

## Spectrophotometric Microdetermination of Tretinoin, Isotretinoin using Iodine and Tazarotene Microdetermination Via Reaction with Rose-Bengal Reagent

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**T**HE REACTIONS of iodine or rose-bengal (Rbng) reagents with three of retinoid drugs tretinoin, isotretinoin and tazarotene had been studied for the development of simple, rapid, sensitive spectrophotometric methods for microdetermining of these drugs in pure and in their pharmaceutical formulation. These methods are based on the formation of reaction product sbetween the drugs and iodine or rose -bengal reagent. The spectra of the formed reaction products were measured at selected proper conditions of time, temperature, pH and selected wavelength. The analytical parameters such as standard deviation (SD), relative standard deviation (RSD), Sandell's sensitivity (S), LOQ and LOD were calculated in order to check accuracy, sensitivity and precision of the given procedures. The values of SD = 0.1312 - 1.100, RSD = 0.5556 - 1.946 %, S = 0.0229 – 0.0508  $\mu\text{g cm}^{-2}$ , LOQ = 13.17 – 17.45  $\mu\text{g mL}^{-1}$ , LOD = 4.347 – 5.757  $\mu\text{g mL}^{-1}$  obtained of these parameters refer to the accuracy and sensitivity of the suggested procedures and can be applied for analyses of these drugs in their pharmaceutical formulations. Beer's law was valid in the range 9.041 – 29.71, 35.75 – 119.6 and 10.48 – 71.11  $\mu\text{g mL}^{-1}$  with recovery of 97.84 - 102.8 %, 98.67 - 101.8 %, and 98.32 - 102.0 % for Tretinoin, Isotretinoin and Tazarotene respectively, The importance of this research stems from applications of these retinoid derivatives in skin improvements. They are always used as creams and found to be effective for photo-damage and for protection from skin irritation. Therefore, the proposed methods had been applied successfully for the analysis of the studied drugs in pure forms and pharmaceutical formulations. The results obtained were found to be in good agreement with those obtained by official methods. This evaluation had been done by F- and t- tests.

### Introduction

Pharmaceuticals are considered as one of the main pillars in human health. These pharmaceuticals would help if only they are pure and when they are administered in an appropriate amount. These pharmaceuticals may develop impurities at various stages of their development, transportation and storage [1]; which makes the pharmaceutical risky thus they must be detected and quantitated. For this; analytical instrumentation and methods play an important role. It is important to emphasize that each analytical technique has its own characteristics; which will vary from drug to drug

Spectrophotometric methods applied in pharmaceutical analysis are numerous; mostly

all spectral methods are in use. Among these to be mentioned is molecular spectrophotometric method using UV and visible radiation which will be discussed here. The advantages of these methods are low time and labor consumption. The precision of these methods is also excellent. One of these methods has been used here for the micro-determination of retinoids which are a class of chemical compounds that are related chemically to vitamin A. Retinoids are used in medicine; primarily due to the way they regulate epithelial cell growth. It have many important and diverse functions throughout the body including roles in vision, regulation of cell proliferation and differentiation, growth of bone tissue, immune function, and activation of genes. Research is also being done into their ability to treat skin

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cancers. Currently 9-cis retinoic acid may be used typically to help treat skin lesions from Kaposi's sarcoma. Tretinoin (Tret), isotretinoin (Itret) and

tazarotene (Taz) are the scope of this study due to their clinical advantages. The chemical structures of these retinoid drugs are shown in Fig. 1.

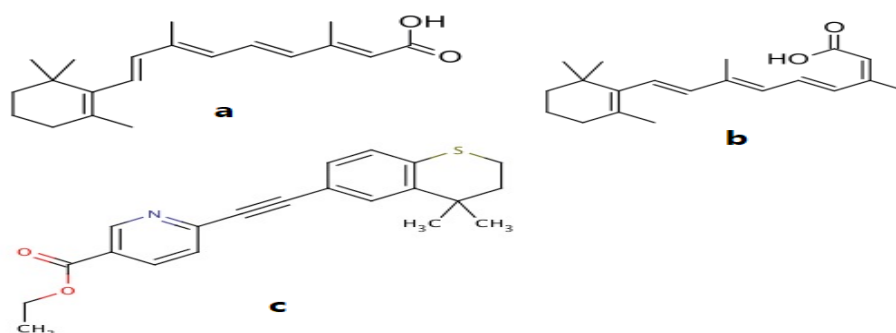


Fig.1. the chemical structures of (a) Tret, (b) Itret and (c) Taz.

