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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF ISOTRETINOIN IN TABLET DOSAGE FORMULATION

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ABSTRACT

Isotretinoin is a topical keratolytic agent which is used in the treatment of skin diseases including acne vulgaris. This paper deals with a simple, feasible and sensitive reverse-phase high performance liquid chromatographic method and ultraviolet spectrophotometric method for quantitative determination of Isotretinoin in pharmaceutical dosage form. The chromatography and spectrum was carried out by using HPLC system and UV spectrophotometry. The mobile phase consisting of Buffer and Methanol in the ratio 80, 20. The detection made at 232 nm and the mobile phase flowed 1.2 ml/min. Validation parameters included system suitability, specificity, linearity, accuracy precision, robustness, ruggedness were determined according to the ICH guidelines. The method could be successfully applied for routine analysis of *Isotretinoin* in pharmaceutical dosage forms.

KEYWORDS

Isotretinoin, RP-HPLC, UV and Validation.

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INTRODUCTION

Isotretinoin (Figure No.1**)** chemically3,7-dimethyl-9-(2,6,6trimethylcyclohex-1-enyl)nona-2,4,6,8 tetraenoic acid, is a retinoid classified as vitamin A. Isotretinoin is a topical keratolytic agent which is used in the treatment of skin disease including acne vulgaris. The mechanism of action is believed to inhibit the secretion of sebum and alter the lipid composition of the skin surface. Due to its effect on regulating cell differentiation it has been used for the treatment of cystic and nodular acne and also as an inhibitor of neoplastic cells proliferation[1-5]. Literature review reveals that several analytical methods have been reported for the formulation

containing *Isotretinoin*[6-14]. In these present studies a successful attempt has been made to develop a rapid, precise, accurate and comparatively economical UV and RP-HPLC method for quantitative estimation of Isotretinoin in. The developed method validated and recovery studies were conducted and studied by using various statistical parameters according to ICH guidelines[15].

MATERIALS AND METHOD

Experimental

Chemicals and reagents

Isotretinoin was obtained as a gift samples from Merck laboratories, Mumbai, methanol (HPLC grade) were purchased from Finer Chemicals Ltd, water (HPLC grade) was purchased from Rankem Chemicals.

Instrumentation

The double beam UV-spectrophotometer Elico SL-196 and chromatography was carried out by using HPLC system (Analytical Technologies Ltd) with UV visible detector.Thermohypersil (longer) C18 column. The mobile phase consisting of Buffer and Methanol in the ratio 80, 20. The detection made at 232 nm and the mobile phase flowed 1.2 ml/min. the volume of injection loop was 20µl prior to the injection of the drug solution, the column was equilibrated for at least 10 min. with the mobile phase following through the system.

Preparation of Standard Solution

Accurately weigh and transfer 8 mg of *Isotretinoin* working standard into a 50 ml volumetric flask add about 10 ml of Methanol and sonicate to dissolve it completely and make volume up to the mark with the Methanol.

Preparation of Sample Solution

Weighed 10 *Isotretinoin* tablets and calculated the average weight. Accurately weigh and transfer the sample equivalent to 80 mg of Isotretinoin into a 50 ml volumetric flask. Add about 5 ml of Methanol and sonicate to dissolve it completely and makeup the volume up to the mark with Methanol. Pipette out 5 ml of the above stock solution into 50ml volumetric flask and makeup up to the mark with Methanol. Mix well and filter through 0.45µm filter. Then twenty micro liters of *Isotretinion* standard or sample solutions were injected for six times. A quantitative determination of the active ingredient was made by comparison of the peak area from the sample injection to the corresponding peak area from a standard injection. The amount of *Isotretinion* present in a sample was calculated through the standard calibration curve (80µg/ml-240µg/ml). The retention time of *Isotretinion* was found to be 4.4 min and an aliquot of 5ml of the filtrate was diluted to 50ml with methanol to get the solution of 160mcg/ml by UV.

