

## Research Article



## Simultaneous UV Spectrophotometric Valuation of Isotretinoin &amp; their Promoted Drug

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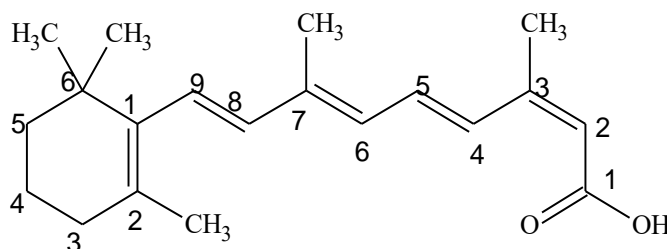
## ABSTRACT

Isotretinoin is a topical drug which is used in the cure of skin diseases including acne vulgaris. These are prescription-only medicines (POM) for the treatment of severe forms of acne, which have not got cure after using other anti-acne treatments. The present research work discusses the development of UV-spectroscopic method for estimation of isotretinoin & their different formulation present in market. It is a fast, reliable, correct & cheap method for the assay of isotretinoin. The assay is based on the ultraviolet UV absorbance maxima at about 344 nm wavelength of isotretinoin using methanol as solvent. A sample of drug was dissolved in methanol to produce a solution containing isotretinoin. Similarly, capsules of different marketed product were extracted with methanol and diluted with the same methanol. The wavelength for maximum absorbance ( $\lambda_{max}$ ) of isotretinoin was observed at 344 nm in methanol. The linearity equation was established as  $0.1233x-0.1093$ ,  $R^2= 0.9994$ , in the range of 1-8  $\mu\text{g/ml}$  for standard. Marketed product of isotretinoin like Tretiva, Acno & Acutret is also evaluated at 344nm. The linearity equation for Tretiva, Acno & Acutret drug was estimated as  $y=0.1198x-0.1145$ ,  $R^2= 0.9996$ ,  $y=0.1198x-0.1096$ ,  $R^2= 0.9995$ ,  $y=0.12x-0.1094$ ,  $R^2= 0.9991$ . Percentage assay was calculated as 98.38%, 99.08% & 98.48% respectively. All of the results are satisfactory. The proposed method will be suitable for analysis of isotretinoin generic drug as well as pharmaceutical formulations present in market. It is thus established that the suggested method is novel, simple, cheap, safe, precise and ecological friendly. Analytical methods have been appropriately validated and are satisfactory for ensuring compliance with the relevant specifications. All reference standards used are appropriate and acceptable.

**Keywords:** Isotretinoin, UV-Visible Spectroscopy, Soft Gelatin Capsule.

## INTRODUCTION

**Name of drug:** Isotretinoin  
**Molecular formula:**  $\text{C}_{20}\text{H}_{28}\text{O}_2$ .  
**Molecular weight:** 300.44.

**Color:** Yellow orange powder.**Odor:** Faint odour resembling vitamin A.**Solubility:** Insoluble in water, soluble in chloroform & methylene chloride, sparingly soluble in ethanol, 2-propanol.**Instability:** Air, heat, light.**IUPAC name:** 3, 7-dimethyl-9-(2, 6, 6-trimethyl-1-cyclohexen-1-yl)-2-cis-(4, 6, 8) - trans-nona-tetraenoic acid, 13-cis-retinoic acid or 13-cis-vitamin A.**Uses:** Treatment of severe obstinate cystic acne.

(2Z,4E,6E,8E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohex-1-en-1-yl)nona-2,4,6,8-tetraenoic acid

**Figure 1:** Chemical Structure of Isotretinoin

## Pharmacokinetics

Isotretinoin is more than 99.9% bound to plasma proteins, primarily albumin.

Metabolism: After oral administration of isotretinoin, 4-oxo-isotretinoin, retinoic acid (tretinoin), and 4-oxo-

retinoic acid (4-oxo- tretinoin) have been found in human plasma.

**Caution:** Isotretinoin is teratogenic. Avoid inhalation & skin contact.<sup>1-4</sup>

**Table 1:** Drug Formulation available in market

Name of marketed product	Pharmaceutical Company	Dose
Accutane	Roche product Ltd.UK	10mg, 20mg, 40mg
Isotretinoin	Ranbaxy	10mg,20mg
Isotretinoin	Merck generics	40mg
Acnetoin	Laeford Health care Ltd.	20mg
Isotino	Kivi Labs Ltd.	5mg
Isort	Organic lab	10mg
Tretiva	Intas Pharmaceutical	5mg
Acno	Ajanta Pharamceutical Ltd	10mg
Acutret	Ipca Industries	5mg

There are different methods have been reported for determination of isotretinoin drug by UV, RP-HPLC method.<sup>5,6</sup>

Assay of Isotretinoin is previously reported in USP by titrating with 0.1N sodium methoxide<sup>7</sup> whereas in BP potentiometric titration with tetra butyl ammonium hydroxide.<sup>8</sup> In previous studies, isotretinoin was also determined by gas chromatography in soft and hard gelatin capsules.<sup>9</sup>

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.<sup>10</sup>

There are several methods reported by UV with isotretinoin but virtual assay related to different brand of isotretinoin is not reported yet. The aim of this study is to investigate the assay of commercially available three brands of isotretinoin in Uttarakhand by using UV spectrophotometry.

