JOTCS A
Journal of the Turkish Chemical Society
Section: A
Chemistry
TURKISH CHEMICAL SOCIETY

Assay of Tretinoin using Safranine-O as a Chromogenic Reagent in Bulk and Dosage Forms

Thuttagunta Manikya Sastry[1]*, Venkata Nagalakshmi Kiilana[1], and Rama Krishna Karipeddi[2]

[1]Gayatri Vidya Parishad College of Engineering (Autonomous), Chemistry, Department of Chemistry, Visakhapatnam-530048, India

[2]GITAM, Deemed to be University, Department of Chemistry, Rushikonda, Visakhapatnam- 530045, India

Abstract: A new, economical and accurate analytical technique was developed for the assay of tretinoin (TTN) in bulk and formulations. While developing the method, it was found that the reaction was carried out due to the formation of ion-pair association complex involving the carboxyl group in the side chain of the TTN with safranine-O. The colored species formined was stable up to 60 minutes. The optical density of color species was measured at 520 nm. All the variables were optimized. The linearity range lies for the developed method within the concentration ranges of 2-10 µg mL^{-1}.The linear correlation coefficient (r) and molar absorptivity (ϵ_{max})values were found as 0.9999 and 1.66 x 10^4 L $mol^{-1}cm^{-1}$. Percentage recoveries were found from 99.2 ± 1.8 to 100.2 ± 0.7.The method was validated as per ICH guidelines.

Keywords: Bioactive compounds, tretinoin, safranine-O(SFN-O), Ion-pair association complex, spectrophotometry.

Submitted: September 17, 2022. **Accepted:** January 04, 2023.

Cite this: Manikya Sastry T, Nagalakshmi KV, Ramakrishna K. Assay of Tretinoin using Safranine-O as a Chromogenic Reagent in Bulk and Dosage forms. JOTCSA. 2023;10(1):139–46.

DOI: https://doi.org/10.18596/jotcsa.1176621.

***Corresponding author. E-mail:** tmsastry@yahoo.com.

1. INTRODUCTION

Bioactive compounds are found in both plant and animal products and can also be synthetically produced. Drugs are an important class of bioactive compounds. The estimation based on the reactions with suitable chromogenic agents are simple and inexpensible. Over-the-counter (OTC) 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 and found in 80-90% of teenagers due to hormonal changes. The acne product namely tretinoin works by replacement of skin cells. Acne vulgaris is considered as common skin related problem that was treated with combination therapy using topical drug clindamycin and tretinoin (TTN) (1). Literature survey revealed that there is an evidence about the determination TTN by HPLC (2-6), RP-HPLC (7-12), UPLC (13), LC (14), and GC (15). Studies on phototoxicity of tretinoin (16), UV–spectrophotometry (17-22), and UV-derivative spectroscopy (23-25) were also carried out by the previous authors. Most of the analytical methods involve sophisticated instruments which are expensive, and require maintenance and are not within the reach of most of the laboratories. Visible spectrophotometry can be chosen as an alternative technique. It is a highly preferable method for routine analysis because of its simplicity, low time and economical advantages. The novelty of this technique lies in its sensitivity and further depends on the nature of reaction and not on the sophistication of the instrument. No evidence is

found in the literature for the determination of TTN by visible spectrophotometry. Hence the authors made an attempt to develop simple and sensitive visible spectroscopic method for the assay TTN in bulk and formulations using safranine-O (SFN-O) as a chromogenic reagent. Figure 1 shows the structural molecule for tretinoin.

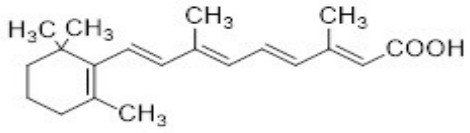


Figure 1: Chemical structure of Tretinoin (2E,4E,6E,8E)-3,7-Dimethyl-9-(2,6,6-trimethylcyclo-hex-1-enyl) nona-2,4,6,8-all-trans-tetraenoic acid).

