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[Research article]

Quantitative estimation and validation of isotretinoin in Pharmaceutical dosage forms by RP-HPLC

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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 for the quantitative determination of isotretinoin in pharmaceutical capsule dosage form. The chromatography was carried out by using HPLC system (Shimadzu LC2010HT) with UV- Visible dual absorbance detector (PDA), C18, 25 cm X 4.6 mm, 5 µm. The mobile phase consisting of 0.3% glacial acetic acid and water in the ratio of 85:15 and tetrahydrofuran and methanol used a diluent in the ratio of 20:80. The detection was made at 355 nm and the mobile phase flowed at 1 ml/min. Validation parameters included system suitability, specificity, linearity, accuracy precision (repeatability & reproducibility), robustness, ruggedness and stability were determined according to the ICH guidelines. The retention time of isotretinoin was found to be 12.33 min. Hence, the method could be successfully applied for routine analysis of isotretinoin in pharmaceutical dosage forms.

Keywords: Isotretinoin, Capsule, RP-HPLC, Validation.

INTRODUCTION

Isotretinoin (figure 1) chemically, 13-cis isomer of retinoic acid, is a retinoid classified as vitamin A. Isotretinoin is a topical keratolytic agent which is used in the treatment of skin diseases 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].

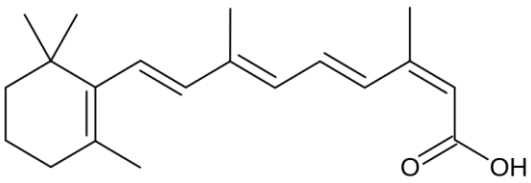

Figure 1: Structure of Isotretinoin

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Literature review reveals that several analytical methods have been reported for the formulation containing Isotretinoin [6-8]. In this present study, a successful attempt has been made to develop a rapid, precise, accurate and comparatively economical RP-HPLC method with UV detection for quantitative estimation of Isotretinoin in soft gelatin capsule dosage form. The results obtained have been statistically validated in accordance with the ICH guidelines.

MATERIALS AND METHODS

Experimental

Chemicals and reagents

Tetrahydrofuran and Glacial acetic acid were purchased from E.Merck (India) Ltd., Mumbai. Methanol was obtained from Qualigens Fine Chemicals Ltd., Mumbai. Isotretinoin was a gift sample by Caplin Point Laboratories Ltd., Chennai – 600 096, Tamil Nadu, India. The commercially available capsules containing isotretinoin were procured from the local market.

Instrumentation and chromatographic conditions

The chromatography was carried out by using HPLC system (Shimadzu LC2010HT) with UV-Visible dual absorbance detector (PDA), C18, 25 cm X 4.6 mm, 5 µm. The mobile phase consisting of 0.3% glacial acetic acid and water in the ratio of 85:15 and tetrahydrofuran and methanol used a diluent in the ratio of 20:80. The detection was made at 355 nm and the mobile phase flowed at 1 ml/min. The volume of injection loop was 20 µl prior to the injection of the drug solution; the column was equilibrated for at least 15 min. with the mobile phase following through the system.

Preparation of Isotretinoin standard solution

Accurately weighed about 50 mg of Isotretinoin WS and transfered into 50 ml volumetric flask. 25 ml of diluent was added and sonicated for 5 minutes until it completely dissolved. Cooled and diluted up to the volume with diluent. 2 ml of the above solution was transferred through pipette into a 50 ml volumetric flask and diluted up to the volume with diluent. Filtered the above solution through 0.45 µm Nylon filter and collected the solution in an HPLC vial after discarding about first 2 ml of filtrate (0.04 mg/ml of isotretinoin).

Preparation of Isotretinoin Sample solution

10 Capsules were randomly collected and weighed; the content was taken carefully from each capsule and mixed well. Accurately weighed 50 mg equivalent of Isotretinoin from the mixed contents and transfered into 50 ml volumetric flask. 25 ml of diluent was added and sonicated for 10 minutes until it completely dissolved. Cooled and diluted up to the volume with diluent. Cooled and diluted up to the volume with diluent. 2 ml of the above solution was transferred through pipette into a 50 ml volumetric flask and diluted up to the volume with diluent. Filtered the above solution through 0.45 µm Nylon filter and collected the solution in an HPLC vial after discarding about first 2 ml of filtrate (0.04 mg/ml of isotretinoin).

