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Quantitative estimation of isotretinoin (13-cis retinoic Acid) in bulk and formulation by UV - visible spectrophotometry

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ABSTRACT

A simple, sensitive and specific UV spectrophotometric method has been proposed for the estimation of Isotretinoin in bulk and soft gelatin capsule for routine analysis. The optimum conditions for analysis were established and validated in conformance with ICH guidelines. It was observed, the absorbance maximum (λmax) for Isotretinoin was 344 nm in methanol and the linearity was in the range of 1-8 µg/ml with coefficient of correlation as 0.9994. The lower limit of detection and the limit of quantification were found to be 0.2519 and 0.7634µg/ml respectively.

Keywords: Isotretinoin, Tretinoin, UV-Visible Spectroscopy, Soft Gelatine Capsule

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INTRODUCTION

Isotretinion, 13-cis retinoic acid is indicated for the treatment of severe recalcitrant cystic acne. The mechanism of action is believed to involve the inhibition of sebaceous gland function and follicular keratinisation [1]. Pure crystalline Isotretinoin is a yellow-orange powder whose faint odour resembles that of vitamin A. It is soluble in chloroform and methylene chloride, sparingly soluble in ethanol, 2-propanol, polyethylene glycol and very sparingly soluble in water [2].

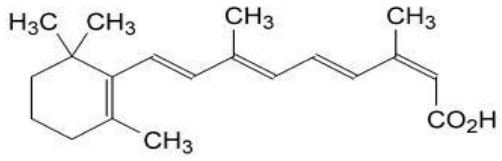

Fig 1 Chemical Structure of isotretinoin

Assay of Isotretinoin is performed by titrating with 0.1N sodium methoxide in USP [3] whereas potentiometric titration with tetrabutyl ammonium hydroxide is reported in BP [4]. In previous studies, Isotretinoin was determined by gas chromatography in soft and hard gelatin capsules [5]. Tretinoin (13-trans retinoic acid) and retinoid were determined in different formulations by reversed-phase HPLC [6, 7 and 8]. Although the reported methods are able to separate and quantify Tretinoin and Isotretinoin, the extraction procedure is quite complex and HPLC method is time consuming.

For determination of Isotretinoin in dermatological formulations, there is no UV-Visible spectroscopic method reported. Therefore, a successful attempt has been made here to develop a simple, sensitive, accurate and specific spectrophotometric method for estimation of Isotretinoin in soft gelatin capsule and validate the same in accordance with the ICH guidelines [9].

MATERIALS AND METHOD

Instruments

The experiments were carried out using a UV-Visible double beam spectrophotometer (UV-1700, Shimadzu Co, Japan) with 1cm matched quartz cells, Digital balance (Sartorius TE214S) and Sonicator (RC systems ,Bangalore).

Chemicals and Reagents

Isotretinoin was procured as gift sample from M/S Strides Arcolab ltd (Bangalore). Analytical grade reagents along with Isotroin (Isotretinoin capsules USP 20mg as commercial formulation) were used.

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EXPERIMENTAL

Preparation of Standard Stock Solution

The standard stock solution was prepared by dissolving 10mg of Isotretinoin in methanol in 100ml amber coloured volumetric flask and volume was made up to the mark (100µg/ml).

Determination of Wavelength of Maximum Absorbance (λmax)

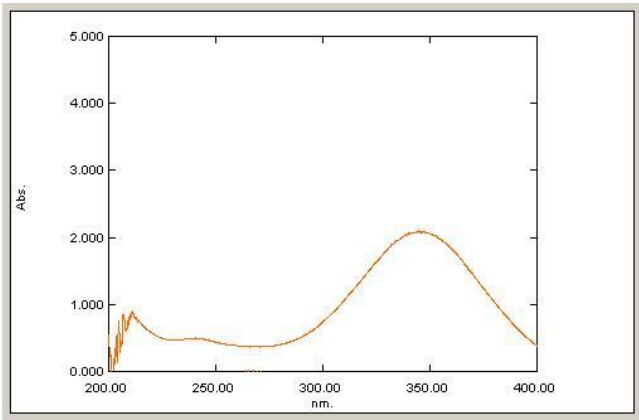

Fig 2 Spectrum of Isotretinoin

The stock solution was further diluted with methanol to obtain concentration of 10µg/ml. This solution was scanned in the range of 200-800nm as shown in Fig 2.

Preparation of Calibration Curve

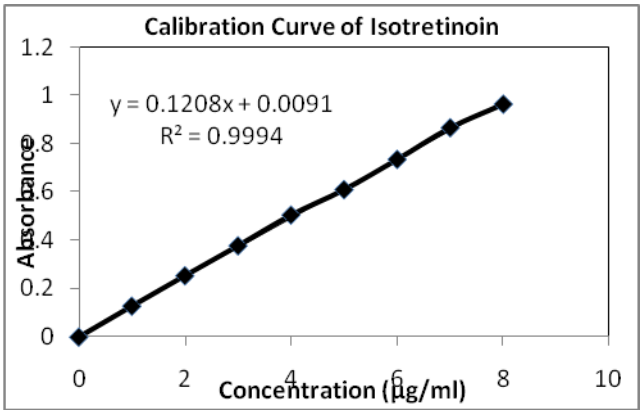

Fig 3 Calibration Curve of Isotretinoin

For the preparation of calibration curve, the stock solution was diluted to concentration 1-8 µg/ml. The absorption of the solutions was measured at 344nm (Table 1). A calibration

curve was prepared by plotting absorbance versus concentration of Isotretinoin as shown in Fig 3.