2. EXPERIMENTAL SECTION

2.1. Materials and Methods

2.1.1. Instrumentation

Shimadzu UV double beam spectrophotometer has been chosen for obtaining precise and accurate optical density measurements. In addition a digital pH meter (equiptronics, India) was used to measure the pH of the samples. An electrical balance (Dhona 200 D, India) was used to weigh all the materials.

2.1.2. Chemicals and Reagents

TTN (Biophore India), Formulations namely Retino-A(EthnorJanssencilag), Airol (Piramal Health care). Avita, Bertek pharmaceutical Inc.) and Eudyna, (German Remdies.) were procured from the registered pharmacy.

Reagents namely SFN-O (Fluka, Mumbai, India, 98% purity), Sodium hydroxide (Qualigens Mumbai, India, 99% purity) and solvent $CHCl_3$ (Qualigens Mumbai, India, 99% purity) were procured for this study are of analytical grade.

Aqueous solutions of SFN-O (Fluka; 0.01%, 2.86×10^{-4} M) was prepared by dissolving 10 mg of safranin-O in 100 mL of distilled water and washed with $CHCl_3$ to remove chloroform-soluble impurities. Buffer solution was prepared by mixing 50 mL of 0.025 M borax solution with 15 mL of 0.1 M of sodium hydroxide and diluted to 100 mL with distilled water and pH was adjusted to 9.8.

2.1.3. Bulk sample solution

Stock solution (mg mL^{-1}) for bulk drug sample was prepared in 100 mL of chloroform by dissolving 100 mg of tretinoin. Working standard solutions of concentration of 40 µg mL^{-1} were prepared from the above stock solution. Further dilution was done using chloroform.

2.1.4. Formulations

Cream equivalent to 50 mg was dissolved in 30 mL of aqueous methanol (3:1). The resulting solution was extracted with solvent $CHCl_3$ (3 x 25.0 mL portions) followed by filtration. 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 was further diluted to a concentration of 40 µg mL^{-1}.

2.1.5. Calibration curve - UV method (Reference method)

Stock solution (mg mL^{-1}) was prepared by dissolving100 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 (Figure 2).

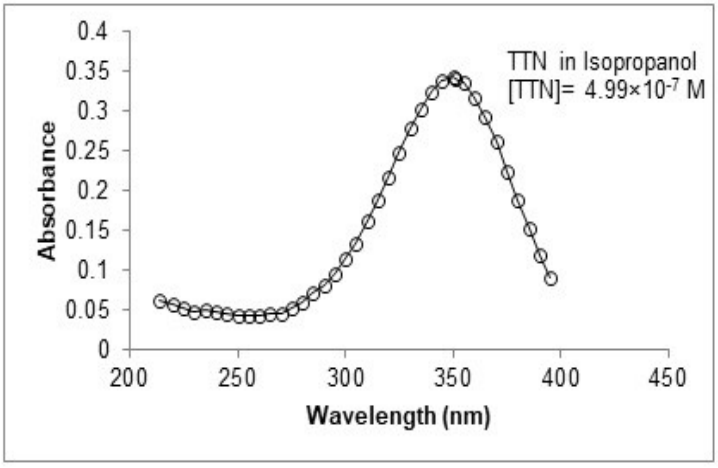


Figure 2: Absorption spectrum of tretinoin ([TTN] = 4.99×10^{-7}M) against (blank).

A series of solutions were prepared by taking 0.5-2.5 mL standard drug solution (10 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 TTN (Figure 3).

