Development and Validation of HPLC Method for the estimation of Triamcinolone Acetonide and 5-Fluorouracil

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ABSTRACT

A sensitive HPLC method has been developed and validated for the determination of Triamcinolone Acetonide and 5-Fluorouracil in topical formulations. The developed method is found to be specific, reproducible and stability indicating. The Phenomenex C_{18}, 250 × 4.6 mm, 5µm particle size column was used and mobile phase contains two solvents: Solvent A contains 10mM phosphate buffer pH 3.0 and solvent B was Acetonitrile. The detector linearity was established from concentrations ranging from 1 µg/ml to 10 µg/ml for both Triamcinolone Acetonide and 5-Fluorouracil with a correlation coefficient of 0.999. The limit of detection(LOD) and limit of quantification(LOQ) for Triamcinolone Acetonide was found to be 0.04 and 0.13 respectively and 5-Fluorouracil was found to be 0.01 and 0.03 respectively. The method was proved to be robust with respect to changes in flow rate, column temperature, mobile phase ratio and detector wavelength.

Keywords: *Triamcinolone Acetonide, 5-fluorouracil, Validation, HPLC method, topical formulations*

INTRODUCTION

Triamcinolone acetonide is a synthetic glucocorticoid that is the 16,17-acetonide of triamcinolone. Used to treat various skin infections. It has a role as an anti-inflammatory drug and an anti-allergic agent. It is an 11beta-hydroxy steroid, a 20-oxo steroid, a 21-hydroxy steroid, a 3-oxo-Delta (4) steroid, a glucocorticoid, a cyclic ketal, a fluorinated steroid and a primary alpha-hydroxy ketone. It derives from a triamcinolone. It derives from a hydride of a pregnane.[1,2]

Steroid tapes, intralesional steroid injection and steroid ointments is widely used in the treatment of keloids and hypertrophic scars. Although intralesional injections are highly in demand in market, steroid tapes and ointments are still gaining popularity. [3]

Fluorouracil is a cytotoxic chemotherapy medication used to treat cancer. By intravenous injection it is used for treatment of colorectal cancer, oesophageal cancer, stomach cancer, pancreatic cancer, breast cancer, and cervical cancer. It can also be administered in the form of cream and lotions for the treatment of basal cell carcinoma and actinic keratosis [4,5,6]. Studies have also shown the efficiency of 5-fluorouracil for the treatment of keloids. [7]

To the best of our knowledge, none of the currently available analytical methods can separate Triamcinolone Acetonide and 5-Fluorouracil in one HPLC run. Furthermore no such validation is reported in the literature that can adequately separate and quantitate both the compounds.

In this paper we report a method for the assay of Triamcinolone Acetonide and 5-Fluorouracil. The method is capable of separating them efficiently. The method was successfully validated

according to the International Conference Harmonization(ICH) guidelines (Validation of Analytical Procedures: Test and Methodology Q2)

Experimental

Reagents and Chemicals

Triamcinolone Acetonide and 5-Fluorouracil were arranged by Oniosome Healthcare private limited(Mohali). All the drugs were used as received and their solutions were prepared freshly every day and used as working standards. All the chemicals and solvents used were of HPLC Grade, procured from different sources.

Instrumentation

Shimazdu Prominence-I LC-2030 C HPLC System equipped with photo-array detector were used for method development. All HPLC systems were equipped with column compartment with temperature control and an on-line degasser. Data acquisition, analysis and reporting were performed by chromatography software.

Selection of Detection Wavelength

The sensitivity of HPLC method that uses UV detection depends upon proper selection of detecting wavelength. An ideal wavelength is the one that gives good response for the drugs that are to be detected. In the present study, the Triamcinolone and 5-Fluorouracil solution of 10 µg/ml was prepared in methanol. This solution was then scanned in the UV region of 200-400 nm and a spectrum was recorded.

Chromatographic Conditions

Stationary Phase	C_{18}, 250 × 4.6 mm, 5µm particle size, Phenomenex
Elution mode	Low pressure gradient system mode (62:38 v/v)
Diluent	Mobile Phase
Mobile Phase	Solvent A was 10mM phosphate buffer pH 3.0 ; Solvent B was Acetonitrile
Column Temperature	45°C
Detection wavelength	250 nm
Flow rate	1 ml/min
Injection volume	20 µl
Run Time	10 minutes

Table 1-HPLC conditions

Standard stock Preparation

An accurately weighed quantity of about 10 mg of Triamcinolone and 10 mg of 5-Fluorouracil was added in 10 ml volumetric flask. About 10 ml of diluent was added and sonicated for 10 minute to dissolve. From this stock solution, suitable amount of sample was further diluted to form mixture containing 10 µg/ml of each Triamcinolone and 5-Fluorouracil.

