ASSAY OF TRETINOIN IN BULK AND DOSAGE FORMS USING PHOSPHOMOLYBDIC ACID

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ABSTRACT

New analytical technique has been developed for quantitative estimation of tretinoin in bulk and formulations. Tretinoin (acne product) was estimated by using visible spectrophotometric absorption method at λ_{max} of 700 nm. While developing the method, it was found that the colored species formed were due to the formation of molecular complex between tretinoin and heteropoly acid such as phosphomolybdic acid (PMA). All the regression analysis parameters were validated statistically according to ICH guidelines. The limits of linearity lie in the concentration range of 4 - 12 μg mL^{-1} for tretinoin. Limit of detection, limit of quantification, molar absoptivity, intra-day and inter-day precession (% RSD), values are 6.12×10^{-2} μg mL^{-1}, 1.855×10^{-1} μg mL^{-1}, 1.2346×10^{4} L^{-1}mol^{-1}cm^{-1}, 0.57, and 0.50, respectively. Recovery studies found to be in the percentage range of 99.12 to 99.87(± 1.2 to ± 0.23) for tretinoin. The proposed method is suitable for the quantitative determination tretinoin in quality control laboratories and can be applied as a substitute to advanced instrumental methods. A novel and inexpensive visible spectrophotometric method was established for quantitative estimation of tretinoin in formulations.

Keywords: *spectrophotometry, tretinoin, phoshomolybdic acid, OTC products, ICH guidelines.*

INTRODUCTION

Bioactive compounds are found in plant and animal products and can be produced synthetically. Analysis of bioactive compounds is a vital part of life today. Drugs are bioactive compounds that need to develop more specific and sensitive procedures for their estimation based on the reactions of specific functional groups with suitable chromogenic agents. OTC (Over-the-counter) drugs used for skin care are classified as dry skin products, acne products, sunscreen and suntan products, and foot care products. Acne occurs most commonly during adolescence. It was found in 80 - 90 % of teenagers between the ages of 12 - 25 years due to hormonal changes. The acne product, namely tretinoin, works by replacing skin cells. It was the first retinoid developed for this type of topical use. Acne vulgaris is a common skin-related problem treated with combination therapy using topical drugs clindamycin and tretinoin [1]. Chemical structure of tretinoin is 2E,4E,6E,8E) - 3,7- Dimethyl - 9 - (2,6,6 - trimethylcyclo - hex -1-enyl) nona-2,4,6,8-all-trans-tetraenoic acid. The drug is listed in the Merck Index [2]. There is little evidence about the determination of tretinoin (TTN) in the literature by HPLC [3 - 5], RP-HPLC [6 - 9], GC [10], UV - Vis spectrophotometry [11 - 15], and derivative spectrophotometry [16, 17].

However, significant functional groups in tretinoin (TTN) have not been exploited efficiently in developing visible spectrophotometric techniques. Very few spectrophotometric methods are reported for the determination of tretinoin (TTN). It is necessary to

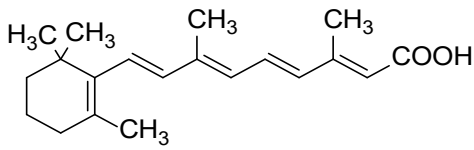

Fig. 1. Chemical structure of tretinoin.

develop more specific and sensitive procedures for its determination by exploiting the analytically significant functional groups. Hence, the authors proposed a simple method using safranine - O (SFN - O) as a chromogenic reagent. Chemical structure of tretinoin [12] is given in Fig. 1.

EXPERIMENTAL

Instrumentation

A Shimadzu UV double-beam spectrophotometer has been chosen for obtaining precise and accurate optical density measurements. In addition, an electrical balance (Dhona 200 D, India) was used to weigh all the materials.

Chemicals and Reagents

Formulations of Retino-A (EthnorJanssencilag), Airol (Piramal Health Care), Avita (Bertek Pharmaceuticals, Inc.), and Eudyna (German Remedies.) were procured from the registered pharmacy. Bulk drug tretinoin (Biophore India) was chosen for this study. Phosphomolybdic acid (E. Merck), glacial acetic acid (E. Merck), and solvent acetic anhydride (E. Merck) used in this study are of analytical grade. An aqueous solution of phosphomolybdic acid (PMA) is prepared by dissolving 200 mg of phosphomolybdic acid in 100 ml of glacial acetic acid (0.2 %).

Bulk sample solution

The stock solution (mg mL^{-1}) for bulk drug sample was prepared in 100 mL of glacial acetic acid containing 2 % Ac_2O solvent by dissolving 100 mg of tretinoin. Working standard solutions of concentration of 20 µg mL^{-1} were prepared from the above stock solution. Further dilution was done using chloroform.

