DEVELOPMENT AND VALIDATION OF UV-SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF TRETINOIN.

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Abstract- The main objective of this study is to estimate the concentration of tretinoin in cream tretin and to characterize its standard curve, intra and inter day precision, accuracy, and robustness. Tretinoin has been widely used in anti-acne creams available throughout the world. The methods used in the study were strictly followed under Indian Pharmacopeia. The methodology part includes the method employed for the process of experimentation and revealed a source of information regarding the characteristics and amount of tretinoin in the cream. The maximum absorption wavelength was found by taking 10 µg/ml solution and scanning it using a spectrophotometer (Thermo Fisher Scientific) within a wavelength region of 400-200 nm against ethanol as a blank which is found to be 352 nm. The intraday and interday precision studies indicated good precision of the method. The analyzed samples of tretinoin in three concentrations show a percentage accuracy of 98.35% to 103.81%, which is higher than 100%, indicating the accuracy of the method. The quality of the sample preparation was good to changes in temperature and wavelength. The results obtained showed the values to be under the stipulated values.

Index Terms- Tretinoin, Linearity and range, Precision, LOD, LOQ.

I. INTRODUCTION

Tretinoin (Molecular formula= C20H28O2) is the Trans isomeric form of retinoic acid, which is generally used for the treatment of acute promyelocytic leukemia acne vulgaris, and keratosis pilaris. It is a retinoid, meaning it's a vitamin A form also used for the treatment of hair loss, aging, etc. The chemical structure of tretinoin is shown in figure 1. It increases collagen production that helps reduce the stretch marks, which are indications that they can slow skin aging or reduce wrinkle formation. Topical Tretinoin, known to be very prone to degradation under daylight by oxidation of the conjugated double bonds, which is neither retarded nor lessened by the presence of antioxidants, is employed for treating mild to moderate acne, fine wrinkles, and hyperpigmentation. Its chemical structure includes a functional acid group and a side chain with conjugated double bonds, both liable to redox reactions. The ascorbic acid redox system comprises L-ascorbic acid, mono-dehydro ascorbic acid, and dehydroascorbic acid. Each of these substances has different physicochemical properties but possesses an antioxidant capacity for hydrophilic and lipophilic substances. [1]. Tretinoin or all trans' retinoic acid is easily oxidizable, thermally unstable, and randomizes fast when exposed to radiation. Retinoids like tretinoin also help your skin to manage its natural oil (sebum) production, which may prevent future breakouts. They also have anti-inflammatory properties, which clear up active acne pustules [2]. It is sold under a variety of brand names such as Retino-A, Retin-A, Atret, Nexret, Tretin, etc. For the simultaneous estimation of various drugs in pharmaceutical products, UV spectrophotometric methods have been employed. The spectrophotometric method is not reported for simultaneous estimation of tretinoin in cream tretin (Figure 2). In our research work, we try to estimate the concentration of Tretinoin in the cream tretin and to characterize its standard curve, intra and inter day precision, accuracy, limit of detection (LOD), the limit of quantitation (LOQ), and robustness. The methodology part includes the method employed for the process of experimentation and revealed a source of information regarding the characteristics and amount of tretinoin in the cream.

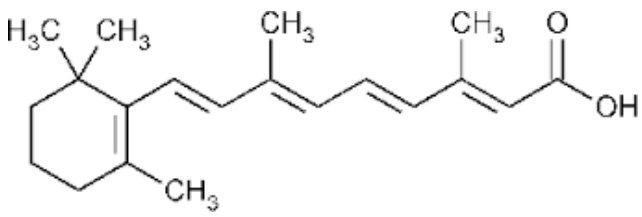

Figure 1: Chemical structure of tretinoin.

Figure 2: Tretin cream.

II. MATERIALS AND METHODS

Chemicals

Tretin, cream was obtained from Hedge & Hedge Pharmaceuticals Ltd. Distilled water, ethanol, and all chemicals used were of analytical grade and purchased from Loba Chemicals and Merck by the Department of Pharmacy, RIPANS; Mizoram.

Instruments and Apparatus

Electronic weighing balance, double beam UV-Vis Spectrophotometer (Thermo Fisher Scientific) with 1 cm matched quartz cell was used for the absorbance measurement, Beaker, Measuring cylinder, volumetric flask, Pipette, Test tubes.

Method Development *[2, 3, 4, 5, 6]*

1. Maximum absorption wavelength

To find the wavelength of maximum absorption (λmax) of tretinoin, a stock solution of 100µg/ml was prepared by taking 10mg of the drug in 100ml of ethanol. A solution of the drug (10µg/ml) in ethanol was scanned using a spectrophotometer within the wavelength region of 400-200nm with ethanol as blank. The resulting spectra were shown in Figure 3 and the absorption curve showed characteristic absorption maxima at 352nm for the drug.