For determination of retinoids in dermatological formulations, there are few UV-Visible spectroscopic methods reported [2-17], but there are several other methods have been reported for the determination like chromatography [18-34,12]. However, most of these methods are complicated and not available at most laboratories. For these reasons, it was worthwhile to develop a new simple and selective spectrophotometric method for the determination of the studied drugs in their pharmaceutical dosage forms. Iodine [35 - 38] and Rose - Bengal [39-41] were used as reagents for various compounds. In the present work, we report the development of accurate and precise spectrophotometric method using these two reagents. The drugs (Tret, Itret and Taz) and reagents (iodine or Rose-bengal) reaction product are studied spectrophotometrically. The proposed methods were applied successfully for the determination of the studied drugs in pure and pharmaceutical forms. These methods are rapid, sensitive and accurate in comparing with reference methods.

## Experimental

### Materials and solutions

All chemicals used were of the highest purity available. They included standards Tret (M.wt = 300.44 g mol<sup>-1</sup>) and Itret (M.wt = 300.44 g mol<sup>-1</sup>) were provided by Medzen pharmaceutical industries Company, Cairo, Egypt. The standard Taz (M.wt = 351.436 g mol<sup>-1</sup>) was provided by Delta pharma quality assurance department, Egypt. The reagents used were Rbng disodium salt and Iodine (I<sub>2</sub>) (M.wt 253.81 g mol<sup>-1</sup>) which, supplied by British Drug House (BDH) Chemicals, Ltd (Poole, England) and sigma- Aldrich respectively.

Chloroform (99 %) and ethanol (95 %) were supplied by Dasit group (Carlo erba reagents S.A.S) and Tetra hydro furan (THF, 99.5 %) was supplied by Rankem. Absolute ethanol was supplied by Scharalu. Distilled water obtained from all glass equipments was usually used in all preparations. Pharmaceutical preparations of the investigated drugs were purchased from the local market; these are :

1. Acetin 0.05 % cream (30 g) was obtained from Jamjoom Pharmaceuticals, Jaddah – Saudi Arabia, labelled to contain (0.05 Tret weight (wt) /wt %).
- 2- Isotretinoin capsules were obtained from Ranbaxy laboratories limited, paonta sahib, India labeled to contain (20 mg Itret capsule<sup>-1</sup>).
- 3- Acnitaz gel (15g) was obtained from Marcyrl pharmaceutical industries, labeled to contain (0.1 % Taz wt/ wt).

### Instruments

The spectrophotometric measurements were carried out using the Thermo Fisher Scientific, Model: EVO 60 in the wavelength range from 190-800 nm. And optizen pop., Model: 5u4701-127022-00 in the wavelength range 200 -800 nm. The pH measurements were performed by using HANNA pH/mV/temperature meter, Model pH S - 3CW. The weights measurement was performed by using Radwag wagi Elektroniczne Sensitive analytical balance 0.0001g, Model: AS 220/C/1. Stirring and heating were performed by using heating magnetic stirrer theromostated hot plate, Model: VELD-Europe. Automatic Micropipettes, Model: Accupipette USA, volume range 100-1000 µL were used to measure the small volumes.

### Procedures

#### a. General procedure

Solution of  $1 \times 10^{-3}$  M  $I_2$  (M. Wt. = 253.81 g mol<sup>-1</sup>), was prepared by dissolving accurate weight (0.0126 g) and complete the volume by ethanol 95% in 100 mL volumetric flask. Solution of  $1 \times 10^{-3}$  M Rbng reagent (M. Wt. = 1017.64 g mol<sup>-1</sup>), was prepared by dissolving weight of (0.0508 g) in appropriate volume of distilled water and the volume completed to 50 mL. Solutions of  $1 \times 10^{-3}$  M (300.44 μg ml<sup>-1</sup>) standard drugs Tret and Itret, were prepared by dissolving the accurately weighed amount of the pure drug (0.0150 g) in the appropriate volume of ethanol (95 %) and the volumes completed to 50 mL in a volumetric measuring flasks. Solution of  $1 \times 10^{-3}$  M (351.46 μg mL<sup>-1</sup>) standard drug of Taz was prepared by dissolving accurately weighed amount of the pure drug (0.0175 g) in the appropriate volume of ethanol 95 % and the volume completed to 50 mL. Dilute solutions were prepared by accurate dilution from the stock solutions to get the desired concentrations. Series of universal buffer solutions covering the range of pH values from 2.00 to 11.00 were prepared as recommended by Britton and Robinson [42]; where a mixture of 0.04 M phosphoric, acetic and boric acids was titrated with 1 N NaOH to adjust the desired pH into the required value in 100 ml of the acid mixture using pH-meter.

