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Determination of Isotretinoin by UV and RP-HPLC

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Abstract

Isotretinoin content was accurately estimated using UV and RP-HPLC methods. A simple, sensitive double-beam spectrophotometric technique was determined for the assessment of Isotretinoin content in pure form as well as its formulations. Standard solution of Isotretinoin showed its highest absorbance at 354 nm. Beer's Law was found to be linear between 1–10 µg/ml, with a correlation coefficient (r^2) of 0.9983, a slope of 0.0791, and an intercept of 0.0119. The method was validated and exhibited good linearity, precision and accuracy for quantitative estimation of Isotretinoin in tablet formulations. The method was optimized using a mobile phase of Acetonitrile and methanol (30:70) at a flow rate of 1.2 ml/min to accomplish good resolution. The present method had a good correlation coefficient (r^2) of 0.9992 and linear in the concentration range 1–64 µg/ml. Study protocol for content estimation was rapid. Retention time of the drug was found to be 2.592 min, with high accuracy (% recovery = 97.7, 97.7, 98.3), precision, and sensitivity. The literature reveals that no systematic scientific evaluation has been carried out for isotretinoin using both the UV and RP-HPLC methods. The process was validated according to the ICH guidelines, ensuring that the technique is reliable and reproducible one.

Keywords: 13-Cis-Retinoic Acid, ICH-Guidelines, Isotretinoin, Method Development, RP-HPLC, Validation.

Introduction

Isotretinoin is a synthetic vitamin A acid derivative. Chemically, it is designated as (2Z, 4E,6E,8E)-3,7-dimethyl-9-(2,6,6-trimethyl-1_cyclohexenyl) nona-2,4,6,8-tetraenoicacid, also known as 13-cis-retinoic acid (1). The molecular weight of the compound is 300.44 and practically difficult to soluble in water (2). It is highly hydrophobic, and its nature pose challenges in dosage design (3). Isotretinoin doesn't dissolve in water but it's freely dissolved in chloroform and methylene chloride, and sparingly soluble in ethanol and 2-propanol (4). It is used for drugs with a less biological half-life and has a narrow therapeutic window. Along with improved patient compliance, this makes it suitable for self-medication by increasing its physiological and pharmacological responses (5). The structure of Isotretinoin (ITN) is given in Figure 1, a natural form of vitamin A and a derivative of retinoic acid, was first introduced in 1979 for treating severe skin problem. ITN is widely used to treat various skin disorders. It also helps in regulating epithelial cell growth and differentiation, sebum and collagen production (6, 7). API is used for systemic treatment of severe acne, targeting all major factors contributing to the pathogenesis of acne (8). Isotretinoin is a highly unstable compound, on storage conditions, especially under various atmospheric conditions like temperature, oxygen, and light. Due to the photo lability, thermal sensitivity and susceptibility to oxidation of retinoids, their quantitative estimation in pharmaceutical products is essential for stability-indicating tests and quality control of final products. Rapid analytical techniques for quantifying tretinoin and related compounds in dermatological formulations are valuable from various literatures. Due to sensitivity to heat and oxidation during storage, precise and accurate estimation is crucial for ensuring the good quality control of final product (9). Isotretinoin belongs to keratolytic drug used topically to treat skin conditions such as acne vulgaris. Its mechanism of action involves suppressing sebum release and modifying the lipid content on surface of the skin. Due to its active role in regulating cell differentiation, it is employed for the treatment of cystic and nodular acne. It also works as an inhibitor of neoplastic cell growth. Isotretinoin belongs to an orally active retinoic acid derivative.

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Pharmacological profile of the drug suggests that it primarily works by reducing sebaceous gland mass size and sebum production, thereby altering skin surface lipid composition (10-12). UV spectroscopy is a versatile, reliable, and cost-effective analytical tool whereas RP- HPLC system performs with tiny porous packing material and operates at extremely high pressure for enhanced efficacy and sensitivity, as well as faster chromatographic analysis to achieve good resolution peaks. HPLC is a sophisticated analytical technique which provides accurate, sensitive, and reproducible for the separations of a wide range of compounds. Literature review reveals that several analytical methods using UV (13, 14), HPLC (15-20) and UHPLC (21), LCMS (22) have been reported for the formulation containing isotretinoin.

