechanized infusion sets, cannulas, or intravascular catheters after the addition of medicines or the disengagement of blood and the sterile hypotonic distilled water for injection (USP) which is employed for diluting or reconstituting injectable drugs for intravenous use both have concentrations of 0.9 percent benzyl alcohol as a preservative[3].

Benzyl alcohol iupac name: phenyl methanol

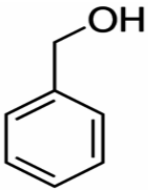

Fig. 2. Benzyl alcohol structure

Molecular Formula: $C_6H_5CH_2OH$
Molecular Weight: 108.14 g/mols

The literature review, reveals that various methods of analysis are available for triamcinolone acetonide[4–7] and benzyl alcohol[8–15] determination with different formulations or drug matrices, only one method is available for the tablet or pure form of simultaneous estimation, in which force degradation and recovery studies are performed on standard prepared in diluent instead of placebo which does not meet FDA or ICH guidelines criteria to show, specificity or interference due to placebo occurs with the main component[16–18] and also in previous methods mobile phase is made by mixing a buffer of adjusted pH with organic to the desired ratio which is a tedious or time-consuming task when analyzing a large number of samples. Buffer in the mobile phase reduces column life and increases the cost of analysis. Still, no method of chromatography is available for direct simultaneous estimation of triamcinolone acetonide and benzyl alcohol in injectable suspension.

This research aims to develop a validated novel, simple, repeatable, linear, specific, high-performance reverse-phase stability-indicating liquid chromatographic method as per ICH guidelines for simultaneously detecting triamcinolone acetonide and benzyl alcohol content in injectable suspension in which a force degradation study performed on sample and recovery study on placebo to show drug matrices impact on components of the drugs and buffer is removed from mobile phase preparation to make method simple and cost-effective.

METHODS AND MATERIALS

Chemical and reagents

Ancalima Lifesciences Ltd India provided the triamcinolone acetonide API, benzyl alcohol excipient, and placebo with triamcinolone acetonide and benzyl alcohol for market sample analysis. Ancalima life sciences Ltd.'s market sample Pcort 40 mg per 1 mL used for sample preparation. Merck limited provided the gradient grade acetonitrile, methanol, and the AR grade, sodium hydroxide, hydrogen peroxide, and hydrochloric acid. The Millipore Milli-Q water purification device was used to create ultra-pure water.

Instrumentation

Equipment and instruments were used in the development and validation experiments as follows: The chromatography was carried out utilizing a water alliance 2695 high pressure liquid chromatographic system with 2996 photodiode array detector, degasser, quaternary pump, and autosampler system, as well as Empower[3] Software (Waters corporation, milford, USA). Thermo lab precision water bath was employed for basic, acidic, and oxidative degradation experiments. In a photostability chamber (Thermolab), a study on photostability was conducted. Thermo hygrometers were used to measure humidity in humidity desiccators. A vacuum oven (Thermolab) was used to conduct the thermal analysis.

Table 1: Mobile phase and Chromatographic conditions

Parameters	Conditions
Column	Inertsil ODS-2 (100 x 4.60 mm, 5µm)
Mobile phase	Water : Acetonitrile (70:30 v/v)
UV-detection, nm	254nm for Triamcinolone Acetonide and 215nm for Benzyl alcohol
Flow rate, mL/min	1.5
Injected volume, µl	10
Column Temperature °C	40

Diluent Preparation

Filtered and degassed mobile phase mixed with methyl alcohol in the proportion of 50 : 50 v\v is used as diluent.

Standard solutions preparation

Prepared a standard stock solution of benzyl alcohol (1000 µg/mL) and Triamcinolone acetonide (4000 µg/mL) using diluent, further dilute 5 mL of both the stock solution to 250 mL to prepare a standard solution of benzyl alcohol (20µg/mL) and triamcinolone acetonide (80 µg/mL).

Sample preparation

Take 2 vials of triamcinolone acetonide injectable suspension, shake well, and transfer contents into 100 mL glass volumetric flask with adequate washing of vials from diluent, again added 100 mL diluent and sonicate for 2 min use diluent to mark it up to volume and mixed. 5 mL of this solution was again diluted to a 50 mL volumetric flask with diluent.