## MATERIALS AND METHOD

### Instruments & Chemical reagent

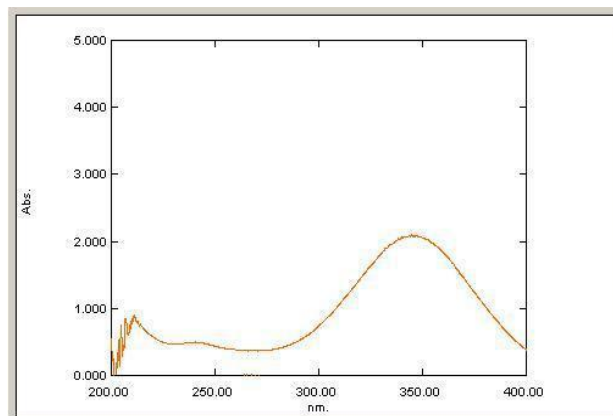
Ultra Violet visible (1601 Systronics) double beam spectrophotometer with 1cm matched quartz cells, digital balance (SF-400) and sonicator (IID, Delhi) was available in department of UIPS, Uttaranchal University, Dehradun.

### Standard Stock solution

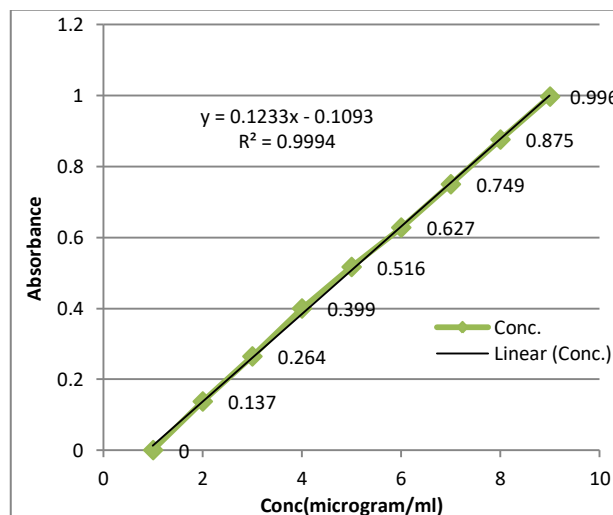
The standard stock solution was prepared by dissolving 10mg of isotretinoin in sufficient methanol to produce 100ml by using amber colored volumetric flask. Resulting solution was sonicated for 5min. to dissolve it.

### Wavelength Selection of Maximum Absorbance ( $\lambda_{max}$ )

100 ppm of isotretinoin was precisely prepared in spectroscopic-grade methanol. These solutions were scanned in the 200-400 nm UV regions. The maximum wavelength ( $\lambda_{max}$ ) was detected at 344 nm and this wavelength was using for the further investigation (Figure 1).

**Figure 1:** Spectrum of Isotretinoin

### Preparation of Calibration Curve

**Figure 2:** Calibration Curve of Isotretinoin

For the preparation of calibration curve, the stock solution was diluted to make concentration 1-8  $\mu\text{g/ml}$ . 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 Figure 2.

### Sample preparation

The three different marketed product of isotretinoin i.e. Tretiva (5mg), Acno(10mg), Acutret(5mg) belongs to Intas pharmaceuticals, Ajanta Pharma Ltd., & Ipca industries purchased from different medical store located in Uttarakhand. All of brands used in experiments have 5 year shelf life.

### Extraction of isotretinoin from soft gelatine capsule

Twenty soft gelatin capsules were cut with a sharp knife blade and added in about 50ml of methanol. By calculating the average weighed sample powder equivalent to 10 mg of isotretinoin was transferred into a volumetric flask containing 10mL water. This was sonicated in sonicator for about 5 minutes and then filtered by using whatman filter paper no. 41. The resulting solution diluted with purified water up to 100ml.

### Procedure

After preparation of standard and sample solutions (strength of solution 100 ppm in 100 ml), observed the absorbance of the sample and standard preparation in 1cm cell at the selective maximum wavelength at about 344nm, by using the blank solution. Calculate the quantity in mg, of isotretinoin as per capsule.