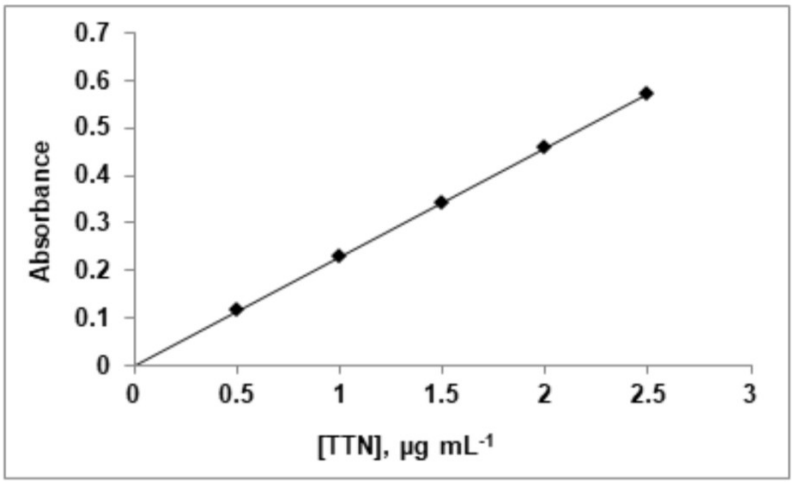


Figure 3: Calibrated curve of tretinoin ([TTN] = 4.99×10^{-7} M).

2.1.6. Method development

A series of solutions were prepared by taking aliquots of standard drug solution (0.5 – 2.5 mL, 40 µg mL^{-1}), 2.0 mL of 2.85 x 10^{-4} M safranine solution and 1.0 mL of buffer solution (pH 9.8) into 50.0 mL separating funnels. The volume of each sample was diluted to 15.0 mL with distilled water and 10.0 mL chloroform was added. The contents of the separating funnel were shaken for two minutes. The two layers were separated and the absorbance of organic layer was measured at 520 nm against blank. The optical density of colored species was observed to decrease after 60 min indicating the decomposition of colored complex. The concentration of the drug was deduced from its calibration curve drawn between optical density and concentration of TTN (Figure 4).

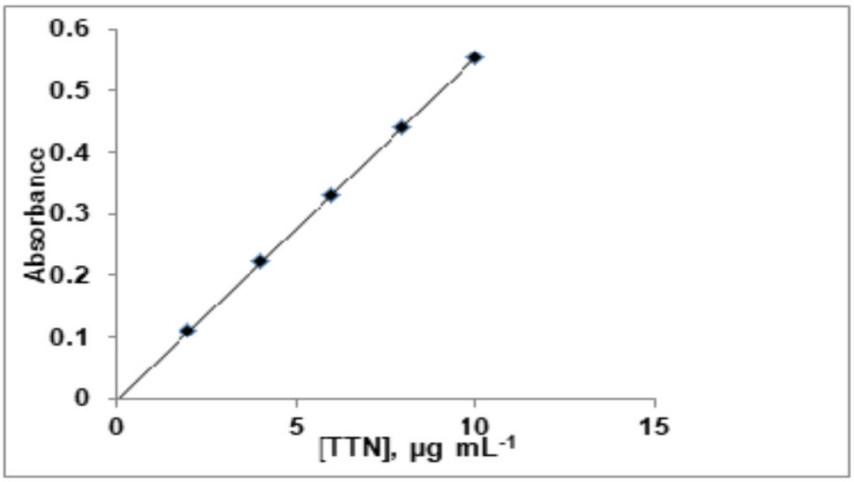


Figure 4: Calibrated curve of TTN - SFN-O method.

3. RESULTS AND DISCUSSION

3.1. Selection of Analytical Wavelength

The sample solution containing fixed amount of TTN, SFN-O (basic dye), buffer and other furnished variables as mentioned in the 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 ion-pair association complex showed maximum absorbance

at 520 nm and this wavelength has been selected for the analysis. The spectrum of the safranine-O (basic dye) showed maximum absorbance whereas blank solution against chloroform showed negligible absorbance at this wavelength (Figure 5).