Preparation of standard curve

Aliquots of standard solution of *Isotretinoin* ranging from 0.5-1.5ml were transferred into a series of 25ml volumetric flasks. The volume in each flask was made up to 25ml with methanol and absorbance was measured at 243nm against solvent blank. The obtained absorbance value when plotted against the concentration of *Isotretinoin* gives the calibration graph. The concentration of unknown sample was determined by calibration graph (Figure No.2, 3).

METHOD OF VALIDATION

The developed analytical methods are validated for the parameters as per ICH guidelines.

Validation of Spectrophotometry Method

Accuracy

Accuracy is the closeness of the test results obtained by the method to the true value. The recovery technique was performed to judge the accuracy of the proposed method. For this known quantities of the *Isotretinoin* solution were mixed with definite amounts of pre-analyzed formulations and the mixtures were analyzed. The total amount of Isotretinoin was determined by using the proposed method and the amount of added drug was calculated by the difference.

Precision

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of homogenous samples. It provides an indication of random error results and was expressed as coefficient of variation (CV).

Intra and inter-day precision

A variation of results within the same day (intra-day), variation of results between days (inter-day) was analyzed. Intra-day precision was determined by analyzing Isotretinoin for five times in the same day at 243nm. Inter-day precision was determined by analyzing the drug once for five days at 243nm.

Linearity

The linearity of the method was demonstrated over the concentration range 0.5-1.5mcg/ml of target concentration. Accurately weighed 8mg of pure drug was taken in clean, dry 50ml volumetric flask and dissolved in small volume of methanol and made up the volume with methanol.

Concentrations of 0.5,0.75,1,1.25,1.5mcg/ml were prepared from above solution, calibration curve was plotted and the correlation coefficient was calculated.

Ruggedness and Robustness

The solutions were prepared and analyzed with change in the analytical conditions like different laboratory conditions and different analysts.

Validation of HPLC method

Above HPLC method for the determination of Isotretinoin has been validated for Precision, Accuracy (Recovery), Linearity, Limit of Detection, and Limit of Quantitation, Ruggedness.

Precision

The precision of an analytical method is the degree of agreement among individual test results, when the method is applied repeatedly to multiple sampling of homogenous samples. It provides an indication of random error results and it's expressed as relative standard deviation (% RSD). Precision of the method was demonstrated by repeatability.

Repeatability (Method precision) of Isotretinoin

Repeatability expresses the analytical variability under the same operating conditions over a short interval of time. 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 to be within the specified limits.

Accuracy studies of Isotretinoin

Accuracy is the closeness of the results obtained by the method to the true value. Recovery studies were carried out at 50%, 100% and 150% by adding known amount of standard drug solution of Isotretinoin. The % recovery was calculated and reported**.**

Linearity

Linearity of the analytical method for assay by injecting the linearity solutions prepared in the range of 80 µg/ml to 240 µg/ml of test concentration, into the chromatograph, covering minimum 5 different concentrations. Inject each level into the chromatographic system and measure the peak area. A calibration curve was plotted for concentration v/s peak area and calculates the correlation coefficient.

Robustness of Isotretinoin

Robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The standard solution was injected for four times with different analysts and column measured the area for all four injections in HPLC. The % RSD for the area of six replicate injections was found to be within the specified limits.

Limits of detection and limits of quantification of Isotretinoin

The parameters LOD and LOQ were determined on the basis of response and slope of the regression equation. As per ICH Q2 R1 guidelines LOD and LOQ are following equations.

$$LOD = 3.3 \times \sigma/S$$

$$LOQ = 10 \times \sigma/S$$

Where

σ = the residual standard deviation of the response and

S = slope of the calibration curve

RESULTS AND DISCUSSION

Selection of wavelength

The maximum absorbance was found at 269nm with characteristic peak as shown in the figure and was selected PDA detector in HPLC analysis of Isotretinoin λ_{max} of Isotretinoin, 232 nm.