### RESULTS AND DISCUSSION

Pharmaceutical assay of isotretinoin was carried out by using UV spectrophotometer. Three different marketed

product of isotretinoin i.e. Tretiva (5mg), Acno (10mg) and Acutret (5mg) were taken and prepared their solutions of 100ppm, 50ppm, 25ppm, 12.5 ppm and 6.25 ppm for linearity study. Their percent assay is calculated and regression equation is obtained to predict further availability of drug. Figure 2 shows the calibration curve of isotretinoin as standard and Figure 3-5 shows linearity of different marketed product at level 100ppm, 50ppm, 25, 12.5ppm and 6.25 ppm. Correlation coefficient was found 0.996 for tretiva, 0.9995 for acno and 0.9991 for acutret. According to guidelines it should not be less than 0.99. Therefore, regression correlation coefficient ( $R^2$ ) of all brands found within the limit<sup>10</sup>.

### CONCLUSION

Linear relationship was observed for different marketed product of isoretonoin i.e. Tretiva (5mg), Acno (10mg) and Acutret (5mg) in the concentration ranges of 100, 50, 25, 12.5 and 6.25 ppm with correlation co efficient < 0.99. Correlation coefficient was found 0.9996 for tretiva, 0.9995 for acno and 0.9991 for acutret.