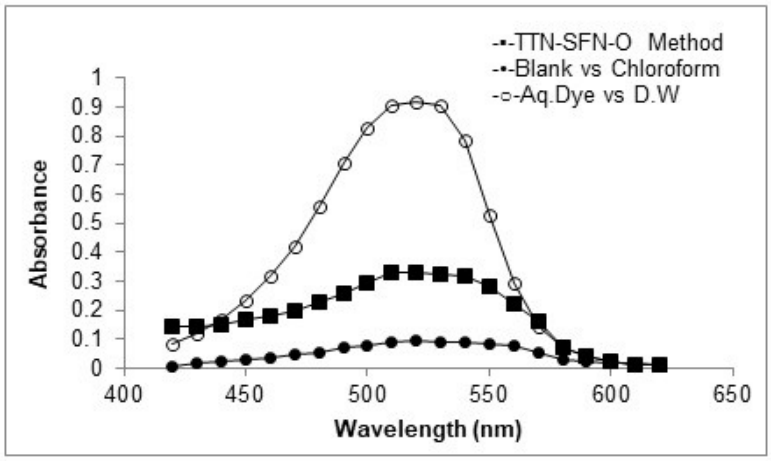


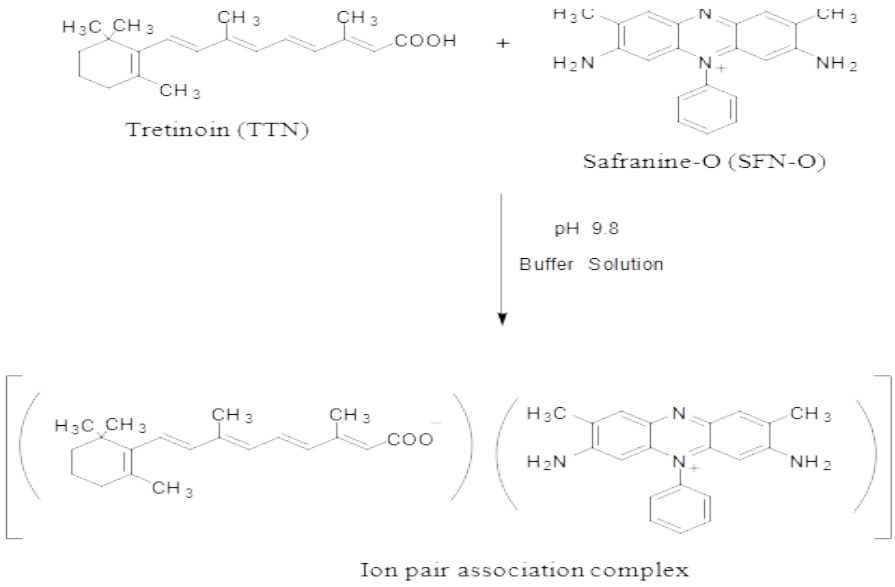
Figure 5: Absorption spectrum of TTN-SFN-O method.

3.2. Optimum Conditions

The responses of several factors like concentration of SFN-O (0.29 – 0.72×10^{-4} mol L^{-1}) the volume of extracting solvent chosen, time of stability of ion-pair association complex formation (1-60 minutes), intenseness of colored species produced, the ratio of aqueous to $CHCl_3$ were studied (26). The following optimum conditions were fixed for the proposed technique are; 2.0 mL (0.57×10^{-4} $molL^{-1}$) SFN-O,1.0 mL borax buffer (pH=9.8) with 2 min agitation time at 28 ± 2 °C. The ion pair association complex is stable up to 60 minutes afterwards the absorbance slowly decreased indicating the decomposition of the complex.

3.3. Mechanism of Ion-pair Association Complex

In TTN, the carboxyl group in the side chain involved in the formation of the ion pair association complex with SFN-O in alkaline medium. Based on the analogy studies, the ion-pair association complex formation mechanism is explained. The negative charge appeared on the carboxylate anion of the drug (TTN) molecule and the positive charge of SFN-O held together through electrostatic force of attraction. The obtained product behaved as a single molecule. The probable sequence of mechanism of the reaction is given in Scheme 1.



Scheme 1: Ion pair association complex of tretinoin with safranine-O at pH 9.8.