Spectrophotometric determination of Tret and Itret with  $I_2$  and Taz with Rbng reagent must be carried out within the concentration range in which Beer's law is valid. To determine the concentration ranges of the drugs under investigation, a series of solutions were prepared in which constant concentration of  $I_2$  ( $1 - 4 \times 10^{-4}$  M solutions) was added to the variable volumes of drugs (Tret and Itret) ( $10^{-4}$  M); then 2 ml universal buffer of pH 6 or 2 was added respectively. Solutions were pale brown in both Tret and Itret mixtures. For Rbng method the reagent was kept constant ( $2 \times 10^{-4}$  M); while that of the drug within the range ( $0.3 - 2 \times 10^{-4}$  M) was varied then 2 ml of buffer with pH 9 was added which, yields red solution. The absorbance values were measured at their  $\lambda_{max}$  (295 nm for Tret and Itret and 285 nm for Taz) under the optimum conditions of molar ratio (1:1), time of 20, 40 and 10 min, temp of 50, 40 and 35 °C and pH of 6, 2 and 9 for Tret, Itret and Taz respectively and plotted against concentration.

#### b. Procedures for analyses of pharmaceutical preparations

For analysis of Tret using iodine reagent: 7.5 g Acretin cream (0.05%) was weighed and

dissolved in 15 ml THF, stirred for 15 minutes then centrifuged for 10 minutes at 4000 round per minute (rpm) which led to form two layers. The upper resulting THF layer was transferred to 25 ml measuring flask and the volume completed to the mark with ethanol 95 % leading to  $0.5 \times 10^{-3}$  M active ingredient in mixed solvent ratio (Ethanol: THF, 1:1.5). 1 ml of  $10^{-3}$  M  $I_2$  was added to different aliquots of 1.1- 1.95 mL of  $0.5 \times 10^{-3}$  M, taken from this solution. Then 2ml buffer of pH = 6 was added. The appeared turbidity was overcome by addition of 3ml THF to the reaction mixture and completed in 10 ml volumetric flask. The reaction mixture without drug had been used as the blank.

In case of Itret analysis: five soft gelatin capsules were cut with a sharp blade and dissolved in about 15 ml of THF. Then stirred for 15 minutes and filtered by using Whatman filter paper no. 41. The filtrate was taken and completed with ethanol 95% in 25 ml volumetric flask. 4 ml of  $10^{-3}$  M  $I_2$  was added to each of different aliquots, 0.6- 2.0 mL of  $2 \times 10^{-3}$  M taken from this solution. Then 2ml buffer of pH = 2 was added. The appeared turbidity was overcome by addition of 3 ml THF to the reaction mixture and all the reaction mixture completed in 10 ml volumetric flask. The reaction mixture without drug had been used as the blank.

In case of Taz application : The applicability of the proposed method for the determination of Taz has been tested on available pharmaceutical formulation, where 4.37 g Acnitaz gel was weighed and dissolved in 20 ml 95 % ethanol, 5 ml THF and 0.365 N HCl stirred for 15 minutes then centrifuged for 10 minutes at 4000 rpm. The stock solution of  $0.5 \times 10^{-3}$  M was prepared by supernatant transferred into 25 ml volumetric flask and completed to the mark with 95 % ethanol. Constant conc of Rbng  $2 \times 10^{-3}$  M was added to variable conc of Taz  $0.5 - 1.6 \times 10^{-3}$  M then 2ml buffer of pH = 9 was added. Turbidity may appear which is overcome with THF addition, all these aliquots completed in 10 ml volumetric flask with ethanol 95 %. The reaction mixture without drug had been used as the blank.

All solutions used are freshly prepared during the whole work, avoiding direct light.

### Results and Discussion

#### Absorption Spectra

These methods are based on the formation of reaction products between drugs and the

reagents ( $I_2$  or Rbng). The absorption spectra of the reaction product were scanned at 200-400

nm against reagent as a blank. Reaction products show maximum absorption at 295 nm for Tret and