CALCULATIONS

Content of Isotretinoin in mg

$$\frac{\text{Sample Area}}{\text{Std area}} \times \frac{\text{STD wt in mg}}{50} \times \frac{2}{50} \times \frac{50}{\text{Sample wt}} \times \frac{\text{STD Purity in ASB}}{100} \times \text{Avg. wt}$$

Content of Isotretinoin in %

$$= \frac{\text{Isotretinoin in mg}}{\text{The Label claim contains in mg}} \times 100$$

RESULTS AND DISCUSSION

All of the analytical validation parameters for the proposed method were determined according to International Conference on Harmonization (ICH) guidelines [9].

System Suitability

It is essential for the assurance of the quality performance of chromatographic system. Five injections of standard drug solutions, isotretinoin was given separately to the system. The system suitability parameters such as retention time, peak area response and number of theoretical plates and their Mean, Standard deviation & % RSD were also be calculated for the standard drug solutions and mentioned in Table 1. It was observed that all the values are within the limits.

Table 1: System suitability for isotretinoin

S.No.	Standard	System suitability parameters		
		Peak area response	Number of theoretical plates	Retention time (min)
1.	Standard -1	101309288	10471	12.80
2.	Standard -2	101367777	10519	12.82
3.	Standard -3	101355055	10568	12.81
4.	Standard -4	101347771	10608	12.80
5.	Standard -5	101328656	10609	12.80
		Mean		**12.806**
		Standard deviation		**0.0089**
		RSD in %		**0.07**

Specificity

The specificity of the HPLC method is illustrated in Fig. 2, where a complete separation of isotretinoin was noticed in presence of other inactive excipients used in capsules. In addition, there was no any interference at the retention time of in the chromatogram of placebo solution. In peak purity analysis with PDA, purity angle was always less than purity threshold for the analyte. This shows that the peaks of analyte were pure and excipients in the formulation does not interfere the analyte. The data were presented in the Table 2.

Table 2: Specificity for Isotretinoin

S.No.	Name	No. of Injections	Area
1	Blank	1	Nil
2	Placebo	1	Nil
3	Standard	1	101309288
4	Sample	1	101347772

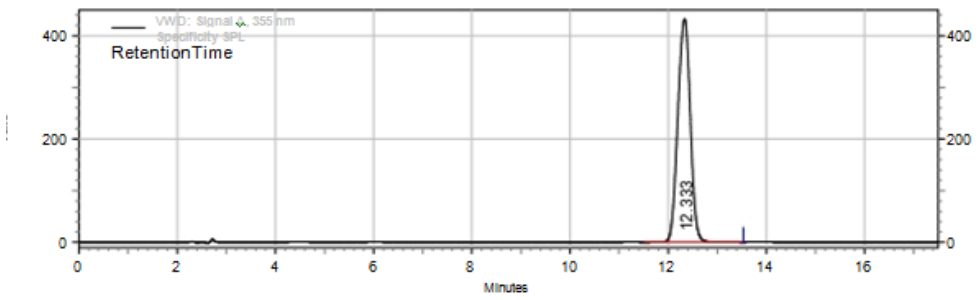


Figure 2 : Typical HPLC Chromatogram of Sample Capsules-(Isotretinoin)

Linearity and Range

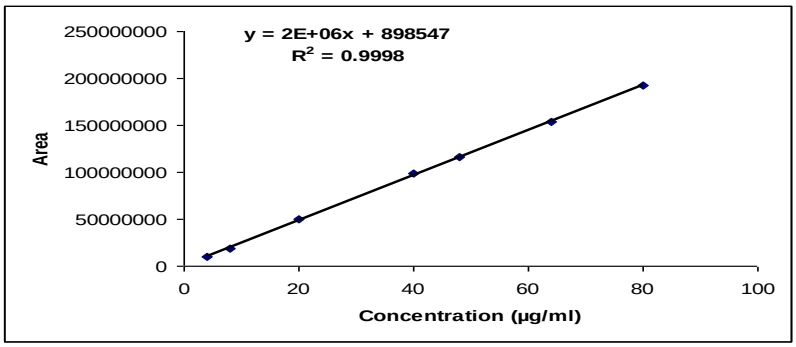
The Linearity of this method was determined at five levels from 10%– 200% of operating concentrations for isotretinoin and it was shown in Table 3. The plots of peak area of each sample against respective concentrations of Isotretinoin were found to be linear (Figure 3) in the range of 10%– 200% of operating concentrations. Beer's law was found to be obeyed over this concentration range. The linearity was evaluated by linear regression analysis using least square method. The regression equations were found to be Y= 2E+06X+898547 for isotretinoin and correlation coefficient of the standard curves were found to be 0.9998 for isotretinoin. It observed that correlation coefficient and regression analysis are within the limits.