Preparation of placebo solution

Take 2 vials of triamcinolone acetonide injectable suspension, shake well and transfer contents into 100 mL glass volumetric flask with adequate washing of vials from diluent, again added 50 mL diluent and sonicate for 2 min use diluent to mark it up to volume and mixed. 5 mL of sample solution was again diluted to a 50 mL volumetric flask with diluent.

Forced degradation studies[19]

Acid degradation: Take 2 vials of triamcinolone acetonide injectable suspension, shake well, and transfer contents into 100 mL glass volumetric flask with adequate washing of vials from diluent, again added 50 mL diluent and sonicate for 2 minutes. Further added 5 mL of 1N HCl and mixed well then for 30 min the contents were heated in a hot water bath of thermolab 70°C. Cooled the sample solution and neutralized it with 5 mL of 1N NaOH, after neutralization use diluent to mark it up to volume and mixed. 5 mL of degraded sample solution was again diluted to a 50 mL volumetric flask with diluent. Fig. 3 & Fig. 4 shows the chromatogram obtained.

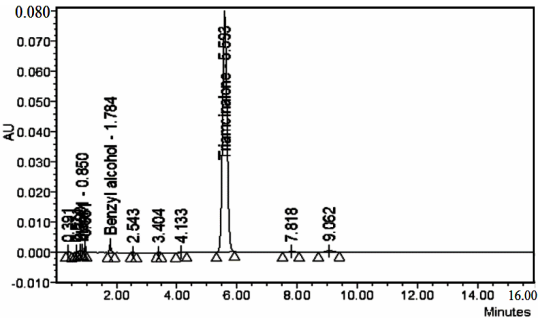

Fig. 3. Acid degradation sample chromatogram at 254nm

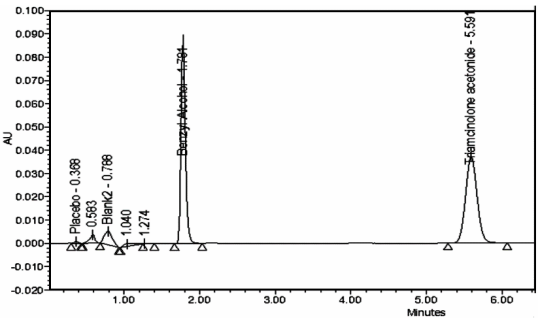

Fig. 4. Acid degradation sample chromatogram at 215nm

Alkali degradation: Take 2 vials of triamcinolone acetonide injectable suspension, shake well, and transfer contents into 100 mL glass volumetric flask with adequate washing of vials from diluent, again added 50 mL diluent and sonicate for 2 minutes. Further added 5 mL of 1N NaOH and mixed well. For 30 min, the contents were heated in a hot

water bath of thermolab at 70°C. Cooled the sample solution then neutralized it with 5 mL of 1N HCl, after neutralization use diluent to mark it up to volume and mixed. 5 mL of degraded sample solution was again diluted to a 50 mL volumetric flask with diluent. Fig. 5 & Fig. 6 shows the chromatogram obtained.