3.4. Validation of Analytical Data

Following (ICH) requirements (27), the TTN-SFN-O system developed was 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 1.66 $x10^4$ and 520, respectively. The limits of linearity range for TTN-SFN-O system was found to have 2 – 10 µg mL^{-1} .The calibrated curve drawn at specified concentration levels consisting of linearity with linear correlation coefficient(r) value 0.9999. Limit of detection (LOD) and Limit of quantification (LOQ) for the developed method were calculated. Sensitivity of developed method is explained on the basis of molar absoptivity values. Precision 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 Tables 1 and 2.

Table 1: Optical conditions for the proposed technique.

Optical condition	TTN-SFN-O method
Wavelength (λ_{max})	520 nm
Molar absorptivity (ϵ_{max})	1.66 $\times10^4$ L $mol^{-1}cm^{-1}$
Limits of linearity range	2-10 µg mL^{-1}
LOD	7.19 $\times10^{-3}$ µg mL^{-1}
LOQ	2.18 $\times10^{-2}$ µg mL^{-1}
Standard error of estimation (S_e)	1.23 $\times10^{-3}$
Sandell's Sensitivity	1.81 $\times10^{-2}$ µg cm^{-2}

Table 2: Validation parameters for the proposed technique.

Validation parameter	TTN-SFN-O method
Slope (b)	5.51 $\times10^{-2}$
Standard deviation on slope (S_b)	2.00 $\times10^{-4}$
Intercept (a)	1.80 $\times10^{-4}$
Standard deviation on intercept (S_a)	1.20 $\times10^{-4}$
Linear correlation coefficient (r)	0.9999
Intra -day precision (%RSD)*	0.65
Inter- day precision (%RSD)*	0.68
0.01 Level of confidence limits	1.07
0.05 Level of confidence limits	0.68

y = a +bC where C is the concentration of analyte in µg/mL and y is the absorbance unit.
Calculated from six determinations.

The accuracy of the analytical procedure was checked in terms of % recovery. 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 were found to be 99.2 - 100.2% (± 0.8 to ±0.7) for considering three determinations (n=3). The results of proposed method (formulations) and UV reference method were compared through student t- and F- tests. 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 and "F"-test respectively. The results are given in Table 3. The proposed method was compared with literature method (20) and found to be more sensitive with reference to molar absoptivity, linear correlation coefficient (r), LOD and LOQ values. Precision and accuracy was calculated in terms of relative standard deviation (%RSD) and %Recovery values. The results are given in Table 4.

4. CONCLUSION

An accurate analytical technique developed for the assay of TTN in bulk and formulations. The developed technique is found to be best among the literature methods in terms of stability, sensitivity and cost. The sensitivity of developed method lies only in the reaction's nature with the reagent chosen but not on the instrument's sophistication. The excipients commonly found in formulations did not intervene in the assay. Hence the developed method is specific and suitable and found as an alternative to instrumental methods such as LC-MS, HPLC, GLC and GC-MS, in quality control laboratories.

Table 3: Assay of TTN in Pharmaceutical Formulations (TTN-SFN-O Method).

Formulation Proprietary name	Labeled Amount (g)	Amount found by proposed method (g)(n=6)[a]	95% Confidence limit values F –Test[b]	95% Confidence limit values t-Test[c]	Quantity found by UV absorption method (g)(n=3)	% Recovery developed method[d]
Cream (Retino-A)	20.0	20.04	0.15	1.55	20.1 ± 0.1	100.2 ± 0.7
Cream (Airol)	20.0	20.03	3.07	1.19	20.1 ± 0.2	100.1 ± 0.7
Cream (Avita)	20.0	19.84	2.99	0.03	19.9 ± 0.2	99.2 ± 1.8
Cream (Eudyna)	20.0	19.94	1.07	0.90	19.9 ± 0.1	99.7 ± 0.6

[a]Average value of six observations. [b]Tabulated value of 5.05 for "F" –Test. [c]Tabulated value of 2.57 for student "t" –Test. [d]Average value of three measurements (n=3).

Table 4: Comparison of proposed method with literature (20) method.