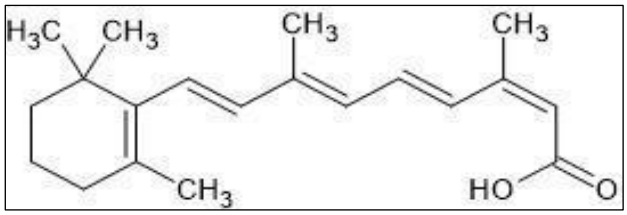

Figure 1: Structure of Isotretinoin

The reported methods were costly and time consuming with greater retention time. In other words, no systematic scientific method development has been made in previous work. So, an attempt has been carried out to quantify isotretinoin in formulation with good recovery. Hence an attempt has been made to develop a precise, sensitive and comparatively economic RP-HPLC technique with PDA detection for the quantification of Isotretinoin in solid dosage form.

Methodology

Method for the Estimation of Isotretinoin by UV Method

For UV analysis reagents, such as methanol, acetone, and distilled water were used. Instruments employed included a Shimadzu UV-visible spectrophotometer for absorbance measurements, a Shimadzu Sonicator for sample preparation, and a Saritorius analytical balance for accurate mass determination. The drug was dissolved in various solvents like water, methanol, methanolic sodium hydroxide etc. The solubility parameter was checked for turbidity, miscibility etc. Isotretinoin solubility in methanol was investigated, and the UV spectra were recorded. To determine the λ max of Isotretinoin, prepare standard solution containing 10µg/ml and it was scanned in double-beam UV visible spectrophotometer (200- 400nm).

Preparation of Standard Solution: Accurately weigh a standard of 10 mg isotretinoin and dissolved using methanol in a 10 ml flask to produce 1 mg/ml solution. From the above stock solution, pipette out 1ml from the above solution and dilute with methanol to make a concentration of 100µg/ml in a 10ml volumetric flask as a working solution.

Preparation of Sample Solution: About 20 tablets were weighed and crushed it in a glass mortar. A quantity of powder equivalent to 10mg was weighed and transferred into a 10 ml standard flask. To this add 5ml of methanol and sonicate for about 10 minutes, dissolving the contents by shaking. Final volume is made up to 10ml with methanol solution to give 1mg/ml. Further dilution will be carried out for the assay.

Linearity: Prepare a standard solution of Isotretinoin containing 1mg/mL, and determine the linearity at 1-10µg/ml. A calibration curve was achieved by plotting concentration on X-axis and absorbance on Y-axis. Calibration curve was used to examine the validated method.

[Y= mx+ b] [1]

LOD and LOQ: Lowest quantity of analyte presents in the test sample that may be identified is known as LOD. Lowest quantity of analyte that may be measured quantitatively is represented by LOQ.

The slope and the response's standard deviation are used to compute the parameters using LOD=3.3σ/S and LOQ =10σ/S.

Precision

Inter-Day Precision: Prepare a drug concentration - standard solution of 5 µg /ml and three consecutive readings were recorded and the results were tabulated.

Method Precision (Intraday Precision): Prepare a drug concentration - standard solution of 5 µg /ml

and intraday precision was carried out on three consecutive days and the results was tabulated.

Accuracy: For the UV the recovery was carried out in different amounts of 80%, 100%, and 120%, The solutions were suitably diluted in the range (4 µg,5 µg, 6 µg) of Isotretinoin and the absorbance for each of these dilutions was measured (23).

Method for the Estimation of Isotretinoin by RP-HPLC Method

Preparation of Standard Solution: Transfer about 10mg of standard into a 10ml standard flask and about 3ml of acetonitrile and 7ml of methanol mixture were added as a diluent and sonicate the solution for few minutes and the final volume was made up to 10 ml - 1000µg/ml.

Sample Preparation for Isotretinoin: 0.068 g of sample was weighed and dissolved with 3ml of acetonitrile and 7ml of methanol in 10 mL volumetric flask. Kept in sonicator for about 15 mins and then filter it. From this solution, 1 mL was pipetted out and transferred into 10 ml flask to this add a mixture of 3mL of acetonitrile and 7ml of methanol as diluent.

Linearity: It produces test results that are directly proportional to the drug concentration within a stated range is termed as linearity. HPLC linearity was assessed using the standard solution in 1-64 µg/ml. A Plot of average peak areas against the concentration.

Range: It is the range between the lowest and highest concentrations of a drug that has been validated to produce accurate, precise, and linear results.

Precision

Intraday Precision: Intraday precision, also known as repeatability, measures the consistency of the method within the same day. It assesses the variation in results when multiple samples are analysed by the same analyst using the same equipment over a short period.