Formulations

A cream equivalent to 50 mg was dissolved in 30 mL

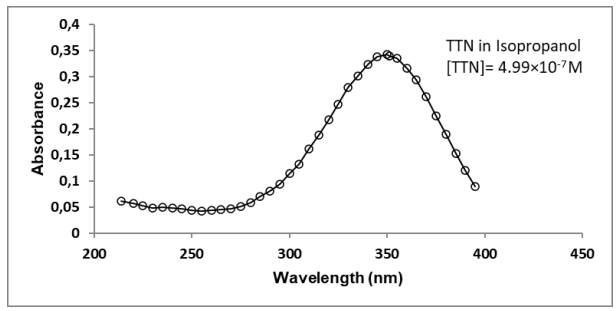

Fig. 2. Absorption spectrum of tretinoin ([TTN] = 4.99×10^{-7}M) against blank.

of aqueous methanol (3:1). The resulting solution was extracted with $CHCl_3$ (3 x 25.0 mL portions) and filtered. The total chloroform extract was kept for drying with 5 g of anhydrous Na_2SO_4 and then filtered. This filtrate was made up to 200 mL with chloroform to obtain the stock solution of 250 µg mL^{-1}. The stock solution is further diluted to concentration of 20 µg mL^{-1}.

Calibration curve by UV method

Stock solution (mg mL^{-1}) was prepared by dissolving 100 mg of bulk drug sample in 100 mL isopropanol. From this stock solution, working standard solution of concentration 10 µg mL^{-1} was prepared using the same solvent. The absorption spectrum of bulk drug sample was recorded against a reagent blank within the UV region using Shimadzu double beam spectrophotometer (Fig. 2). A series of solutions were prepared by taking 0.5 - 2.5 mL standard drug solution (20 µg mL^{-1}) into 20.0 mL calibrated tubes. These are diluted to 10.0 mL with double distilled water. The optical densities of all the sample solutions were measured at 352 nm against reagent blank (isopropanol). The concentration of the drug was deduced from its calibration curve drawn between optical density and concentration of tretinoin (Fig. 3). This UV absorption method was chosen as a reference method.

Selection of analytical wave length

For selecting analytical wavelength, the sample solution containing fixed amount of tretinoin, phosphomolybdic acid (PMA) solution, and other furnished variables as mentioned in the a procedure has been scanned in the visible region (350 - 750 nm) against the reagent blank. The absorption spectrum of the colored species formed on the basis of the molecular

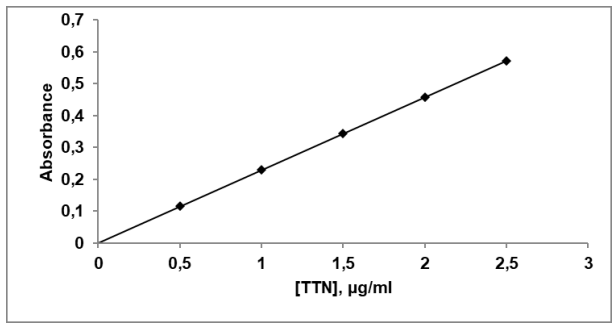

Fig. 3. Calibrated curve of tretinoin ([TTN] = 4.99×10^{-7}M).

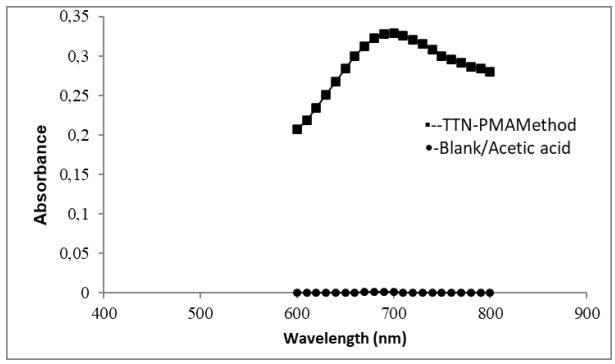

Fig. 4. Absorption spectrum of proposed method (TTN - PMA system) and blank.

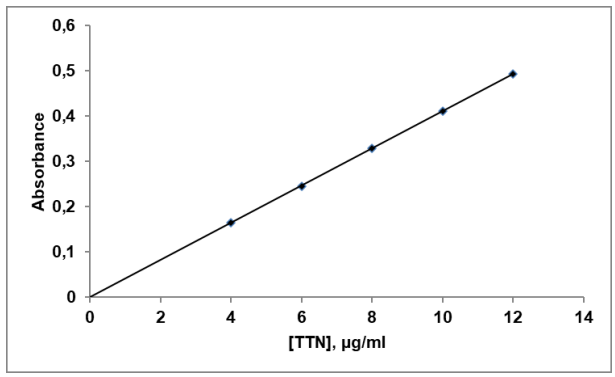

Fig. 5. Calibrated curve of tretinoin (TTN) - phosphomolybdic acid (PMA) method.

complex shows maximum absorbance at 700 nm and this wavelength has been selected for the analysis. The spectrum of reagent blank solution against acetic acid shows negligible absorbance at this wavelength (Fig. 4).