Reagent used	Iodine (I_2)	SFN-O
Wavelength(λ_{max}) nm	295	520
Molar absorptivity(ϵ_{max}) L $mol^{-1}cm^{-1}$	1.31×10^4	1.66×10^4
Limits of linearity range(µg mL^{-1})	9.04 – 29.71	2-10
Linear correlation coefficient (r)	0.9974	0.9999
Relative standard deviation (%RSD)	1.95-0.88	Intraday(0.65) Interday(0.68)
% Recovery	97.84 – 102.80	99.7 - 100.2
LOD µg mL^{-1}	4.35	0.007
LOQ µg mL^{-1}	13.17	0.022
Method	Zayed MA and Abdel-Basset MH (20)	Present method

5. CONFLICT OF INTEREST

None.

6. ACKNOWLEDGMENTS

The authors (Dr. T. Manikya Sastry and Dr.K. V. Nagalakshmi) are grateful to the organisation, GVPCOE (A) for their encouragement and support.

7. REFERENCES

1. Fu LW, Vender RB. Newer approach in topical combination therapy for acne. Skin Therapy let. 2011;16(9):3-6.

2. Platzer DJ, White BA. Development and validation of a gradient HPLC method for the determination of clindamycin and related compounds in a novel tablet formulation. J Pharm Biomed Anal. 2006;41:84-88. DOI: <URL>.

3. Tashtoush BM, Jacobson EL, Jacobson MK. A rapid HPLC method for simultaneous de-termination of tretinoin and isotretinoin in dermatological formulations. J Pharm Biomed Anal. 2007;43(3):859-864. DOI: <URL>.

4. Fawzia I, Mohie KSE , El-Deen AK, and Kuniyoshi S. A New HPLC-DAD Method for the Concurrent Determination of Hydroquinone, Hydrocortisone Acetate and Tretinoin in Different Pharmaceuticals for Melasma Treatment. Journal of Chromatographic Science. 2019;57(6):495–501. DOI: <URL>.

5. Abdelghani M, Mohamed SB. Simplified HPLC method for simultaneous determination of erythromycin and tretinoin in topical gel form. Sep Sci plus. 2020;1–8. DOI: <URL>.

6. Sadaf S, Shahid H , Mohsin J, Ali R, Shahid I,Hamad A, Samar OA, Eslam B, HamoudH. Somaily, Rami AP, EmanA and Abd-E F. Simultaneous HPLC Determination of Clindamycin Phosphate, Tretinoin, and Preservatives in Gel Dosage Form Using a Novel Stability-Indicating Method. Inorganics.2022; 10(10):1-15. DOI: <URL>.

7. Yuenong RY, Eden B, Richard B, Robert H, Barry H. Simultaneous determination of tretinoin and clindamycin phosphate and their degradation products in topical formulations by reverse phase HPLC. J Sep Sci. 2004;27:71-77. DOI: <URL>.

8. Vaidya V, Baing MM, Joshi SS. Reverse phase high performance liquid chromatographic determination of tretinoin in bulk material, Indian Drugs. 2005; 42(1):42-45.

9. Sheliya K, Shah K, Kapupara P. Development and validation of analytical method for simultaneous estimation of mometasonefuroate, hydroquinone and tretinoin in topical formulation by RP-HPLC. J Chem Pharm Res. 2014: 6(4):934-940.

10. Vasanthi R, Rajitha N, Raja MA, Shrisha V, Banji D, Kumar DS. Analytical method development and validation

of Isotretinoin in tablet dosage formulation. Asian J Pharm Anal Med Chem. 2015;3(3):145-153.

11. Ashish A, Manoj S ,Reverse-phase High-performance Liquid Chromatography Estimation of Methotrexate and Tretinoin in Bulk and Pharmaceutical Dosage Forms. International Journal of Pharmaceutical & Biological Archives 2019; 10(2):146-153.