Accuracy

The percentage recovery of Isotretinoin was found to be 99% for accuracy 50%, 100% and 150% samples respectively. The % RSD of the samples

was found to be less than 2. The results are summarized in Table No.1.

Precision

The precision studies were studied by five replicate injections of Isotretinoin. The % RSD value indicates a good degree of precision within the specified range. The results are summarized in Table No.2.

Linearity

Linearity is determined by calculating the regression line using a mathematical treatment of the results (i.e. least mean squares) vs. analytes concentration. The Correlation coefficient value for calibration plot of Isotretinoin was 0.999. The values are summarized in Table No.3.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

LOD and LOQ were calculated by using standard deviation and slope values obtained from calibration curve. The LOD and LOQ value was found to be 0.0006µg/mL and 0.002µg/mL respectively. The results are summarized in Table No.4 and 5.

Ruggedness

Ruggedness is the degree of reproducibility of results obtained by the analysis of the same sample under a variety of normal test conditions i.e. different analysts.

There is no significant effect on the result by doing small deliberate changes in the system as well as in method parameters. The results are summarized in Table No.6.

System Suitability Parameters

All system suitability parameters are within the specified range. The results are summarized in Table No.7.

Specificity

There is no interference of excipients used in formulation at the retention times of Isotretinoin the results are summarized in Table No.8.

Determination of λmax

λmax is the wavelength at which maximum absorption takes place. The λmax is the characteristic of drug molecules. From this figure No.3, 8 absorption maxima (λmax) for Isotretinoin were observed at 243nm. Figure No.4.

Linearity

The linearity of calibration curves (Absorbance Vs concentration) in pure solution was checked over the concentration ranges of about 100-300µg/ml for Isotretinoin and Values l were shown in the following Table No.8. The relationship between the concentration and the peak response of Isotretinoin was linear in the specific range and the regression coefficient was found to be 0.999 respectively (Figure No.5). The results are summarized in Table No.8.

LOD and LOQ

The LOD and LOQ values for the Isotretinoin were calculated. The LOD and LOQ of Isotretinoin is 0.08 and 0.2. The method is reliable as the LOD value should not exceed LOQ and should obey the linearity range. The results are summarized in Table No.9.

Precision

The %RSD should not be more than 2%. For the precision study in the present work, the % RSD for Isotretinoin was found to be 0.05, at 243nm. The % RSD value indicates a good degree of precision within the specified range. The results are summarized in Table No.10.

Accuracy

Accuracy of the method was determined by recovery experiments. To the formulation, the reference standards of the drug were added at the level of 50%, 100%, 150%. The recovery studies were carried out three times and the percentage recovery and percentage relative standard deviation of accuracy data of Isotretinoin were calculated and shown in Table No.11.

The obtained percentage recovery of both drugs was found to be within the limit. This indicates the proposed method was more accurate.

Table No.1: Recovery Studies of Isotretinoin

S.No	% Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	%Recovery	Mean Recovery
1	50%	3591292	78.89	79.01	100	99.67
		3582814		78.83		
		3598209		79.17		
2	100%	7094768	157.78	156.09	99	
		7132310		156.92		
		7053366		155.18		
3	150%	10752060	236.67	236.56	100	
		10796428		237.54		
		10740148		236.30		

Table No.2: Method Precision of Isotretinoin

S.No	Sample Name	Area of Isotretinoin
1	Precision-1	7196572
2	Precision-2	7186134
3	Precision-3	7191769
4	Precision-4	7191208
5	Precision-5	7140815
6	Precision-6	7151266
Mean	---	6096294
S.D	---	23895.97
% RSD	---	0.33

Table No.3: Linearity Studies of Isotretinoin

S.No	Concentration	Area
1	80 µg/ml	3597648
2	120 µg/ml	5395900
3	160 µg/ml	7194368
4	200 µg/ml	8992268
5	240 µg/ml	10929304
Correlation co efficient		0.999
Slope		45356
Intercept		-29277