Inter-Day Precision: Interday precision (also known as intermediate precision or ruggedness) measures the consistency of the method when repeated measurements are taken under different conditions over multiple days.

System Precision: System precision refers to the consistency and reliability of the HPLC system itself. It measures the repeatability of the chromatographic system and ensures that the system is functioning properly. This is usually assessed by multiple injections of a standard solution to evaluate the precision of the system.

$$RSD\ (\%) = (Mean/SD) \times 100 \qquad [2]$$

Robustness: It's a measure of a system's ability to withstand little but intentional variations in method parameters. It indicates a system's dependability within a certain range. Three duplicates of the sample were used to examine the robustness techniques at concentration level of 8 µg/ml for HPLC.

Ruggedness: Its (also known as intermediate precision) refers to the degree of reproducibility of the test results under diverse conditions such as different analysts, different laboratories, different instruments and different days. Testing for ruggedness ensures that the method produces consistent results across different scenarios.

Accuracy: It's a measure of the closeness of the measured value to a standard or true value. Analyse samples with known amounts of analyte (typically spiked samples). Calculate the recovery percentage.

Recovery: Recovery refers to the accuracy and reliability of an analytical method in estimating the actual quantity of analyte in the sample. Recovery studies involve spiking a known amount of analyte into a matrix (e.g., a sample) and then measuring the amount of analyte recovered after analysis.

LOD and LOQ: Signal to noise ratio was used to get the metrics LOD and LOQ. Lowest quantity of analyte present in the test sample that may be identified is known as LOD. Lowest quantity of analyte that may be measured quantitatively is represented by LOQ. The slope and the response's standard deviation are used to compute the parameters using $LOD=3.3\sigma/S$ and $LOQ=10\sigma/S$

Assay Method: Ten tablets were precisely weighed and crushed into a powder. Powdered drug was extracted using acetonitrile and methanol to get 100 µg/ml. Test sample and standard percentages purity were examined using RP-HPLC method (24).

Optimized Chromatographic Conditions

Various trials have been conducted by modifying the ratios of organic solvents like ethyl acetate, Acetonitrile and Methanol (90:10 and 50:50). In HPLC technique, the solvent's evaporation rate directly influences the efficiency of the separation process. Acetonitrile has comparatively low boiling

point which enables rapid evaporation, allowing for shorter analysis time without any compromising in resolution of peak. Another benefit of Acetonitrile is its compatibility with several detection methods used in HPLC such as ultraviolet (UV) and mass spectrometry (MS). It has low UV cut off and also has got minimal background noise makes it an exceptional choice for complex detection of analyte. Isotretinoin is well-known to be soluble in a variety of organic solvents like Acetonitrile, DMSO and DMF. It is considered to have low solubility in water. Hence, Acetonitrile is preferred choice of solvent for the determination of Isotretinoin by HPLC. The trials depicted chromatograms with broader peak, double peak with low resolution which was later optimized by varying the mobile phase composition. Chromatographic separation was determined using a mobile phase as acetonitrile: methanol (30:70), stationary phase C18 (250 x 4.6 mm, 5 µm), flow rate of 1.2 ml/min, pressure of 2140 psi, injection volume of 20 µl with a run time of 10 min. Detection was done at 345nm. Isotretinoin eluted at 2.6 min with a theoretical plate of 6544 and tailing factor of 1.2. The optimized method for isotretinoin was depicted in Figure 2. Various system suitability parameters were assessed as mentioned in Table 1.

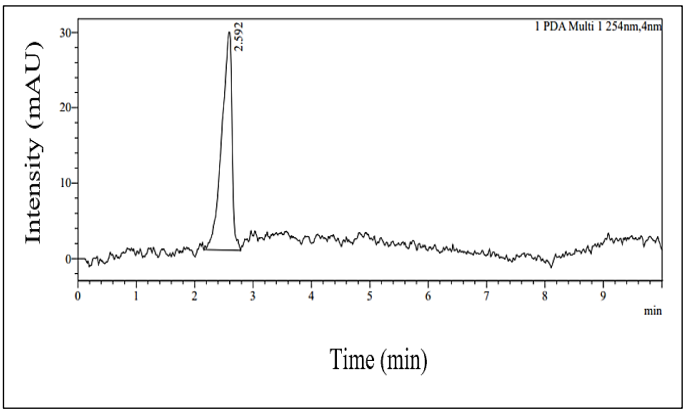

Figure 2: Isotretinoin Optimized Method

Table 1: System Suitability Parameters

S.no	Parameter	Result
1	Standard concentration	8 (µg/ml)
2	Mobile phase	30:70 (Acetonitrile: methanol)
3	Elution	Isocratic
4	Detection wavelength	354 nm
5	Column	C18 (250 x 4.6 mm, 5 µm)
6	Detector	PDA detector
7	Flow rate	1.2 ml/min
8	Run time pressure	2140 psi
9	Retention time	2.6
10	Run time	10 min