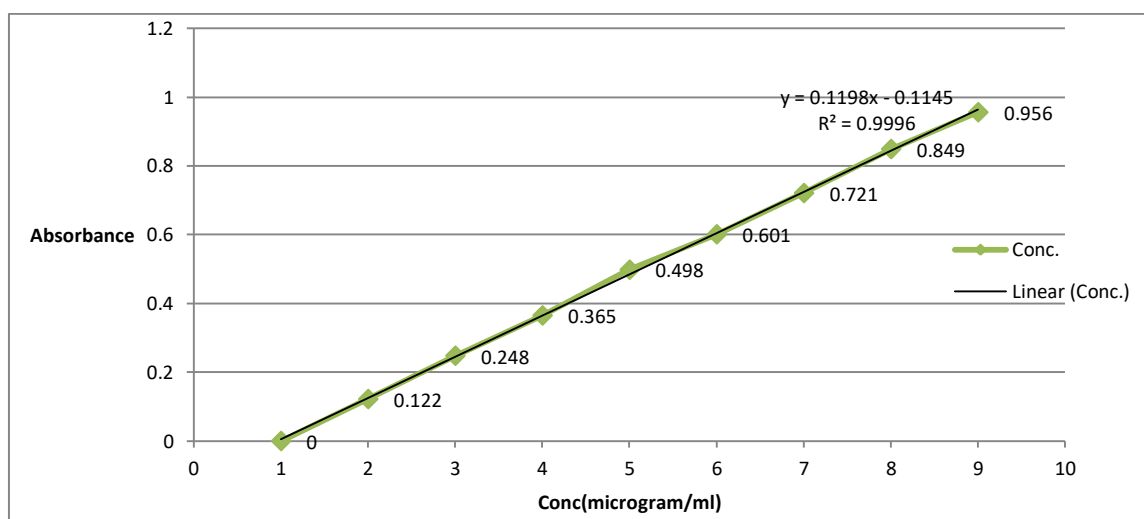


Figure 3: Linearity of Tretiva

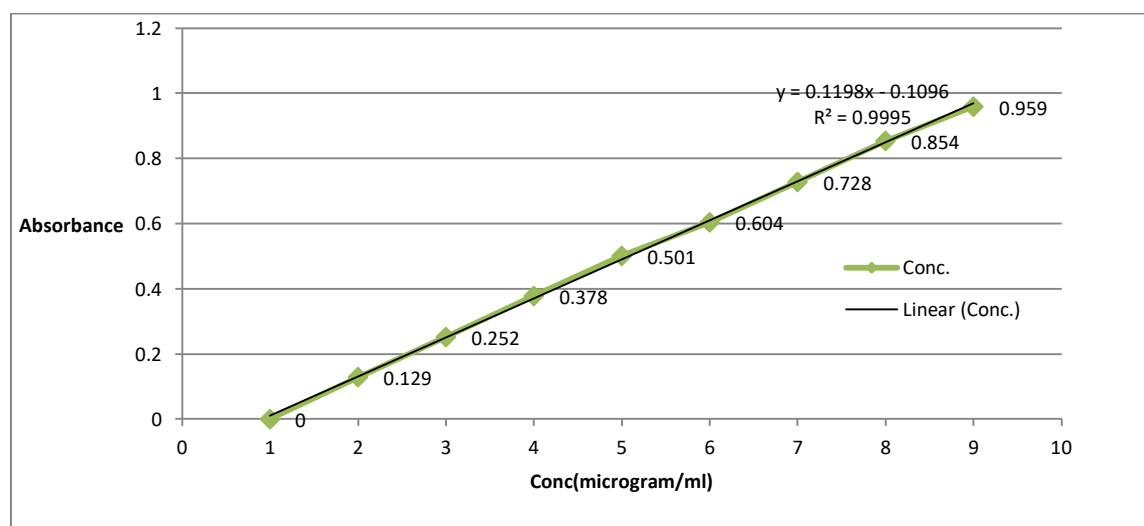


Figure 4: Linearity of Acno

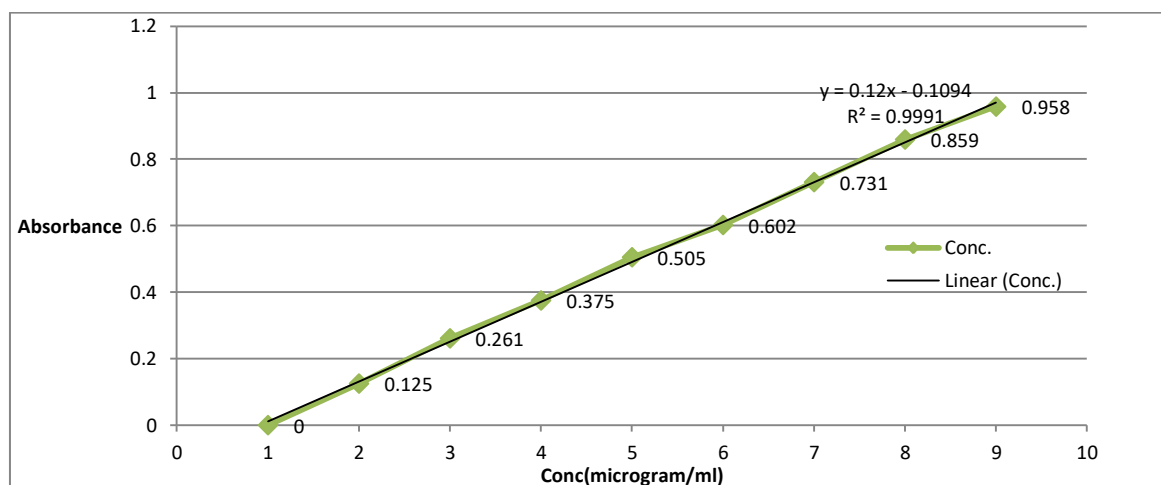


Figure 5: Linearity of Acutret

Table 2: Percentage assay of marketed product

Name of marketed product	Average weight of tablet(in mg)	Weight for 100 ppm	Absorbance at 344nm	Percentage assay
Tretiva	10.5	10.5	0.956	98.38
Acno	17	17	0.959	99.08
Acutret	15.8	15.8	0.958	98.48

## RESULT AND DISCUSSION

An absorption maximum 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.

The method was applied to commercially available brand of isotretinoin and the results were found to be within the official limits. Percentage assay of tretiva, acno & acutret brand is 98.38%, 99.08% & 98.48% respectively (Table 2). These results were found to be within the official limit<sup>7,8</sup>.

Therefore, in conclusion, the proposed method is interpreted as simple, accurate, precise, fast as well as satisfactory. The same method can be effectively applied for other available marketed product of isotretinoin.

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## REFERENCES

- Mangelsdorf DJ, Umesono K, Evans RM, the Retinoids: Biology, Chemistry, and Medicine, 1994, 319–349.
- Singh H, Kapoor VK, Medicinal and Pharmaceutical Chemistry, 3<sup>rd</sup> edition, New Delhi, 2012.
- Beale JM, Block JM, Wilson & Gisvold's: Textbook of Organic Medicinal & Pharmaceutical Chemistry, 12<sup>th</sup> edition, New Delhi, 2011.
- Tripathi KD, Essentials of Medical Pharmacology, 7<sup>th</sup> edition, New Delhi, 2013.
- Patel P, Kabra P, Kimbahun R, GH U, Quantitative estimation of isotretinoin (13-cis retinoic Acid) in bulk and formulation by UV - visible spectrophotometry. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 2(1), 2011, 167-171.
- Vasanthi R. *et al*, Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry, 3(3), 2015, 145 – 153.
- United States of Pharmacopoeia 22 and NF 17, US Pharmacopoeias Convention, Rockville, MD, 22, 1990, 742-743.
- British Pharmacopoeia, Her Majesty Stationery Office, London, 1, 1998, 755-756.
- Lucero MJ, Vigo J, Leon M J. Int J Pharm 1994, 110: 241–248.
- International Conference on Harmonization (ICH), Harmonized Tripartite Guidelines on 'Validation of Analytical Procedure: methodology', Operational from June 1997, The European Agency for the Evaluation of Medicinal products, Human Medicines Evaluation Unit.

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