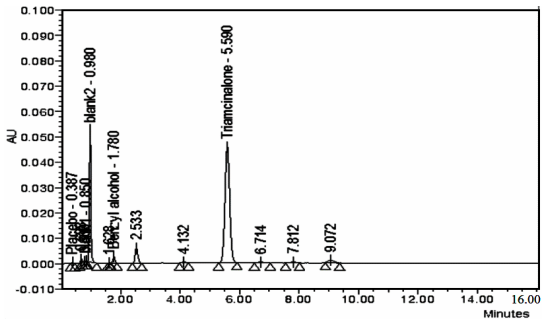

Fig. 5. Base degradation sample chromatogram at 254nm

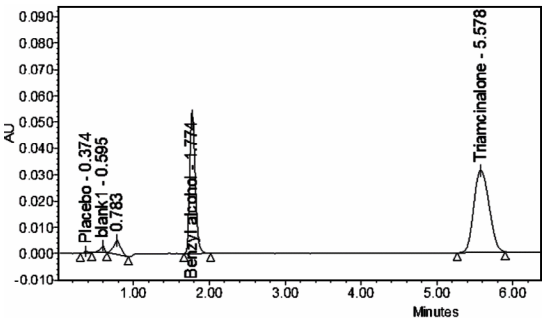

Fig. 6. Base degradation sample chromatogram at 215nm

Peroxide degradation: Take 2 vials of triamcinolone acetonide injectable suspension, shake well, and transfer contents into 100 mL glass volumetric flask with adequate washing of vials from diluent, again added 50 mL diluent and sonicate for 2 minutes. Further added 5 mL of 3.0% H_2O_2 and mixed well. For 30 min the contents were heated in a hot water bath of thermolab at 70°C. Cool the sample solution up to room temperature then use diluent to mark it up to volume and mix. 5 mL of degraded sample solution was again diluted to a 50 mL volumetric flask with diluent. Fig. 7 & Fig. 8 shows the chromatogram obtained.

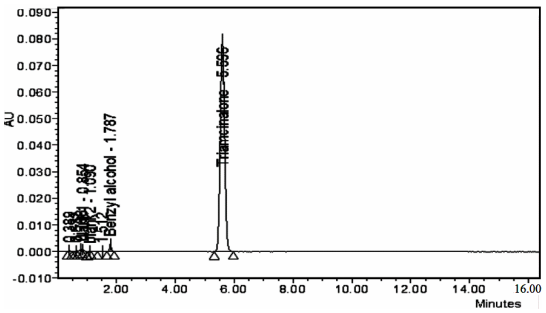

Fig. 7. Oxidative degradation sample chromatogram at 254nm

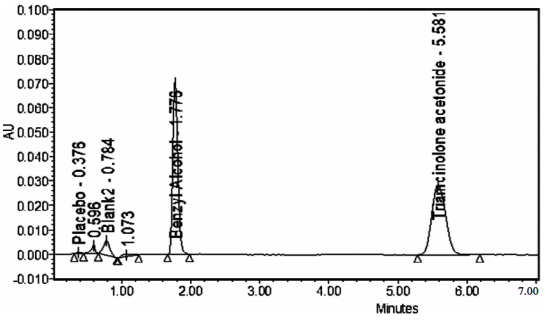

Fig. 8. Oxidative degradation sample chromatogram at 215nm

UV-degradation: Take 2 vials of (previously kept in UV-light for 24 h) triamcinolone acetonide injectable suspension, shake well and transfer contents into 100 mL volumetric glass flask with adequate washing of vials from diluent, again added 50 mL diluent and sonicate for 2 min and use diluent to mark it up to volume and mix. 5 mL of degraded sample solution was again diluted to a 50 mL volumetric flask with diluent. Fig. 9 & Fig. 10 shows the chromatogram obtained.

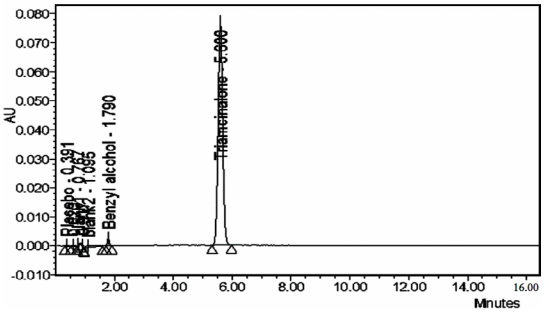

Fig. 9. UV-degradation sample chromatogram at 254nm

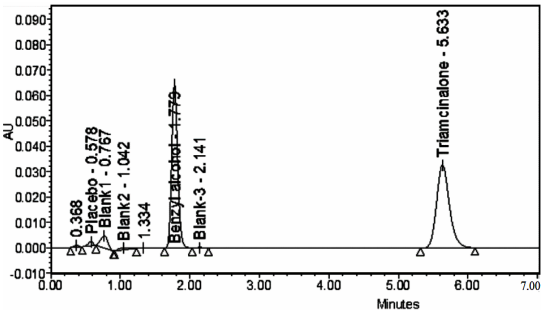

Fig. 10. UV-degradation sample chromatogram at 215nm

Thermal degradation: Take 2 vials of triamcinolone acetonide injectable suspension, shake well, and transfer contents into 100 mL glass volumetric flask with adequate washing of vials from diluent, again added 50 mL diluent and sonicate for 2 minutes. For 30 min the contents were heated in a hot water bath of thermolab at 70°C. Cool the