Recommended procedures

A series of solutions were prepared by taking aliquots of standard drug solution (1.0 - 3.0 mL, 20 µg mL^{-1}), 0.7 mL of 1.09 x 10^{-3} M phosphomolybdic acid (PMA) solution into calibrated graduated test tubes. The resulting solutions were kept for 90 minutes and diluted to 5.0 mL with acetic acid. The absorbance of test solution was measured at 700 nm against a reagent blank. The color was stable for 40 min. The concentration of the drug was deduced from its calibration curve drawn between optical density and concentration of tretinoin (Fig. 5).

RESULTS AND DISCUSSION

The method proposed is a new one, and the results are statistically validated. Optimum conditions for the method are developed. The most probable mechanism and validation of the data are discussed in this section.

Optimum conditions

The responses of several factors like concentration of phosphomolybdic acid (PMA) (0.11 - 0.22 x 10^{-3} M), the addition of volume of acetic acid containing 2 % Ac_2O for final dilution, time period of the reaction (75 - 110 min), stability of molecular complex formation (1 - 40 min), intenseness of colored species produced, order of addition of reagents and temperature of the reaction were studied [18]. The following optimum conditions were fixed for the proposed technique are: 0.7 mL of 1.09 x 10^{-3} M phosphomolybdic acid, final dilution made up to 5.0 mL with acetic acid solution, observed reaction time is 90 minutes at room temperature. The molecular complex obtained is stable up to 40 minutes afterwards the absorbance slowly decreases indicating the decomposition of the complex. The optimum conditions were established by measuring the absorbance at 700 nm and results are presented in Table 1.

Mechanism of molecular complex formation reaction

Heteropoly acids (HPA) are also known as heteropolymetalates which consist of three or more transition metal oxyanions linked together by shared oxygen atoms to form closed 3-dimensional molecular framework. The transition metal atoms are usually Mo, W, or V in their highest oxidation states. Heteropoly acids are widely used as catalysts in wide range of organic reactions. They are usually colorless to orange. The heteropoly acids namely phosphomolybdic acid ($H_3Mo_{12}PO_{40}\cdot12H_2O$), silicotungstic acid (STA) or tungstosilicic acid (TSA) ($H_4SiW_{12}O_{40}\cdot nH_2O$) and phosphotungstic acid (PTA), or tungstophosphoric acid (TPA),($H_3PW_{12}O_{40}\cdot nH_2O$). In the present investigation, the authors have chosen

Table 1. Results of optical, linearity, precision and accuracy of proposed method.

Optical condition	TTN - PMA Method
Wavelength(λ_{max}) (nm)	700
Molar absorptivity(ϵ_{max}) (L mol^{-1} cm^{-1})	1.2346×10^4
Limits of linearity lie in the concentration range(µg mL^{-1})	4 - 12
Regression equation (y = a +bC), where C is the concentration of analyte in µg*/ml and y is the absorbance unit. Slope(b)*	0.0413
Standard deviation on slope(S_b)	0.000115
Intercept(a)	- 0.0018
Standard deviation on intercept(S_a)	0.0007659
Linear correlation coefficient(r)	0.9999
Limit of detection(LOD) (µg mL^{-1})	6.12×10^{-2}
Limit of quantification(LOQ) (µg mL^{-1})	1.855×10^{-1}
Standard error of estimation(S_e)	7.303×10^{-4}
Sandell's Sensitivity	2.439×10^{-2}
Intra -day precision(%RSD)(n=6)	0.57
Inter- day precision(%RSD) (n=6)	0.50
0.01 Level of confidence limits	0.9374
0.05 Level of confidence limits	0.5978

heteropolyacid containing Mo for their study. The chemistry of molybdenum is complicated. It forms compounds corresponding to oxidation numbers + 2 to + 6. The most stable and commonly encountered compounds of molybdenum are derived from its oxide MoO_3. The molybdenum compounds corresponding to the oxidation states ranging from + 2 to + 5 are mostly complex species. The complex may be represented as a resonance hybrid of the type ($D^+A^- \leftrightarrow D^+ + A^-$), where DA is a close association of electron donor molecule (Drug) and the electron acceptor molecule Lewis acid (heteropoly acid - PMA), where D^+A^- an electron has been transferred from D to A. There are reports that these heteropoly acids yield colour species with π-electron donors such as vitamin A. The investigations were carried out based on the formation of molecular complex between the drug (TTN) and phosphomolybdic acid (PMA). Heteropolyacid which acts as electron acceptor (Lewis acid) whereas drug acts as electron donor molecule. A stable-colored complex is formed.