Results and Discussion

The development of HPLC methods for the determination of drugs has received considerable attention the past years due to their importance in Quality control of drugs and pharmaceutical products. Column chemistry, solvent type, detection wavelength, temperature and flow rates were carried to determine the chromatographic conditions giving the best suitable results as shown in Table 1.

Different mobile phase was examined on different columns. At last the elute consists of Solvent A- 10mM phosphate buffer pH 3.0; Solvent B - Acetonitrile was selected and maintained a flow rate of 1 ml/min applied in C_{18}, 250 × 4.6 mm, 5µm particle size column were found to be as optimal for obtaining well defined peaks. The elute was detected at wavelength of 250 nm and it was found to be the optimum wavelength for detection and quantification of Triamcinolone Acetonide and 5-Fluorouracil.

With these optimized chromatographic conditions, typical chromatogram of Triamcinolone Acetonide and 5-Fluorouracil (Fig. 1) was obtained and was found to be a very sharp peak with better resolution.

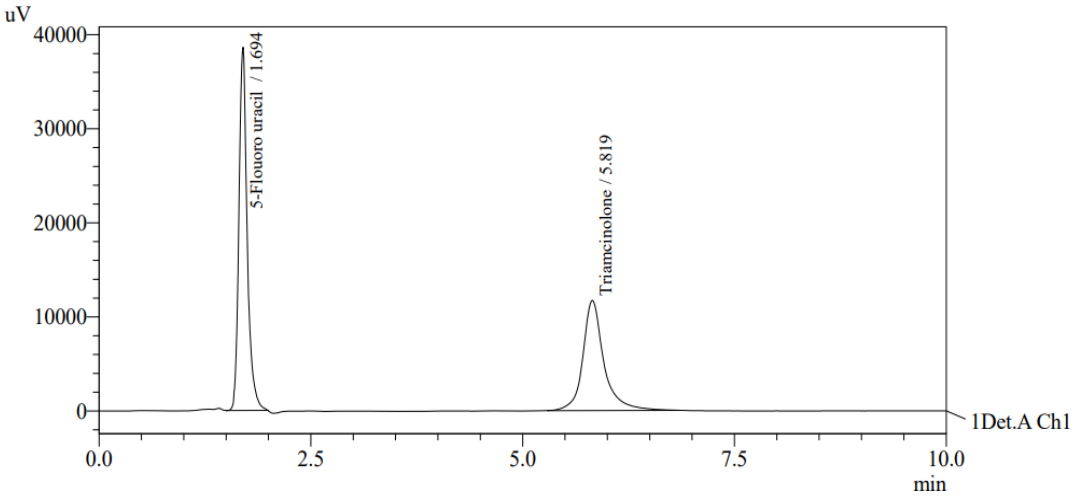

Figure 1-Typical Chromatogram(10ug/ml)

Validation of Method

Linearity and Range

The linearity solutions for Triamcinolone Acetonide were prepared in diluent. Analyte solution has shown linear response for concentration levels ranging from 1 to 10 µg/ml. The correlation coefficient value was found to be 0.9998 and 0.9979 respectively. The linearity plot is shown in Figure 2 for Triamcinolone Acetonide and Figure 3 for 5-Fluorouracil.

Sr. No.	Concentration µg/ml	Area Under Curve	
		Triamcinolone Acetonide	5-Fluorouracil
01.	1	21486	36732
02.	2	39751	59136
03.	3	61084	85430
04.	4	81699	112320
05.	5	101989	136828
06.	6	120908	165824