11	Peak area	335145
12	Purging valve pressure	140psi

Results and Discussion

Method Validation for UV Method

Determination of λ Max: Isotretinoin (standard solution, 10 µg/ml) exhibited maximum absorbance at 354 nm using methanol as the solvent, as depicted in Figure 3. Isotretinoin was linear in 1 to 10 µg/ml range. A promising linear relationship (r^2- 0.9983) was observed. The linearity equation was found to be y = 0.0791x+0.0119, as showed in Figure 4 and in Table 2.

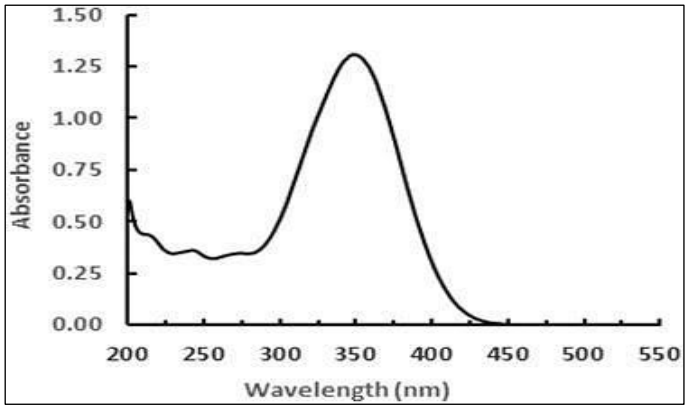

Figure 3: UV Visible Spectrum of Isotretinoin in Methanol

Table 2: Linearity Table of Isotretinoin by UV Method

S.no	Concentration(µg/ml)	Absorbance
1	2	0.165
2	4	0.331
3	6	0.484
4	8	0.660
5	10	0.791
6	Correlation coefficient r^2	0.9983

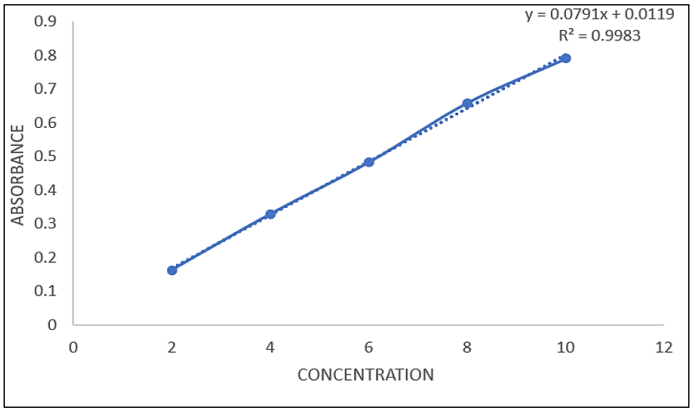

Figure 4: Linearity Graph of Isotretinoin by UV Method

Table 3: Interday Precision of Isotretinoin by UV Method

Day	Absorbance	Mean	Standard Deviation	% RSD
Day-1	0.384 0.394 0.391	0.389	0.00282849636	0.72710208743
Day-2	0.406 0.399 0.408	0.404	0.00259272486	0.6417636038
Day-3	0.406 0.400 0.408	0.404	0.00353578309	0.63175364984

The % RSD values for all three days were well below 1%, indicating a high degree of precision in the measurements and the data provided in Table 3. The mean absorbance values were consistent across the three days, with very small variations. The standard deviations were also low, further supporting the precision of the UV absorbance measurements.

Table 4: Intraday Precision of Isotretinoin by UV Method

S.no	Conc (µg/mL)	Absorbance	Mean	SD	% RSD
1	5	0.384	0.389	0.0035355339	0.908877609
2	5	0.394		0.0035355339	0.908877609
3	5	0.391		0.0014144213	0.36355104431

The % RSD value of 0.9088% indicates good precision for intraday measurements. A % RSD value below 1% is generally considered acceptable in analytical methods, indicating low variability and high repeatability within the same day. The mean absorbance value of 0.389 is consistent with the individual measurements, with minimal variations. The data for intraday precision is provided in Table 4.