Table 1 Absorbance at different concentrations

Concentration (µg/ml)	Absorbance (± %RSD) (n=9)
1	0.127(±0.14)
2	0.253(±0.14)
3	0.377(±0.05)
4	0.503(±0.03)
5	0.608(±0.04)
6	0.733(±0.02)
7	0.864(±0.03)
8	0.961(±0.01)

Extraction of Isotretinoin from Soft Gelatine Capsule

Ten soft gelatine capsules were cut with a sharp blade and added in about 50ml of methanol. This was sonicated for about 15 minutes and then filtered by using whatman filter paper no. 41.

Assay of Marketed Formulation

An extracted solution of soft gelatin capsule was diluted with methanol to get concentration of 6 µg/ml of Isotretinoin and absorbance of the solution was measured at 344nm. The percent of Isotretinoin in soft gelatin capsule was calculated using1208 as [$A_{1\%, 1cm}$] at 344nm.

Method Validation

Table 2: Analytical validation parameters

Parameter	Observations
Absorption maxima(nm)	344
Linearity Range (µg/ml)	1-8
Standard Regression Equation	y = 0.1208x + 0.0090
Sandell's Sensitivity(µg/ml^2/0.001AU)	0.00828
Correlation Coefficient (R^2)	0.9994
Precission (% RSD) Intraday Interday Repeatability Reproducibility	 1.8-3.2 0.7-3.8 0.006-0.06 0.01-0.06
LOD (µg/ml)	0.25
LOQ (µg/ml)	0.76
Assay of marketed formulation ±SD (n=6)	101.5% ±1.09

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As per the ICH guidelines, accuracy, precision, LOD, LOQ, linearity and range were determined. Precision of the proposed method was determined by measuring the absorbance of standard solutions at different time intervals on same day (intra-day), on three different days (inter-day) and results are shown in Table 2.

Table 3: Results of Recovery Study

Amount of drug extracted from formulation (mg)	% Addition of Standard drug	% Recovery ±SD (n=3)
20	80	100.1(±0.115)
20	100	98.6 (±0.092)
20	120	101.3 (±0.075)

Accuracy was determined by adding 80%, 100% and 120% of standard Isotretinoin into the preanalysed extracted capsule solution. The absorbance of the solution was measured and the recovery was calculated as shown in Table 3.

RESULT AND DISCUSSION

Isotretinoin solution was prepared in different solvents like dichloromethane (DCM), dimethyl formamide (DMF), methanol, 0.1N sodium hydroxide and 0.1N hydrochloric acid. These solutions were scanned in the range of 200-800nm. Comparatively, the absorption of the drug was higher in DCM, DMF and methanol. The extracted solution of the drug from soft gelatin capsule in DMF was turbid; hence a further study was carried out in methanol.

An absorption maxima of Isotretinoin in methanol was found to be at 344nm. Isotretinoin obeyed Beer's law within the concentration range of 1-8µg/ml. It was found that per cent RSD of calibration curve was less than 2, which indicated high reproducibility of the method.

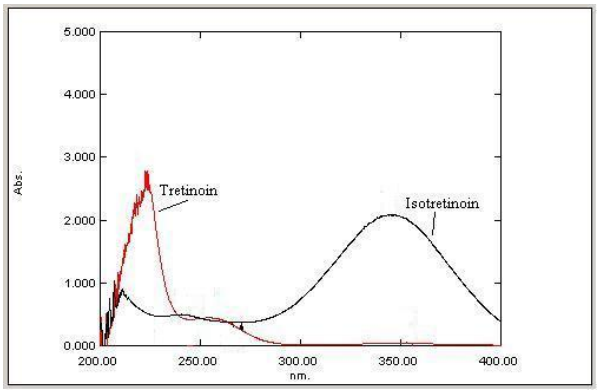

Fig 4 Spectra of Isotretinoin and Tretinoin

During the experiment, it was suspected that Tretinoin, major related substance [3, 4] present in Isotretinoin may interfere with the analysis of Isotretinoin. In order to overcome this suspicion, overlay spectra of Tretinoin and Isotretinoin at same concentration levels in methanol was noted and there was no absorbance of Tretinoin at the λmax of Isotretinoin (Fig 4).

The method was also applied to commercially available brand of Isotretinoin and the results were found to be within the official limits [3, 4], as tabulated in Table 2.

Finally, to confirm with ICH guidelines, the accuracy was assessed by standard addition method and the per cent recovery was found to be in the range of 98.5 to 101.3. The RSD levels were found to be very low in terms of repeatability, reproducibility, intraday and interday variability.

Therefore, in conclusion, the proposed method is construed as simple, accurate, precise, fast and satisfactory and the same can be effectively applied for quantitative estimation of Isotretinoin in soft gelatin capsule dosage form.

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