3. Linearity

The linearity of this method was determined at concentration levels ranging from 2µg/ml and 10µg/ml. The plot of absorbance v/s concentration (Figure 4) of tretinoin was found to be linear in the range in Table 1. Beer's law was obeyed over this concentration range.

TABLE 1: Data for Standard curve of Tretinoin

Concentration (µg/ml)	Absorbance at 352 nm
2	0.069
4	0.178
6	0.298
8	0.401
10	0.508
Standard Deviation (S.D) =0.004	

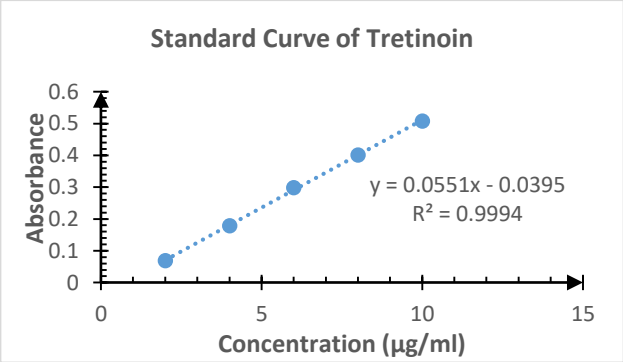

Figure 4: Calibration curve of Tretinion

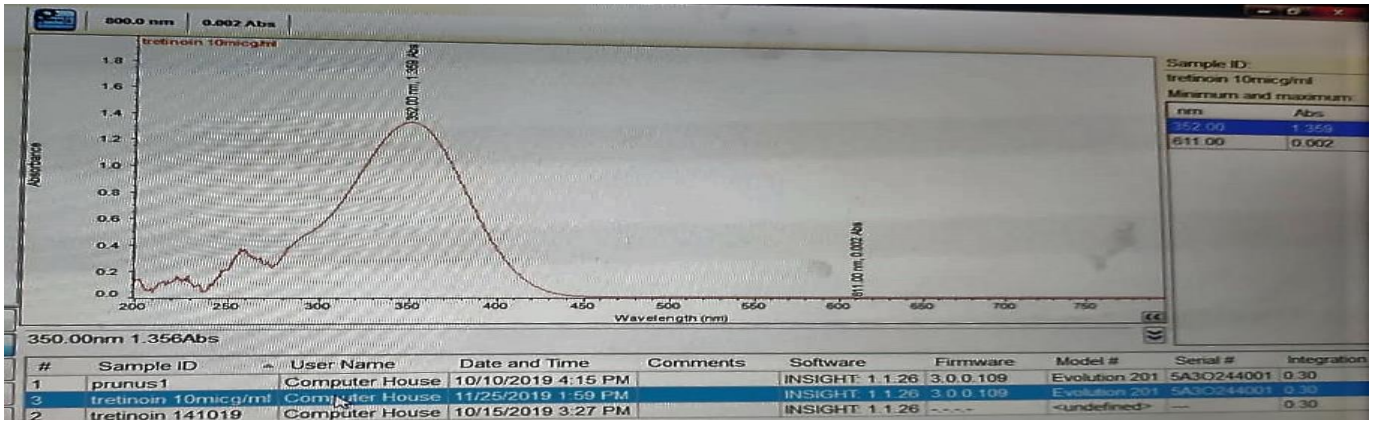

Figure 3*:* UV Spectrum of Tretinoin in ethanol.

2. Preparation of standard stock solution

The standard stock solution was prepared by dissolving tretinoin in ethanol to form a final concentration of 10µg/ml. Different aliquots were taken from the stock solution and diluted with ethanol to arrange a series of concentrations from 1-9 µg/ml. The λmax was found to be 352 nm in ethanol. Absorbance was measured at 348.6 nm with ethanol as a blank. The calibration curve was prepared by plotting the absorbance versus concentration of tretinoin.

4. Intraday precision

The precision of the strategy was carried out by repeatability (intraday precision) of the standard solution in three different concentrations, i.e., 3, 5, and 9µg/ml. The absorbance was taken thrice times each day and the average was taken. The actual concentrations were also calculated using the linearity slope (y=0.0551x – 0.0395) as found in Figure 4.

5. Interday precision

Another intermediate (interday) precision method was performed. In this method, the values of the intraday were taken as the first-day precision followed by continuous two days of determining the absorbance of the same concentrations three times each for the subsequent two days. The average absorbance was taken.

6. Accuracy

Accuracy is defined as the closeness of agreement between the actual (true) and analytical value and is obtained by applying the test method several times. The accuracy was carried out by calculating the % accuracy of different concentrations: 18µg/ml (80%), 20µg/ml (100%), and 22µg/ml (120%).In this method, the absorbance was taken and the actual concentrations were calculated. From this, the % accuracy was determined.