sample solution up to room temperature then use diluent to mark it up to volume and mix. 5 mL of degraded sample solution was again diluted to a 50 mL volumetric flask with diluent. Fig. 11 & Fig. 12 shows the chromatogram obtained.

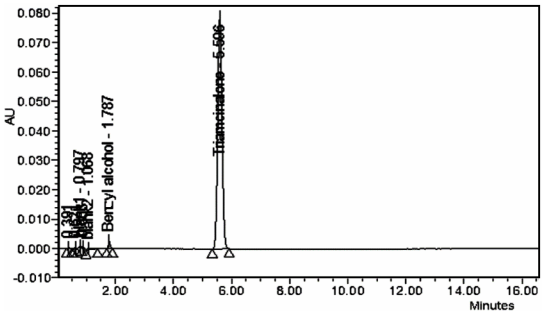

Fig. 11. Thermal degradation sample chromatogram at 254nm

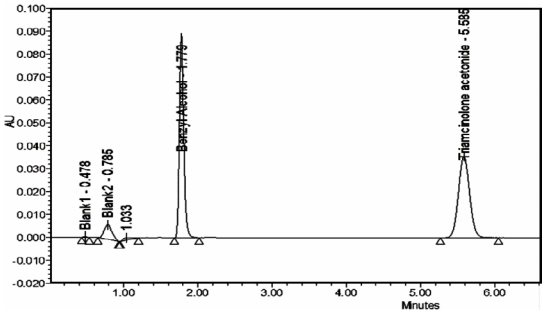

Fig. 12. Thermal degradation sample chromatogram at 215nm

Method validation

Using international council for harmonisation guidelines, the newly designed reverse-phase chromatographic technique was validated for linearity, accuracy, precision, specificity, robustness, ruggedness, the limit of detection and limit of quantification.[16]

System suitability

To verify the system's suitability criteria, before every validation parameter initiation standard preparation injected in five replicates, the system is only suitable for analysis when the %RSD of peak areas and tailing should not be more than 2.0 and theoretical plates should not be less than 2500 for triamcinolone acetonide and benzyl alcohol respectively.

Specificity

Establish specificity of the method by demonstrating

(a) Blank, placebo, and as such sample injections will be used to establish that there is no interference from excipients in the solution.

(b) This was demonstrated by forcing the sample to deteriorate with 1N HCl, 1N NaOH, 3.0% H_2O_2, heated at 70°C for 30 min in a hot water bath of thermolab and exposing for 24 h in ultraviolet light. According to the test methodology, the samples were prepared. and then injected into a high-performance chromatographic system equipped with water's 2996 photodiode array detector. The purity angle for the triamcinolone acetonide peak and benzaldehyde peak was less than the threshold value in each case.

Precision

To evaluate system precision, five replicates of the standard preparation were injected into the system as specified in the method of study, and the percent relative standard deviation (RSD) value derived from peak areas was utilized as a measure. For method precision six sample solutions of triamcinolone acetonide injectable suspension prepared as per method and analyzed on the same day. For intermediate precision six prepared sample solutions of triamcinolone acetonide injectable suspension as per method and analyzed using a separate column from the same manufacturer, the different instruments on a different day of method precision. For six preparations, the percent RSD of the assay was determined.

Linearity

Prepared a series of standard preparations of triamcinolone acetonide and benzyl alcohol in the range of 50% to 150% of the target concentration. for triamcinolone acetonide & 12% to 150% of benzyl alcohol. By plotting the absorbance vs. concentration of the drugs the linearity calibration curves were obtained demonstrating linearity over the concentration range of 10-120 µg/mL for Triamcinolone acetonide and 2.5-30 µg/mL for Benzyl alcohol (Table 5). MS-Excel was used to compute the slope, intercept and coefficient of correlation.