LOD and LOQ: LOD and LOQ were determined to be 1.1503µg/ml and 3.4858µg/ml respectively

Table 5: Assay for Isotretinoin UV Method

S.no	Drug	Label Claim (mg)	Concentration (µg/mL)	Absorbance	%Purity
1	Isotretinoin	10	5	0.390	98.2%

The samples were analysed using both proposed methods under identical experimental conditions and the analyte content was found within the limits as specified by ICH guidelines. The results are tabulated in Table 5.

Table 6: Recovery of Isotretinoin by UV Method

Percentage	Std	Sample	% Recovery	Average
80%	4µg/ml	3 µg/ml	97.81%	97.77%
	4µg/ml	3 µg/ml	98.50%	

	4 µg/ml	3 µg/ml	97.0%	
	6µg/ml	3 µg/ml	96.6%	
100%	6 µg/ml	3 µg/ml	96.90%	97.0%
	6 µg/ml	3 µg/ml	97.50%	
	8 µg/ml	3 µg/ml	98.40%	
120%	8 µg/ml	3 µg/ml	98.80%	98.03%
	8 µg/ml	3 µg/ml	96.90%	

The recovery and accuracy of Isotretinoin by the UV method were evaluated at three concentration levels such as 80%, 100%, and 120% and it was shown in Table 6. The results indicate that the UV method for determining Isotretinoin concentration demonstrates high accuracy and precision across all tested concentration levels, with average recoveries close to 100%. The consistency of the recovery percentages across the different concentration levels suggests that the method is reliable to quantify the content of Isotretinoin in its formulations.

Method Validation for Isotretinoin by HPLC Method

Table 7: Linearity of Isotretinoin by HPLC Method

S.no	Concentration (µg/ml)	Rt (min)	Peak Area
1	1	2.560	117080
2	4	2.598	268780
3	16	2.759	737117
4	32	2.830	1323767
5	64	2.939	2473299
	Correlation coefficient (r^2)	0.9992	

Linearity of proposed technique showed regression coefficient of r^2=0.9992 over a concentration range of 1–64µg/ml. Linearity equation was found to be y=37100x+115861 as showed in Figure 5 and the corresponding data were shown in Table 7.

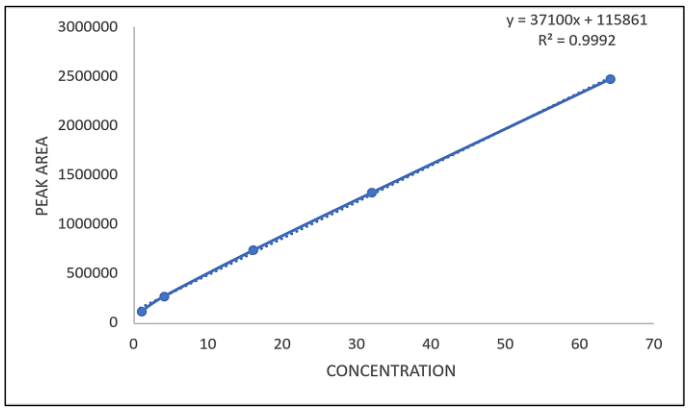

Figure 5: Linearity Graph of Isotretinoin by HPLC Method

Limit Of Detection and Limit of Quantification

LOD and LOQ for Isotretinoin using proposed HPLC technique were determined as 1.412µg/ml and 4.2787µg/ml respectively. The % RSD values, which range from 0.1069% to 0.7575%, are well below 1%, indicating excellent precision for the HPLC system. % RSD below 1% typically demonstrates high reproducibility and reliability of the measurement system. The values for system precision are provided in Table 8. The peak area values are consistent, with minor variations around the mean. This consistency further supports the robustness and precision of the HPLC system.

Table 8: System Precision of Isotretinoin by HPLC Method

S.no	Peak area	Mean	SD	RSD	% RSD
1	347819	345518	1029.03848	0.00297825	0.29782485
2	346759		474.493625	0.00137328	0.13732819
3	346344		369.384894	0.00106908	0.10690757
4	343692		816.612024	0.00236344	0.23634479
5	349009		1561.22266	0.0045185	0.45184988
6	339655		2617.54117	0.0075757	0.75757013

Table 9: Inter-day Precision of Isotretinoin by HPLC Method

S.no	Peak area	Mean	SD	RSD	%RSD
1	346759	343368	2397.7991	0.00698	0.698317
2	3243692		231.223917	0.00067	0.067339
3	339655		2625.48748	0.00746	0.764627

Acceptance Criteria:

Method Precision: RSD of assay results from 6 individual sample preparations should be ≤ 2.0%.