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

LOD (k =3.3) and LOQ (k=10) of the method were established in step with ICH definitions. The LOD and LOQ were estimated from the set of 5 calibration curves that previously used to determine method linearity.

$$LOD= 3.3\times\sigma/S \text{ and } LOQ= 10\times\sigma/S$$

Where σ = the standard deviation of y-intercepts of regression line

S = the slope of the calibration curve

8. Robustness

To determine the robustness of the current method, the samples used in the precision study (3, 5 & 9 µg/ml) were kept in the refrigerator for one hour, and the absorbance was taken at 352nm and 350nm respectively.

III. RESULTS AND DISCUSSION

Validation of Method *[7]*

The method was validated concerning linearity and range, accuracy and precision, and robustness.

Linearity and range

The prepared solution of tretinoin was scanned between the range of 400nm to 200nm to find the λmax of which was found to be 352nm. From Table1, it was discovered that tretinoin obeys linearity within the concentration ranges of 2µg/ml and 10µg/ml. The regression of the curve was y= 0.0551x – 0.0395. Results of linearity and range are showcased in Figure 3.

Precisions

The intraday and interday precision studies indicated good precision of the method as shown in Tables 2 & 3.

Accuracy: The analyzed samples of tretinoin in three concentrations given in Table 4 show a % accuracy of 98.35% to 103.81% which is higher than 10 and thus indicates the accuracy of the method.

Robustness: The refrigerated samples for a short interval time along with the changes in wavelength at 352nm and 350nm confirmed a slight change in the absorbance as shown in Table 5. Thus, indicating the quality of the sample preparation was good to the changes in the temperature and wavelength.

Limit of Detection and Limit of Quantitation: LOD and LOQ were found to be 0.24 µg/ml and 0.727 µg/ml, respectively, as shown in Table 6.

IV. CONCLUSION

The developed method was found to be simple, sensitive, accurate, precise, reproducible, rapid, and most important cost-effective that can be used for routine quality control analysis of tretinoin. The proposed method was found to be specific while estimating commercial formulations without the interference of excipients.

V. DISCUSSION

This developed method may also be useful in daily lab analysis of these drugs in several prepared formulations. The different kinds of parameters like linearity, accuracy, precision, limit of detection, and the limit of quantification were calculated. Hence, from the above validated parameters and the analysis of their positive results, we concluded that the developed simultaneous equation method for spectrophotometric analysis of drug tretinoin is the best for simultaneously analytical studies.

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Table 2: Results for Inter-day precision

Conc. (µg/ml)	Absorbance at 352 nm	Actual concentration(µg/ml)
3	*0.137*	*3.2*
	0.134	*3.14*
	0.135	*3.16*
Average±SD	*0.13±0.001*	*3.167±0.025*
5	*0.258*	*5.39*
	0.258	*5.39*
	0.259	5.41
Average±SD	0.25±0.0004	5.397±0.009
9	0.433	8.75
	0.443	8.77
	0.453	8.75
Average±SD	0.44±0.0004	8.757±0.009

Table 3: Results for Intra-day precision

Conc. (µg/ml)	1st day	2nd day	3rd day	Average absorbance
3	0.134	0.117	0.127	0.127
	0.134	0.117	0.128	0.126
	0.135	0.117	0.128	0.126
			Average	0.12±0.0008
5	0.258	0.231	0.238	0.242
	0.258	0.232	0.239	0.243
	0.259	0.23	0.2396	0.242
			Average	0.24±0.0004
9	0.443	0.482	0.459	0.461
	0.443	0.481	0.461	0.461
	0.443	0.482	0.458	0.461
			Average	0.46±0.005

Table 4: Results of Accuracy

Concentration	Absorbance mean	Actual concentration	Percentage Accuracy
18 µg/ml (80%)	0.94	17.77	98.72
	0.936	17.7	98.35
	0.937	17.72	98.45
Average±SD		17.73±0.029	98.51±0.001
20 µg/ml (100%)	1.098	20.6	103.67
	1.103	20.7	103.67
	1.103	20.7	103.52
Average±SD		20.67±0.047	103.52±0.21
22 µg/ml (120%)	1.212	22.7	103.24
	1.218	22.8	103.73
	1.219	22.8	103.81
Average±SD		22.77±0.047	103.59±0.251

Table 5: Results for robustness

Conc. (µg/ml)	Absorbance mean	Absorbance at 350 nm
3	0.089	0.08
	0.090	0.08
	0.089	0.079
5	0.167	0.148
	0.169	0.148
	0.165	0.148
9	0.262	0.251
	0.262	0.251
	0.262	*0.251*

Table 6: Summary of validation parameters of simple UV spectroscopy

Sl. No.	Parameters		Results
1.	λmax		352nm
2.	Regression line equation		Y=0.055X+0.0395
3.	Correlation coefficient (R2)		0.9994
4.	Precision (%RSD)	Intra-day precision	0.13-0.44
		Inter-day precision	0.12-0.46
5.	Accuracy (%Recovery)		98.35% to 103.81%
6.	LOD		0.24 µg/ml
7	LOQ		0.727 µg/ml

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