The Limit of detection and quantitation

Prepared a series of triamcinolone acetonide and benzyl alcohol standards of different concentrations equal to or below the specified limits using standard stock solutions and injected in triplicate into the HPLC. Limit of detection(LOD) and Limit of quantification (LOQ) was calculated by using the value of slope and intercept of the calibration curves of both the drugs.

Accuracy(Recovery)

The accuracy was carried out by spiking triamcinolone acetonide and benzyl alcohol in the placebo samples at concentrations of 50%, 100, and 150 percent of the target concentration in triplicate (for a total of nine measurements) followed by sample preparation as indicated under the procedure. Mean %assay and %RSD value were calculated for all triplicate preparations.

Robustness

As per the method of analysis, three sample solutions were prepared of triamcinolone acetonide injectable suspension USP and analyzed using varied chromatographic conditions as below.

(i) Change in flow (± 0.2 mL/min)
(ii) Change in column oven temperature (± 2.0)
(iii) Change in organic phase composition in the mobile phase (± 5%)
(iv) Change in wavelength (± 2 nm)

At each variable condition mean assay percent and %RSD for assay value were calculated along with system suitability criteria for Triamcinolone acetonide and benzyl alcohol.

Solution stability

Solution stability was established by freshly prepared standard and sample keeping it at room temperature and then injecting it freshly and at varied time intervals. %Deviation in the peak area of standard and the sample solution from the initial area was calculated.

RESULTS AND DISCUSSION

Method development chromatographic parameters optimization

The main purpose of this method optimization is to quantitate triamcinolone acetonide and benzyl alcohol content simultaneously, accurately, and precisely without any interference of blank or placebo matrices and for that prepared mobile phase with simple composition without using buffer or adjusting pH. The pKa value for triamcinolone acetonide and benzyl alcohol is 13.4 and 15.4 respectively and both the contents are practically insoluble in water but soluble in methanol hence the approach for development started from the use of water, acetonitrile, and methanol in the mobile phase and diluent preparation. Many columns are used after studying various parameters of development and started with water, acetonitrile ratio (50:50 v/v, 55:45 v/v) with column Hypersil BDS (150 X 4.6mm, 5 µm), at 254nm but the peak of benzyl alcohol elute too early and peak shape was not good hence column changed to Inertsil ODS-2 (100 X 4.6 mm) 5 µm with mobile phase ratio water: acetonitrile (50:50) but again peak of benzyl alcohol eluted too early in comparison to triamcinolone acetonide peak and the response of benzyl alcohol peak is not satisfactory hence need to change wavelength for benzyl alcohol peak from 254 nm to 215 nm to get a better response from the detector at lower levels.

In a final optimize method the mobile phase ratio of water: acetonitrile(70:30 v/v) using column Inertsil ODS-2 (100mm X 4.6mm, 5 µm) thermostated at 40°C isocratically pumped with a flow rate of 1.5mL/min and 10 µL of injection volume. The wavelength for detection of benzyl alcohol and triamcinolone acetonide set at 215 nm and 254 nm respectively.

Force degradation behavior

Chromatography of triamcinolone acetonide and benzyl alcohol samples under suggested conditions shows degradation behavior

Acid degradation

At 70°C for 30 min in 5 mL of 5N hydrochloric acid, moderate deterioration was detected in the sample. (Table 2)

Alkali degradation

In 5 mL of 1N sodium hydroxide solution at 70°C for 10 min the drug was very unstable but excipient benzyl alcohol is stable. (Table 2)

Peroxide degradation

In 5 mL of 3.0 percent H_2O_2 at 70°C for 30 min the sample was stable. (Table 2)

UV-degradation

The sample should be relatively stable when exposed in 1.2 million lux hours visible and 200 Watt-hour per square meter UV-light. (Table 2)

Thermal degradation

In response to Thermal conditions, the sample revealed no substantial deterioration. (Table 2)

Table 2: Force degradation summary of Triamcinolone acetonide(254nm) and Benzyl alcohol(215nm)