07.	8	161326	223322
08.	10	204027	262628

Table 2:Linearity of Triamcinolone and 5-Fluorouracil

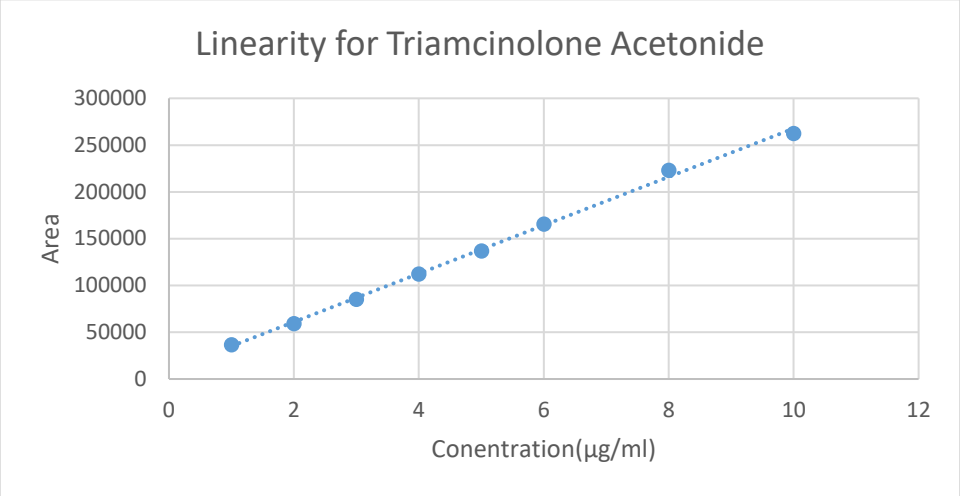

Figure 2-Linearity for Triamcinolone Acetonide

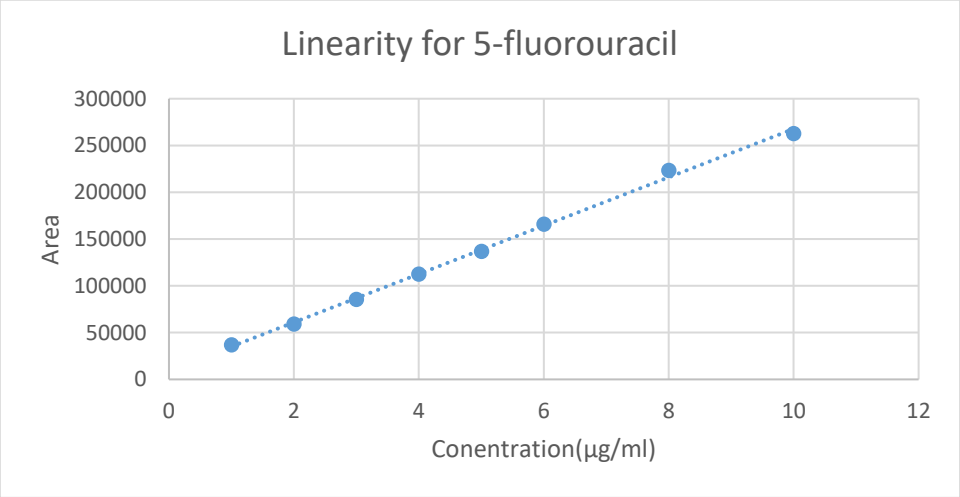

Figure 3-Linearity for 5-fluorouracil

Precision

The intra-day and inter-day variation for determination of Triamcinolone and 5-Fluorouracil were carried out three times for definite concentrations i.e. 5 µg/ml of Triamcinolone and 5 µg/ml of 5-Fluorouracil for six consecutive days for inter day variation. The %RSD of Triamcinolone Acetonide and 5-Fluorouracil was found to be less than 2.0%.

S.no.	Level	Concentration	% Recovery	% Mean Recovery	%RSD
01.	Repeatability (5µg/ml)	4.990	99.80	99.30±0.66	0.66
		4.927	98.54		
		4.977	99.54		
02.	Interday (5µg/ml)	4.958	99.16	99.58±0.37	0.37
		4.984	99.69		
		4.994	99.89		
3.	Intraday (5µg/ml)	4.984	99.68	99.21±0.41	0.41
		4.952	99.05		
		4.944	98.89		

Table 3 : Repeatability and inter-intraday precision study of Triamcinolone Acetonide

S.no.	Level	Concentration	% Recovery	% Mean Recovery	%RSD
01.	Repeatability (5µg/ml)	4.965	99.30	99.27±0.29	0.29
		4.948	98.97		
		4.978	99.56		
02.	Interday (5µg/ml)	4.947	100.89	99.40±0.38	0.39
		4.983	100.28		
		4.979	100.67		
3.	Intraday (5µg/ml)	4.973	99.47	99.72±0.21	0.21
		4.993	99.87		

Table 4 : Repeatability and inter-intraday precision study of 5-Fluorouracil

The method was found to be precise due to low values of the %RSD.

Accuracy

Accuracy of the method was determined in terms of % recovery of standard. Recovery studies were carried out by analysing of standard drug solution at the level of 80%, 100% and 120% in triplicate. Results of the recovery study found to be within the acceptance criteria 100 ±10 %, which indicates a good degree of sensitivity of the method towards detection of analytes in sample.