Table No.4: LOD of Isotretinoin

S.No	Sample name	LOD value (µg/ml)
1	Isotretinoin	0.0006

Table No.5: LOQ of Isotretinoin

S.No	Sample name	LOQ value (µg/ml)
1	Isotretinoin	0.002

Table No .6: Component Summary of Ruggedness for Isotretinoin

S.No	Injection	Area
1	Analyst-1	7141769
2	Analyst-2	7281208
3	Column-3	7100815
4	Column-4	7081266
5	**Average**	7151265
6	**Standard Deviation**	90223
7	**% RSD**	1.3

Table No.7: System Suitability Parameters of Isotretinoin

S.No	Drug name	Theoretical plates	Resolution	Tailing factor	% RSD
1	ISO	11157	NA	1.101	0.1

Table No.8: Linearity of Isotretinoin

S.No	Concentration in µg/ml	Isotretinoin Absorbance at 243nm
1	100	0.3129
2	150	0.4771
3	200	0.6364
4	250	0.7974
5	300	0.9564
	Slope:0.003 Intercept:-0.002 Correlation Coefficient:0.999	

Table No 9: LOD and LOQ of Isotretinoin

S.No	Parameters	Isotretinoin Absorbance at 243nm
1	LOD	0.08µg/ml
2	LOQ	0.2µg/ml

Table No.10: Intraday Studies

S.No	Sample weight	Isotretinoin Absorbance at 243nm
1	924	0.6361
2	924	0.6365
3	924	0.6367
4	924	0.6367
5	924	0.6371
6	924	0.6368
RSD:0.05		

Table No.11: Recovery of Isotretinoin at 243nm

S.No	% of Recovery	Sample weight Mg	Sample area	Amount added	Amount found	% of Mean
1	50%	462	0.3129	78.893	78	99
		462	0.3128	78.893	77.97	
		462	0.3133	78.893	78.10	
2	100%	924	0.6368	157.785	158.73	101
		924	0.6364	157.785	158.63	
		924	0.6362	157.785	158.58	
3	150%	1386	0.955	236.678	238.05	101
		1386	0.9554	236.678	238.15	
		1386	0.9558	236.678	238.25	

Table No.12: Assay Results of Isotretinoin

S.No	Brand name	Available form	Label claim	Concentration	Amount found	% Assay
1	Isotroin-20	Capsules	ISO-20mcg	160µg/ml	158.73	101