Conditions and time	Triamcinolone acetonide at 254nm			Benzyl alcohol at 215nm		
	%Assay	Purity angle	Purity threshold	%Assay	Purity angle	Purity threshold
Sample as is	99.19	0.100	0.292	100.2	0.169	0.365
Acid degradation (5 mL 1N HCl, heat 70°C for 30 mins)	95.36	0.092	0.283	98.21	0.174	0.369
Alkali degradation (5mL 1N NaOH, heat 70°C for 10 mins)	60.94	0.160	0.349	98.08	0.178	0.377
Peroxide degradation (5 mL 3.0% H_2O_2, heat 70°C for 30 mins)	99.42	0.096	0.287	99.23	0.183	0.379
UV-degradation (1.2 million lux hours visible and 200 Watt hour per square meter UV-light)	99.14	0.098	0.293	99.80	0.228	0.369
Thermal degradation (heat 70°C, 30 mins)	100.61	0.094	0.288	99.20	0.211	0.388

Method Validation

According to International Council for Harmonisation recommendations.[16]

System suitability

As specified in the method of analysis, system suitability was checked by injecting five replicates of the standard solution on different days of the validation study. The tailing factor, theoretical plates, and peak area %RSD of Triamcinolone acetonide and benzyl alcohol peak from standard solution were computed using the system software The summary of system suitability was found within the acceptance criteria. (See Table 3)

Table 3: Summary of system suitability criteria

Parameters	Specification	Observed values			
		Triamcinolone Acetonide		Benzyl alcohol	
		Method precision	Intermediate precision	Method precision	Intermediate precision
Area [RSD (%), n = 5]	≤2.0%	0.43	0.55	0.47	0.76
USP tailing	≤2.0	1.05	1.11	1.19	1.31
Theoretical plates	NLT 2500	6559	6249	4381	4245

Precision

For System precision %RSD for replicate injections of triamcinolone acetonide is 0.43 and for benzyl, alcohol is 0.47, respectively (Table 3).

For method precision and intermediate precision %RSD for assay of triamcinolone is 0.21 and 1.26 while for benzyl alcohol is 0.41 and 1.32 respectively (Table 4). Overall %RSD for twelve test preparations (six from method precision and six from intermediate precision) (Table 4) of triamcinolone acetonide is 0.90, and for benzyl alcohol is 0.98, which is less than 2.0%. The low RSD value indicates that the technique is precise.

Table 4: Summary of method or intermediate precision

Sample no.	Triamcinolone Acetonide Assay (% of label claim)		Benzyl alcohol Assay (% of label claim)	
	Method precision	Intermediate precision	Method precision	Intermediate precision
1	99.33	100.36	100.26	100.62
2	99.47	99.99	100.78	100.44
3	99.87	98.23	101.42	98.76
4	99.34	101.16	100.56	101.34
5	99.62	101.36	101.13	101.80
6	99.42	98.80	100.91	98.58
Mean	99.51	99.98	100.84	100.26
SD	0.21	1.26	0.41	1.32
%RSD (≤2%)	0.21	1.26	0.41	1.32
Overall Mean	99.75	100.55		
Overall SD	0.894	0.984		
Overall %RSD (≤2%)	0.90	0.98		

Linearity and range

Equations of Linear regression were found to be y = 9431.2x + 5589.6 and y = 17613x + 2358.7, Fig. 13 & Fig. 14 whereas the values of the regression coefficients (r) were 0.9999 for Triamcinolone acetonide and Benzyl alcohol respectively Table 5 shows the proposed method is linear in the given concentration range.

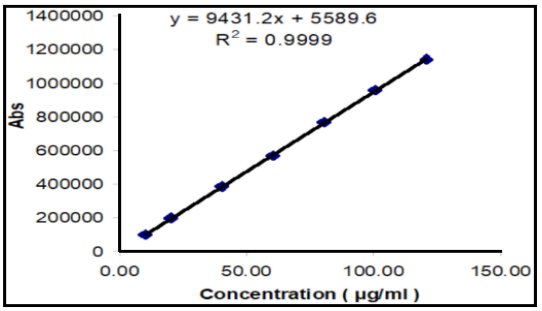

Fig. 13. Triamcinolone acetonide Linearity graph

The Limit of detection and quantitation

In this study, the LOD and LOQ values for triamcinolone acetonide were determined to be 0.2012 μg/mL and 0.4020 μg/mL, respectively, while for benzyl alcohol, the values were 0.0205 μg/mL and 0.0512 μg/mL, respectively.