Validation of analytical data:

Following (ICH) requirements [19], the developed method has been validated statistically. Validation parameters like slope (b), intercept (a), linear correlation coefficient(r), inter and intraday precision (% RSD) were studied. Optical and regression characteristics like ε_{max} ($Lmol^{-1}cm^{-1}$) and λ_{max} (nm) values were found to be 1.2346×10^4 L mol^{-1} cm^{-1} and 700 nm. The limits of linearity range for the developed method system were found to have 4 - 12 µg mL^{-1}. The calibrated curve is drawn at specified concentration levels consisting of linearity with linear correlation coefficient (r) value 0.9999. Limit of detection (LD) and limit of quantification (LQ) for the developed method were calculated using the formulas (LD) = $3.3 \times S_a / b$ and (LQ) = $10 \times S_a / b$, where b is the slope of the calibrated curve and S_a is the standard deviation of the intercept. Sensitivity of developed method was explained on the basis of molar absoptivity values. Precision of the proposed method was explained in terms of relative standard deviation (% RSD) considering from six (n = 6) determinations of the sample solution under optimum conditions. The results are given in Table 2. Analytical procedure accuracy was checked in terms of % recovery of the drug. Recovery experiments were carried out by introducing a calculated quantity of drug to the pre-analyzed formulations at different levels and determining the accuracy of the techniques proposed. Values of % Recovery ± SD were found in between 99.12 to 99.87 (± 1.2 to ± 0.23) for considering three determinations (n = 3). The results of proposed method (formulations) and

Table 2. Results of assay of tretinoin in marketed dosage forms.

Formulation, Propritery name& Pharmaceutical company concerned	Labelled Amount (g)	Quantity found by proposed method(g) (n=6)*	95 % Confidence limit values F -Test (Tabulated value = 5.05)	95 % confidence limit values t-Test (Tabulated value = 2.57)	Quantity found by Ultraviolet absorption method (mg)	%Recovery by developed method (n=3)•
Cream(A Ret-HC) Shalaks Pharmaceuticals.	20	19.93 ± 0.1	2.51	0.14	19.95 ± 0.15	99.63 ± 0.48
Cream(Retino-A) Janssen-Cilag Pharmaceuticals	20	19.97 ± 0.05	1.87	1.30	20.00 ± 0.06	99.87 ± 0.23
Cream (Airol) Piramal Health care	20	19.91 ± 0.19	1.36	0.02	19.91 ± 0.22	99.53 ± 0.96
Cream(Avita) Bertek Pharmaceutical Inc.	20	19.8 ± 0.23	3.95	0.84	19.94 ± 0.12	99.12 ± 1.2

** n = 6 Average of six determinations,•n = 3 Average of three determinations*

Table 3. Optimum conditions of proposed method (TTN-PMA system).

Parameter	Optimum range	Conditions in Procedure	Remarks
λ_{max} (nm)	695 - 705	700	--
Effect of volume of PMA (0.2 %) in CH_3COOH containing 2 % Ac_2O	0.5 - 1.0 mL	0.7 mL	0.7 ml of PMA in CH_3COOH containing 2% Ac_2O was necessary for maximum colour development to cover broad range of Beer's law limits. > 1.0 ml PMA was found no additional advantage.
Time and temperature required for colour development	75 - 110 min Laboratory temperature (28 ± 5°C)	90 min Laboratory temperature (28 ± 5°C)	To produce maximum colour intensity 90 min was found necessary which resulted better sensitivity and reproducibility
Solvent used for final dilution	CH_3COOH	CH_3COOH	Acetic acid was found to be the suitable solvent among the other solvents are ethanol, methanol and 2-propanol
Stability of coloured species after final dilution	Immediate - 40 min.	10 min.	After dilution with CH_3COOH the absorbance was measured after 10 min. The stability of coloured species were stable upto 40 min. Thereafter the absorbance was decreased slowly.

UV reference method were compared through student t- and student F - tests [18]. It was observed that no significant difference was noticed in between developed and UV reference methods as the results are found within the acceptable limits (based on 95 % confidence limit values for student t-test (2.57) and student F-test (5.05), respectively. The results are given in Table 3.

CONCLUSIONS

In this study, investigations were carried out to develop an accurate new analytical technique for assay tretinoin in bulk and formulations. The developed technique is found to be the best among the literature methods in terms of stability, sensitivity, and cost. The sensitivity of the developed method lies only in the reaction's nature with a suitable reagent chosen rather than the instrument's sophistication. The excipients present in formulations did not intervene in the assay. Hence, the results indicate that the method developed is specific and suitable for routine analysis in bulk and formulations and can be used as a substitute for advanced instrumental methods in quality control laboratories.

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