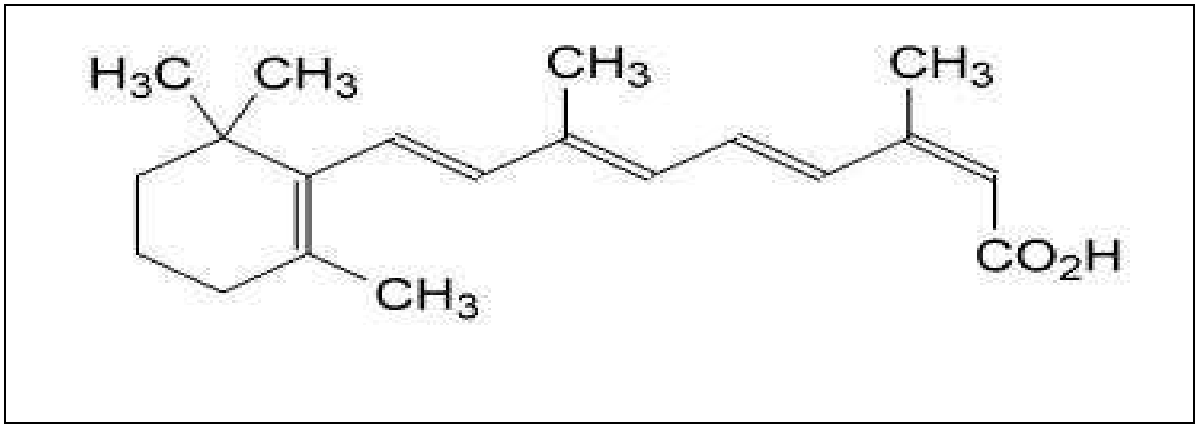

Figure No.1: Isotretinoin

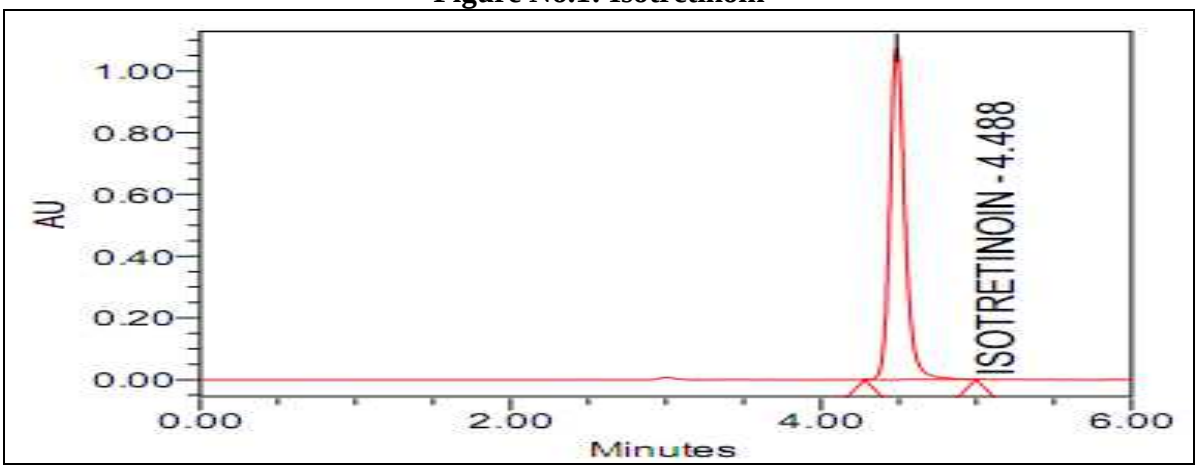

Figure No.2: Standard Chromatogram of Isotretinoin

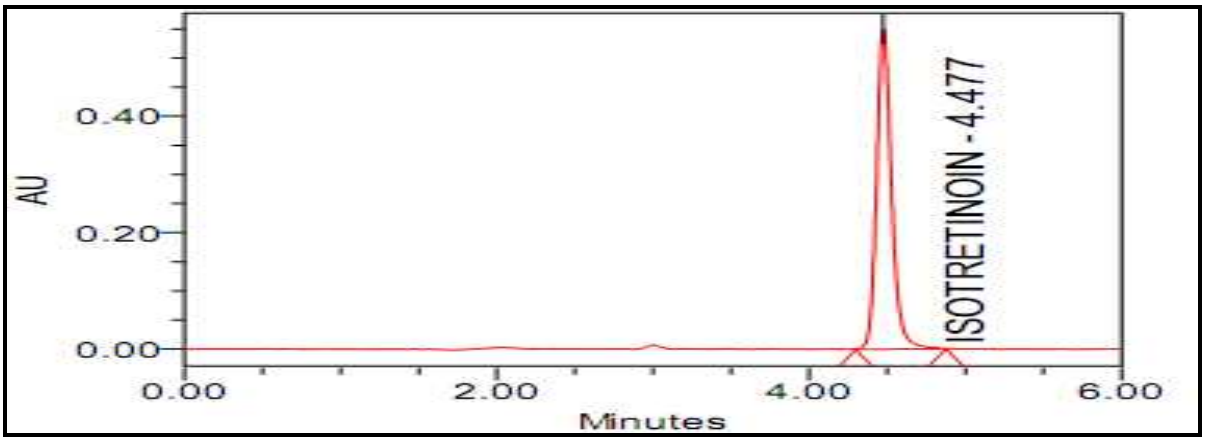

Figure No.3: Sample Chromatogram of Isotretinoin

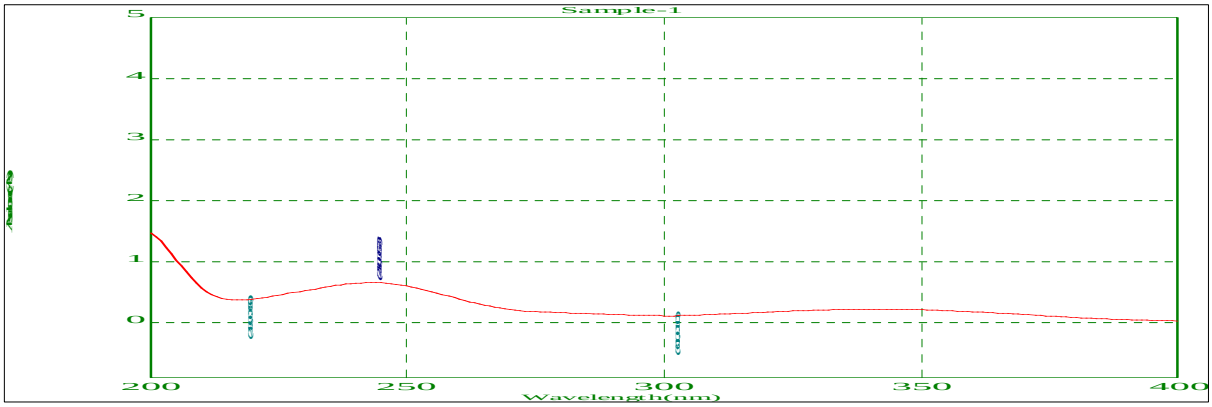

Figure No.4: UV Overlay Spectra of Isotretinoin

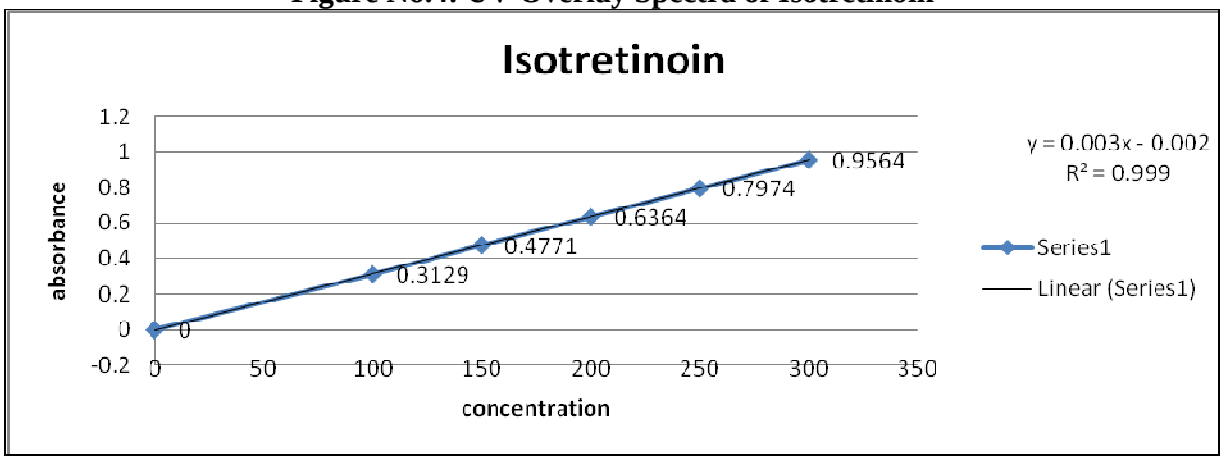

Figure No.5: Calibration of Curve of Isotretinoin

CONCLUSION

The proposed UV as well as HPLC methods for quantitative determination a simple, feasible and sensitive. Validation parameters include system suitability, specificity, linearity, accuracy, precision, robustness, and ruggedness was determined according to the ICH guidelines. The proposed method was showed good linearity, precision and accuracy for sensitive quantitative determination of Isotretinoin in pharmaceutical formulations without interference.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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