Table 3: Linearity of response for Isotretinoin

S.No	Linearity Level Concentration (%)	Concentration (µg/ml)	Area
1.	10	4	10103315
2.	20	8	19051752
3.	50	20	49450608
4.	100*	40	98250411
5.	120	48	116505552
6.	160	64	154208823
7.	200	80	191903905

***** Operating concentration

Figure 3: Linearity of response for Isotretinoin



Accuracy

Accuracy of the method was found out by recovery study by standard addition method. The known amounts of standard, isotretinoin was added to pre-analysed samples at a level from 80% up to 120% and then subjected to the proposed HPLC method individually. The results of recovery studies were shown in Table 4. It was observed that the mean percentage recoveries were found to be for isotretinoin which demonstrated that the method was highly accurate.

Table 4: Accuracy for Isotretinoin

S.No.	Target level	Isotretinoin added (mg)	Isotretinoin recovered (mg)	Drug Recovery (%)
1.	80%	0.0327	0.0324	99.22
2.	80%	0.0319	0.0324	101.66
3.	80%	0.0322	0.0321	99.70
4.	100%	0.0405	0.0408	100.77
5.	100%	0.0407	0.0410	100.84
6.	100%	0.0407	0.0410	100.68
7.	120%	0.0483	0.0487	100.92
8.	120%	0.0484	0.0487	100.71
9.	120%	0.0487	0.0491	100.84
		Mean		**100.59**
		Standard deviation		**0.7161**
		RSD in %		**0.71**

Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the homogenous sample under the prescribed conditions.

Reproducibility

Examines the precision between laboratories and is often determined in collaborative studies. Reproducibility data for isotretinoin was shown in Table 5. This indicated that method was highly precise.

Table 5: Precision - Reproducibility for Isotretinoin

S.No.	Tailing factor	No.of theoretical plates	Retention Time (min)	Area
1.	0.94	10133	12.81	100374850
2.	0.95	10130	12.87	101645305
3.	0.94	10177	12.88	101108356
4.	0.95	10367	12.95	101168422
5.	0.95	10279	12.96	101923323
6.	0.96	10662	12.84	100781757
	Mean		**12.88**	**101167002.16**
	Standard deviation		**0.0595**	**562166.36**
	RSD in %		**0.46**	**0.56**

Repeatability

Repeatability is the precision of a method under the same operating conditions over a short period of time. One aspect of this is instrumental precision. A second aspect is sometimes termed intra-assay precision and involves multiple measurements of the same sample by the same analyst under the same conditions. Repeatability data for isotretinoin were shown in Table 6. This indicated that method was highly precise.

Table 6: Precision – Repeatability for Isotretinoin

S.No.	Sample Name	Area	Amount of drug present (mg)	Drug Recovery (%)
1.	Sample -1	100922382	19.83	99.13
2.	Sample -2	106090198	20.32	101.59
3.	Sample -3	105969786	20.26	101.29
4.	Sample -4	106150450	20.23	101.15
5.	Sample -5	106371562	20.24	101.19
6.	Sample -6	105805825	20.18	100.90
		Mean		**100.87**
		Standard deviation		**0.8836**
		RSD in %		**0.88**

Robustness

Measure of method's capacity to remain unaffected by small, but deliberate variations in method. A deliberate plus and minus changes in the analytical method parameters in wavelength, mobile phase composition and flow rate were altered and the assay was done as per the procedure. It was shown in Table 7. It was observed that there were no marked changes in the chromatograms, which demonstrated that the proposed method was robust.

Table 7: Robustness data for Isotretinoin

S.No.	Change of parameter	Amount of drug present (mg)	Drug Recovery (%)
1.	Wavelength (- 2) 353 nm	20.34	101.70
2.	Wavelength (+2) 357 nm	20.01	100.07
3.	Flow rate (- 2) 0.8mL	20.18	100.89
4.	Flow rate (- 2) 1.2mL	19.94	99.71
5.	Mobile phase Composition (-5 %)	19.81	99.04
6.	Mobile phase Composition (+5 %)	19.91	99.53

Ruggedness

Six sample preparations were analyzed as per the methodology by a different analyst on a different instrument on a different day. The ruggedness data isotretinoin was shown in Table 8. It was observed that there were no marked changes in the chromatograms, which demonstrated that the proposed method was ruggedness.