The calculated %RSD values for individual measurements range from 0.06733% to 0.7646%, indicating good precision for individual days. However, the combined %RSD for inter-day precision is quite high as shown in Table 9, suggesting significant variability across different days.

Table 10: Intra-Day Precision of Isotretinoin by HPLC Method

S.no	Peak area	Mean	SD	RSD	%RSD
1	347819	347724	67.17514	0.000193185239	0.0193185239
2	346344		975.8074	0.00280626979	0.280626979
3	349009		908.6322	0.00261308456	0.261308456

Acceptance Criteria:

Method Precision: The RSD of assay results from 6 individual sample preparations should be ≤ 2.0%.

The % RSD values for the peak areas range from 0.0193% to 0.2806%, which are well below the acceptance criteria of ≤ 2.0%. This indicates excellent intraday precision for the HPLC system across the three different sample preparations within the same day. The peak area values show minor variations around the mean, demonstrating consistent performance of the HPLC system within a single day. The values are provided in Table 10.

Table 11: Robustness of Isotretinoin by HPLC Method

Exp	Parameter variation	Peak shape	RT	Peak area
	Mobile Phase			
1	Mobile solvent B	Symmetrical shape	2.592	344453
2	+2% mobile phase	Symmetrical shape	2.62	339237
3	- 2% mobile phase	Symmetrical shape	2.58	297914
	Temperature			
1	Temp 40^0 C	Symmetrical shape	2.592	344453
2	Temp 42^0 C	Symmetrical shape	2.586	331925
3	Temp 38^0 C	Symmetrical shape	2.592	325193

The robustness study indicates that the HPLC method is reasonably robust under the tested variations in mobile phase composition and temperature. Both parameters are given in the Table 11 generally maintained symmetrical peak shapes, with minor variations in retention time and peak area. These variations were within acceptable limits for method robustness, suggesting that the method is reliable and capable of producing consistent results under slight variations in operational conditions.

Table 12: Ruggedness of Isotretinoin by HPLC Method

Exp	Parameter Variation	Peak Shape	RT	Peak Area
Instrument variation				
1	Analyst 1	Symmetrical shape	2.599	348624
2	Analyst 2	Symmetrical shape	2.592	343256
Mobile phase preparation				
1	Person 1	Symmetrical shape	2.592	343369

2	Person 2	Symmetrical shape	2.592	344453

Acceptance Criteria: Similar to the criteria for precision, with RSD values typically required to be ≤ 2.5%

The ruggedness study indicates that the HPLC method is robust against variations introduced by different analysts and different persons preparing the mobile phase and the values are provided in Table 12. The method consistently produced symmetrical peak shapes with minor variations in retention time and peak area within the acceptance criteria of ≤ 2.5% RSD. This confirms that the method is rugged and reliable under different operational conditions, ensuring consistent and reproducible results across different users and preparation methods.

Table 13: Assay of Isotretinoin by HPLC Method

S.no	Sample(tablet)	Label claim	Concentration (µg/ml)	Amount present	%	Purity
1	Isotretinoin	10mg	8	0.068g		98.4

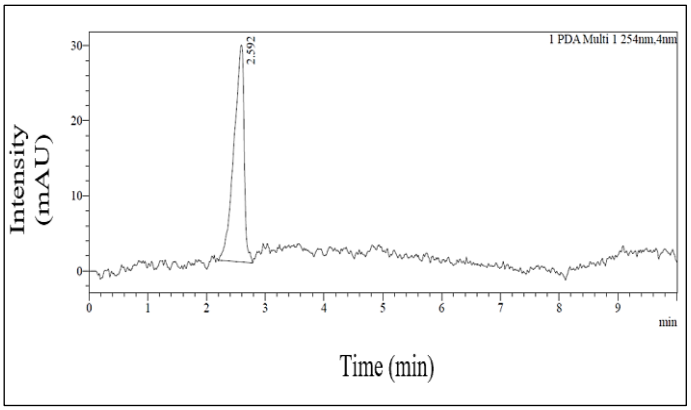

Figure 6: Chromatogram of Isotretinoin Assay by HPLC Method

The purity of Isotretinoin in the tablet (10 mg tablet) is determined to be 98.4%, which suggests high purity and confirms the accuracy of the assay method and the values are provided in Table 13. The assay method accurately quantifies Isotretinoin in the tablet, providing confidence in the reliability of the HPLC analysis for this compound. Chromatogram of Isotretinoin assay by HPLC method is given in Figure 6.