12. Jamel MKA, Gagy AA. Development and validation of analytical spectrophotometric and RP-HPC methods for the simultaneous estimation of hydroquinone, hydrocortisone and tretinoin ternary mixture in topical formulation. International journal of pharmacy and pharmaceutical sciences.2019;11(11):10-16. DOI: <URL>.

13. His SL, Surman P and Al-Kassas R. Development of a stability-indicating UPLC method for determination of isotretinoin in bulk drug. Pharmaceutical Development and Technology, 2018; 24 (2):189-198.

14. Dileusa DO, Diego FDA, Edilene GDO, Ruy CRB. Liquid chromatography method to assay tretinoin in skin layers: validation and application in skin penetration/retention studies. Heliyon. 2020;6:e03098. DOI: <URL>.

15. Martins LE, Danielle G, Almeida DA, Nelson R, Antoniosi F. Development of a gas chromatography method for the determination of isotretinoin and its degradation prod-ucts in pharmaceuticals. J Pharm Biomed Anal.2005;38:678–685. DOI: <URL>.

16. Slade HB, Shroot B, Feldman SR, Cargill DI, Stanfield J. Reappraising the phototoxicity of tretinoin: a report of four controlled clinical trials. Photodermatology, Photoimmunology&Photomedicine. 2009;25(3):146–152. DOI: <URL>.

17. Maryam B, Ali YF, Jahanbakhsh G, Mohammad M, Ahari M, Nahid S, Mohammad Taghi B, Simultaneous spectrophotometric determination of minoxidil and tretinoin by the H-point standard addition method and partial least squares. Chem pap. 2009;63(3):336–344. DOI: <URL>.

18. Patel P, Kabra P, Kimbahune R, Urmila GH. Quantitative estimation of isotretinoin (13-cis retinoic acid) in bulk and formulation by UV-visible spectrophotometry. Res J Pharm BiolChem Sci. 2011;2(1):167-172.

19. Pankti D, Kusum M, Mehul P. Development and validation of UV-visible spectrophotomet-ric method for simultaneous estimation of momentasonefuroate, hydroquinone and tret-inoin from their pharmaceutical dosage form. Int J Pharm Sci Rev Res. 2013;21(1): 296-300.

20. Zayed MA and Abdel-Basset MH. Spectrophotometric Micro determination of Tretinoin, Isotretinoin using Iodine and Tazarotene Micro determination Via Reaction with Rose-Bengal Reagent. Egypt. J. Chem. 61(1);2018:143 – 153. DOI: <URL>.

21. Suraj D, Lobsang TK , Hauzel L, Laldinchhana, Sabir H. Development and validation of UV-spectrophotometric method for the determination of tretinoin. Journal of Xi'an Shiyou University. 2022;18(9):547-553.

22. Kiran D, Deepak N, Arvind N. Simultaneous UV spectrophotometric valuation of isotretinoin& their promoted drug. Int. J. Pharm. Sci. Rev. Res. 2018;49(1):82-85.

23. Barazandeh TM, Namadchian M, Fadaye VS, Souri E. Derivative spectrophotometric method for simultaneous determination of clindamycin phosphate and tretinoin in pharmaceutical dosage forms. DARU. 2013;21(1): 2-7.

24. Mahrous MS, Abdel-Khalek MM, Beltagy YA. Simultaneous Quantitation of Minoxidil and Tretinoin in Magistral and Pharmaceutical Preparations by First Derivative Spectrophotometry. Anal lett. 2006;25(9):1673-1686. DOI: <URL>.

25. Nazira S and Abdulkader S. Development and validation of derivative UV spectroscopic method for simultaneous estimation of nicotinamide and tretinoin in their binary mixtures and pharmaceutical preparations. Sarkis and Sawan BMC Chemistry. 2022;16(15):1-11. DOI: <URL>.

26. Massart DL, Vandeginste BGM, Doming S N, Michotte Y, Kaufman L. Chemometrics, A text Book, Amsterdam: Elsevier. 1988;293; 80.

27. ICH Harmonized Tripartite Guideline; International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, Text and Methodology on Validation of Analytical Procedures, Q2, (R1); 2005.