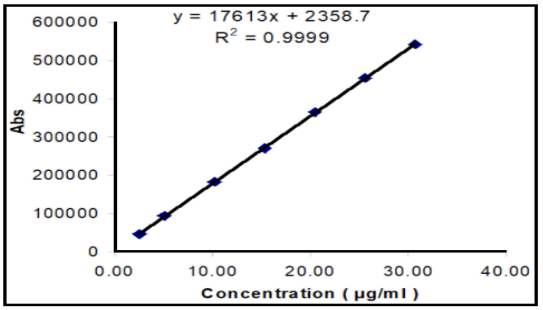

Fig. 14. Benzyl alcohol Linearity graph

Accuracy (Recovery)

Table 6 shows the mean %assay recovery is within the range of 98.0%–102.0%, Individual assay percentage be in the range of 97.0% to 103.0%, and overall %RSD should not be greater than 2.0. The results show that the recovery of triamcinolone acetonide and benzyl alcohol from the placebo is acceptable.

Table 5: Summary of triamcinolone acetonide and benzyl alcoh ol Linearity

Triamcinolone Acetonide (254 nm)		Benzyl Alcohol (215 nm)	
Concentration (μg/mL)	Mean area	Concentration (μg/mL)	Mean area
120.72	1141904	30.72	542507
100.60	956839	25.60	454226
80.48	768329	20.48	364924
60.36	569106	15.36	270251
40.24	385653	10.24	182731
20.12	198718	5.12	94189
10.06	98341	2.56	46530
Slope	9431.23	Slope	17613.05
Intercept	5589.57	Intercept	2358.71
Correlation Coefficient (r)	0.9999	Correlation Coefficient (r)	0.9999

Table 6: Summary of triamcinolone acetonide (254 nm) and Benzyl alcohol (215nm) recovery

Levels	Preparation	Triamcinolone Acetonide at 254nm		Benzyl alcohol at 215nm		Limits
		Amount Recovered%	%Recovery	Amount Recovered%	%Recovery	
50%	1	49.02	98.04	49.52	99.03	Individual assay
	2	49.61	99.23	49.42	98.83	97.0% -103.0%
	3	49.46	98.92	49.59	99.19	
100%	1	100.10	100.10	97.92	97.92	
	2	100.38	100.38	97.24	97.24	
	3	99.94	99.94	97.11	97.11	
150%	1	149.69	99.79	147.46	98.31	
	2	149.20	99.47	147.71	98.47	
	3	150.00	100.00	154.15	102.77	
Mean(98.0%-102.0%)			99.54	Mean	98.76	98.0%-102.0 %
Standard Deviation			0.72	SD	1.67	-
Overall %Relative Standard Deviation≤ 2.0			0.73	%RSD≤ 2.0	1.69	NMT 2.0 %

Robustness

For all the varied chromatographic conditions, the standard solution (theoretical plates, tailing and, %RSD) validity was examined according to system suitability criteria. For each robustness condition, the %assay RSD of Triamcinolone acetonide and benzyl alcohol was computed shown in Table 7 & Table 8, the overall %RSD for each set of data should not be more than 2.0.shows that the robustness of the method.

Table 7: Summary of robustness [Triamcinolone acetonide (254nm)]