Table 14: Recovery and Accuracy of Isotretinoin by HPLC Method

Percentage	Std	Sample	Peak Area	% Recovery	Average
50%	1µg/ml	3 µg/ml	349445	98.50%	97.93
	1µg/ml	3 µg/ml	346707	97.70%	
	1µg/ml	3 µg/ml	346344	97.60%	
100%	5 µg/ml	3 µg/ml	343510	96.80%	97.13
	5 µg/ml	3 µg/ml	344453	97.10%	
	5 µg/ml	3 µg/ml	345881	97.50%	
150%	9 µg/ml	3 µg/ml	343692	96.90%	98.03
	9 µg/ml	3 µg/ml	350339	98.80%	
	9 µg/ml	3 µg/ml	349013	98.40%	

The recovery and accuracy study for the HPLC is given in Table 14. The method shows that it provides reliable and accurate results across different sample concentrations (50%, 100%, and 150% of standard).

The average recovery values for each concentration level are close to the expected value confirming the method's capability to accurately quantify the analyte under different conditions. It ensures confidence in the method performance for routine analysis in pharmaceutical and analytical applications. Recovery of isotretinoin standard chromatogram by HPLC method is provided in Figure (7-10).

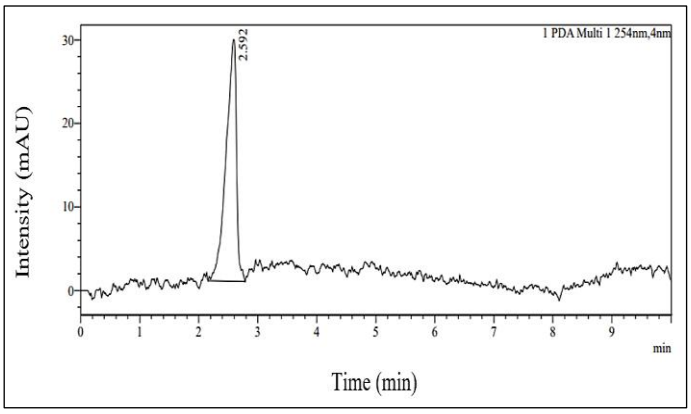

Figure 7: Chromatogram of Isotretinoin Standard

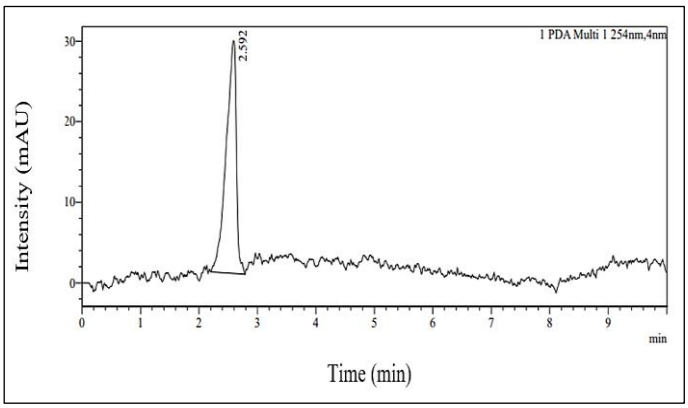

Figure 8: Chromatogram of Isotretinoin Sample 50% Recovery

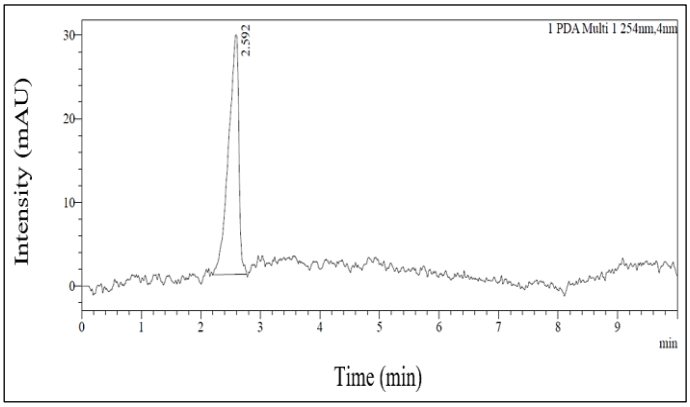

Figure 9: Chromatogram of Isotretinoin Sample 100% Recovery

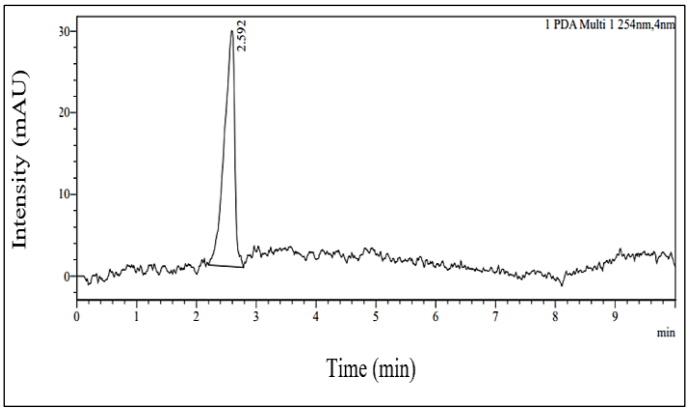

Figure 10: Chromatogram of Isotretinoin Sample 150% Recovery

The current study findings showed that two techniques such as RP-HPLC method and UV spectrophotometric approach aimed at the measurement of Isotretinoin in pharmaceutical formulations have been successfully developed and validated. Both techniques demonstrated an excellent efficacy, sensitivity, and reproducibility, yielding precise isotretinoin content.

UV spectrophotometric method which operates on the principle of Beer's law showed its maximum absorbance at 354 nm and obeyed its linearity in range of 1–10 µg/ml. The correlation coefficient (r^2) of 0.9983 indicates a strong linear relationship and the method demonstrated excellent precision and accuracy. The simplicity of this method makes it an attractive option for predictable quality control in isotretinoin formulations, as it requires minimal instrumentation and can be performed relatively fast.

In comparison, the RP-HPLC method was optimized with a mobile phase comprising of acetonitrile and methanol (30:70) ratio provided excellent resolution of isotretinoin. It showed linearity in 1–64 µg/mL with a high correlation coefficient (r^2 -0.9992). The retention time of 2.6 minutes ensures that the analysis is rapid, making it suitable for both high-throughput analysis and quality assurance testing in pharmaceutical industries. Furthermore, the method accuracy was confirmed with a recovery rate of 97.7%, 97.7%, and 98.3%, indicating that it effectively quantifies isotretinoin without significant interference from other components in the formulation.