S.no.	Level	Concentration	% Recovery	% Mean Recovery	%RSD
01.	80% (4µg/ml)	3.953	98.84	98.58±0.016	0.420
		3.952	98.81		
		3.924	98.10		
02.	100% (5µg/ml)	4.921	98.42	98.25±0.008	0.171
		4.912	98.25		
		4.904	98.08		
3.	120% (6µg/ml)	5.981	99.68	99.25±0.024	0.417
		5.931	98.86		
		5.953	99.23		

Table 5: Accuracy Study of Triamcinolone

S.no.	Level	Concentration	% Recovery	% Mean Recovery	%RSD
01.	80% (4µg/ml)	3.949	98.74	98.67±0.010	0.26
		3.935	98.38		
		3.954	98.87		
02.	100% (5µg/ml)	5.010	100.21	100.04±0.007	0.15
		4.999	100.00		
		4.995	99.91		
3.	120% (6µg/ml)	5.946	99.11	100.50±0.081	1.36
		6.033	100.57		
		6.110	101.84		

Table 6: Accuracy Study of 5-Fluorouracil

Limit of Detection(LOD) and Limit of Quantification

The Limit of detection and limit of quantification of the method were calculated basing on standard deviation of the response and the slope (s) of the calibration curve at approximate levels of the limit of detection and limit of quantification. The results obtained were within the limit.

Sr. No.	Drug	LOD (µg/ml)	LOQ (µg/ml)
01.	Triamcinolone	0.04	0.13
02.	5-Fluorouracil	0.01	0.03

Table 7: LOD and LOQ Data

Robustness

The robustness was studied by analyzing the sample of lower concentration with deliberate variation in the method parameters. The change in the responses of drugs was noted in terms of %RSD. Robustness of the method was studied by change in wavelength & change in flow rate.

Sr. No.	Initial concentration (µg/ml)	Triamcinolone final concentration (µg/ml)	% recovery at 1.3 ml/min	Triamcinolone final concentration (µg/ml)	% recovery at 1.7 ml/min
01.	5	4.942	98.84	4.893	97.87
02.	5	4.977	99.54	4.845	96.91
03.	5	4.931	98.63	4.892	97.84
		Mean	**99.01**		**97.55**
		SD	**0.48**		**0.55**
		%RSD	**0.49**		**0.56**

Table 8: Robustness data of Triamcinolone with deliberate change in flow rate

Sr. No.	Initial concentration (µg/ml)	5-Flurouracil final concentration (µg/ml)	% recovery at 1.3 ml/min	5-Flurouracil final concentration (µg/ml)	% recovery at 1.7 ml/min
01.	5	4.903	98.06	4.897	97.95
02.	5	4.927	98.55	4.848	96.97
03.	5	4.787	95.75	4.863	97.26
		Mean	**97.46**		**97.40**
		SD	**1.49**		**0.50**
		%RSD	**1.53**		**0.51**

Table 9: Robustness data of 5-Fluorouracil with deliberate change in flow rate

Sr. No.	Initial concentration (µg/ml)	5-Flurouracil final concentration (µg/ml)	% recovery at 248 nm	5-Flurouracil final concentration (µg/ml)	% recovery at 252 nm
01.	5	4.970	99.41	4.924	96.49
02.	5	4.925	98.51	4.876	97.52
03.	5	4.937	98.74	4.847	96.94
		Mean	**98.89**		**97.66**
		SD	**0.47**		**0.78**
		%RSD	**0.48**		**0.80**

Table 10: Robustness data of Triamcinolone with deliberate change in wavelength

Sr. No.	Initial concentration (µg/ml)	5-Flurouracil final concentration (µg/ml)	% recovery at 248 nm	5-Flurouracil final concentration (µg/ml)	% recovery at 252 nm
01.	5	4.831	96.62	4.832	96.64
02.	5	4.888	97.76	4.865	97.76
03.	5	4.910	98.20	4.861	98.20
		Mean	**97.53**		**97.07**
		SD	**0.82**		**0.36**
		%RSD	**0.84**		**0.38**

Table 11: Robustness data of 5-Fluorouracil with deliberate change in wavelength

The Percentage RSD should not be more than 2. The %RSD obtained for change of flow rate was found to be below 2, which was within the acceptance criteria. Hence the method was robust.

Conclusion

A new HPLC method was developed for the analysis of Triamcinolone Acetonide and 5-Fluorouracil under same conditions. As shown above the method is effective, accurate, precise and robust proving the reliability of the method. The proposed method was validated showing satisfactory data for all the method validation parameters. The information presented herein could be very useful for quality monitoring of samples as well as employed to check quality during stability studies.

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