Varied chromatographic parameters	System suitability parameters observed in the standard			Triamcinolone acetonide at 254 nm		
	USP tailing ≤2.0	Theoretical plates	%RSD ≤2.0 (n = 5)	%Assay	Mean assay%	%RSD ≤2.0
Column temperature (42°C)	1.05	5954	0.25	98.45 98.21 98.78	98.48	0.29
Column temperature (38°C)	1.07	6857	0.19	99.99 99.85 100.25	100.03	0.20
Flow rate (1.70 mL/min)	1.05	6963	0.98	99.61 100.23 99.81	99.88	0.32
Flow rate (1.30 mL/min)	0.95	5700	0.55	100.91 100.73 101.31	100.98	0.29
Buffer:acetonitrile (664:336)	1.02	6020	0.27	99.97 100.12 100.28	100.12	0.15
Buffer:acetonitrile (696:304)	1.04	6251	0.67	101.31 100.62 100.45	100.79	0.45
Wavelength (256 nm)	1.08	6369	0.25	100.28 100.71 101.4	100.80	0.56
Wavelength (252 nm)	1.03	6757	0.37	100.84 100.71 101.31	100.95	0.31

Table 8: Summary of robustness[Benzyl alcohol (215nm)]

Varied chromatographic parameters	System suitability parameters observed in the standard			Benzyl alcohol at 215nm		
	USP tailing ≤ 2.0	Theoretical plates	%RSD ≤2.0 (n = 5)	%Assay	Mean assay	%RSD ≤ 2.0
Column temperature (42°C)	1.19	3956	0.48	99.01 99.25 99.04	99.1	0.13
Column temperature (38°C)	1.33	4755	0.8	101.11 99.82 101.4	100.78	0.83
Flow rate (1.70 mL/min)	1.42	4876	0.77	99.33 99.52 100.4	99.75	0.57
Flow rate (1.30 mL/min)	1.23	4054	0.57	101.32 99.52 100.94	100.59	0.94
Buffer:acetonitrile (664:336)	1.12	4205	0.65	100.35 101.25 100.94	100.85	0.45
Buffer:acetonitrile (696:304)	1.15	3978	0.49	101.89 101.56 101.73	101.73	0.16
Wavelength (256 nm)	1.2	4235	0.73	100.71 100.78 101.38	100.96	0.36
Wavelength (252 nm)	1.28	4412	0.52	100.88 101.25 100.6	100.91	0.32

Specificity

By injecting a blank, a placebo and a sample prepared from the same method, No peaks were observed during the retention time of triamcinolone acetonide and benzyl alcohol peaks, showing that blank and excipient interference is not present. The purity angle of the triamcinolone acetonide peak and benzaldehyde peak was less than the threshold value in each case of force degradation data shown in Table 2, that no degradation products interfered within the retention duration of the triamcinolone acetonide and benzyl alcohol peak.

Stability of solution

Sample and the standard solution are stable for 48 h at room temperature as the %deviation from the initial area was found to be less than 2.0%. (Table 9)

Table 9: Solution stability of Triamcinolone acetonide and Benzyl alcohol

Time	Triamcinolone Acetonide(254nm)				Benzyl Alcohol (215nm)			
	Area counts		% Deviation from initial area NMT: ±2.0%		Area counts		Deviation from initial area NMT: ±2.0%	
	Standard	Sample	Standard	Sample	Standard	Sample	Standard	Sample
Initial	783472	835244	0	0	363308	361941	0	0
3 h	783924	839262	0.1	0.5	364550	361479	0.3	-0.1
7 h	783470	849318	0	1.7	364001	363468	0.2	0.4
13 h	788259	847903	0.6	1.5	365822	366594	0.7	1.3
22 h	790651	844587	0.9	1.1	368151	364285	1.3	0.6
26 h	793720	847678	1.3	1.5	368900	365873	1.5	1.1
45 h	795720	847295	1.6	1.4	368132	367300	1.3	1.5
48 h	798720	850596	1.9	1.8	369800	368893	1.8	1.9

CONCLUSION

For the assessment of triamcinolone acetonide and benzyl alcohol content within the presence of sample matrices, placebo, and various degradation conditions an economical hydrophobic stationary phase stability-indicating high-performance liquid chromatographic method has been created and validated in compliance with the International Council for Harmonisation requirements, which is simple, quick, rugged, accurate, precise and linear. The validation findings of the analytical technique were determined to be adequate. The short runtime of 10 min is enough to separate each analyte, and the simple mobile phase composition makes this approach cost-effective and suitable for regular testing of many samples of injections in a short time interval.

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Conflict of Interest

The author declares that there is no conflict of interest.

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