The robustness and the accuracy of both approaches were confirmed by validation in compliance with the ICH requirements. Although the RP-HPLC method is more complicated it offers better specificity and sensitivity especially for complex formulations with many active components or excipients. In contrast, the UV approach has the advantages of being inexpensive and simple to use.

Conclusion

The developed UV and RP-HPLC methods provided accurate, precise, and reliable for the determination of isotretinoin in pharmaceutical formulations. The RP-HPLC method, optimized with a mobile phase of acetonitrile and methanol (30:70 v/v) at a flow rate of 1.2 mL/min, demonstrated good resolution and peak symmetry. Method validation confirmed the suitability of the method in terms of linearity, accuracy, precision, specificity, and robustness. Thus, the proposed analytical methods are effective and can be routinely employed for the quality control of isotretinoin in bulk and dosage forms.

However, the analytical studies were evaluated only in pharmaceutical formulations and not in biological matrices such as plasma or serum. Moreover, stability-indicating capability was not assessed through forced degradation studies.

Future research should aim to extend the applicability of these methods to biological samples to support pharmacokinetic studies. Developing a stability-indicating version of the RP-HPLC method would be beneficial for shelf-life determination and regulatory compliance. Additionally, innovative analytical techniques such as UHPLC or LC-MS/MS could further enhance the analytical performance in shorter time.

Abbreviations

API: Active pharmaceutical ingredient, RP HPLC: Reversed-phase high-performance liquid chromatography, ITN: Isotretinoin, LOD: Limit of

detection, LOQ: Limit of quantification, RSD: Relative standard deviation, RT: Retention time RP, SD: Standard deviation.

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Author Contributions

Sheela Rani T: conceptualized the study, designed the experimental framework, worked on acquisition of data, interpretation of data, Bhuvanesh R: contributed towards the administrative, technical or logistic support, Divya M: drafting the article.

Conflict Of Interest

The authors declare that there is no conflict of interest.

Ethics Approval

Not applicable.

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