Table 8: Ruggedness data for Isotretinoin

S.No.	Samples Area			Amount of drug present (mg)	Drug Recovery (%)
	Sample -1	Sample -2	Average		
1.	98796827	98135654	98466241	20.13	100.67
2.	98610509	98853965	98732237	20.12	100.61
3.	98623780	98540901	98582341	20.01	100.04
4.	104933821	105096167	105014994	20.26	101.3
5.	97890701	97681272	97785987	19.89	99.44
6.	104422482	104226011	104324247	20.03	100.17
			Mean		**100.37**
			Standard deviation		**0.6365**
			RSD in %		**0.63**

Stability

Standard and sample solutions to be used in the analytical method were scrutinized for their solution's stability. This study was performed by injecting standard and sample solution for the period of 18 hours and results were presented in the Table 9 and 10. It was found that there were no remarked changes in the system suitability parameters.

Table 9: Stability results obtained for Isotretinoin standard solution

S. No.	Time point	Standard solution area	Results obtained		
			Cumulative % RSD	Tailing factor	Theoretical plate
1.	0 hour	99133256	-	0.938	9941
2.	1st hour	99294350	0.115	0.939	9729
3.	2nd hour	99344968	0.115	0.937	9713
4.	3rd hour	99508490	0.111	0.932	9770
5.	4th hour	99707493	0.220	0.940	9822
6.	5th hour	99940368	0.297	0.938	9848
7.	6th hour	100163575	0.373	0.944	9911
8.	7th hour	100351513	0.440	0.938	9946
9.	8th hour	100585548	0.510	0.945	10023
10.	9th hour	100843793	0.509	0.952	10041
11.	10th hour	101085294	0.663	0.956	10180
12.	11th hour	101292795	0.725	0.955	10170
13.	12th hour	101535917	0.806	0.948	10198
14.	13th hour	101668030	0.865	0.948	10224
15.	14th hour	101837769	0.920	0.952	10243
16.	15th hour	101986826	0.969	0.955	10347
17.	16th hour	102559121	0.969	0.965	10393
18.	17th hour	103026514	1.168	0.965	10464
19.	18th hour	103362521	1.278	0.961	10595

Table 10: Stability results obtained results obtained for Isotretinoin sample solution

S. No.	Time point	Standard solution area	Results obtained		
			Cumulative % RSD	Tailing factor	Theoretical plate
1.	0 hour	118563774	-	0.927	9475
2.	1st hour	119022941	0.273	0.933	9569
3.	2nd hour	119144350	0.258	0.931	9680
4.	3rd hour	119403513	0.295	0.939	9771
5.	4th hour	119680407	0.353	0.936	9752
6.	5th hour	119999011	0.425	0.942	9848
7.	6th hour	120387629	0.518	0.939	9905
8.	7th hour	120844897	0.630	0.941	9957
9.	8th hour	121348780	0.758	0.950	10049
10.	9th hour	121815774	0.886	0.947	10099
11.	10th hour	122259671	0.885	0.957	10161
12.	11th hour	122772349	1.139	0.954	10249
13.	12th hour	123213580	1.262	0.958	10349
14.	13th hour	123613991	1.376	0.961	10427
15.	14th hour	124038840	1.487	0.961	10469
16.	15th hour	124429143	1.592	0.966	10538
17.	16th hour	125595217	1.763	0.970	10561
18.	17th hour	126327078	1.942	0.972	10699
19.	18th hour	126820924	2.106	0.972	10860

CONCLUSION

The Proposed study describes a simple, feasible and sensitive reverse-phase high-performance liquid chromatographic method for the quantitative determination of isotretinoin in pharmaceutical capsule dosage form. Validation parameters included system suitability, specificity, linearity, accuracy precision (repeatability & reproducibility), robustness, ruggedness and stability was determined according to the ICH guidelines. The proposed method was found to be simple, sensitive, accurate and precise. Therefore the proposed method can be successfully used for the routine analysis of isotretinoin in pharmaceutical capsule dosage form without interference.

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