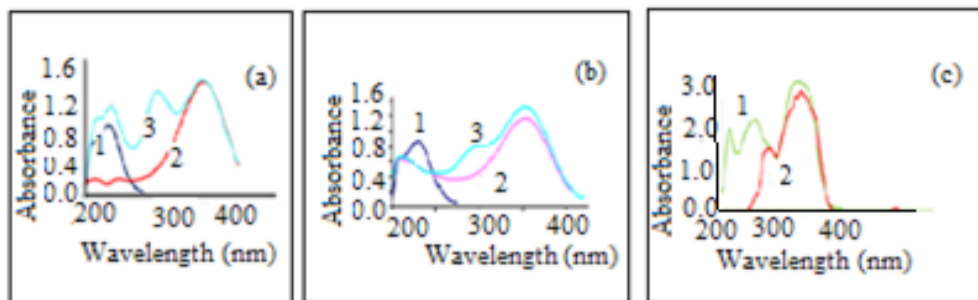


Fig.2. UV Absorption spectra of (a) 1-  $1 \times 10^{-4}$  M  $I_2$ , 2 -  $1 \times 10^{-4}$  M Tret, 3-Product of  $1 \times 10^{-4}$  M Tret and  $I_2$  using  $1 \times 10^{-4}$  M  $I_2$  as a blank (b) Absorption spectra of: 1-  $1 \times 10^{-4}$  M  $I_2$ , 2 -  $1 \times 10^{-4}$  M Itret, 3-Product of  $1 \times 10^{-4}$  M Itret and  $I_2$  using  $1 \times 10^{-4}$  M  $I_2$  as a blank. (c) Absorption spectra of: 1-  $1.3 \times 10^{-4}$  M Taz, 2-  $1.3 \times 10^{-4}$  M Product (Taz-Rbng) in 95 % Ethanol using Rbng as a blank.

Itret -  $I_2$  and 285 nm for Taz-Rbng.

*Optimum reaction conditions for product formation.*

Optimum conditions of the methods were carefully studied to achieve complete reaction formation, highest sensitivity, and maximum absorbance.

*Effect of time and temperature*

The optimum reaction time on the drug-reagent products was studied by measuring absorbance of the reaction product of Tret, Itret and Taz with reagents  $I_2$  or Rbng at 295 or 285 nm, time was investigated from 1 to 60 minutes (Fig.3). From the obtained results, the optimum time for the reaction product was assumed to be 20, 40 and 10 minutes for Tret, Itret and Taz respectively.

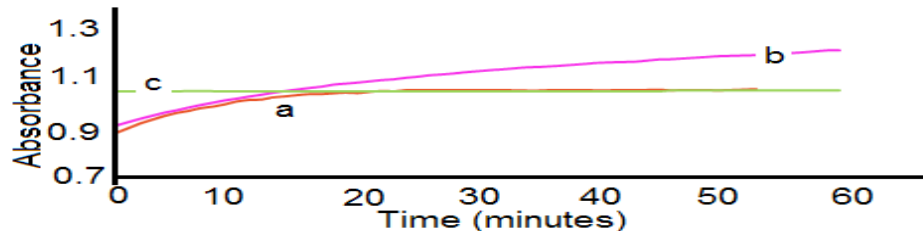


Fig. 3. Effect of Time on reaction product of : a-  $1 \times 10^{-4}$  M Tret and  $I_2$  at Wavelength = 295 nm, b-  $2 \times 10^{-4}$  M Itret and  $I_2$  at Wavelength = 295 nm, c-  $2 \times 10^{-4}$  M Taz and Rbng at Wavelength = 285 nm.

The effect of temp on the drug-reagent complex was studied by measuring absorbance of the reaction product of Tret, Itret and Taz

with reagents  $I_2$  or Rbng at 295 and 285 nm and optimum time to select the optimum temperature suitable for the product formation (Fig 4).

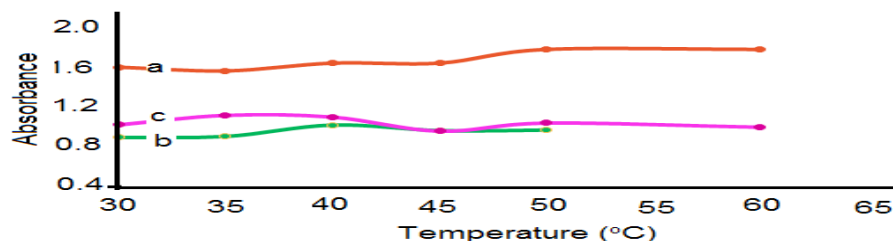


Fig.4. Effect of Temperature on reaction product of: a-  $2 \times 10^{-4}$  M Tret and  $I_2$  at  $\lambda_{max} = 295$  nm, Time = 25 min, b-  $2 \times 10^{-4}$  M reaction product of Itret and  $I_2$  at  $\lambda_{max} = 295$  nm, Time = 45 minutes, c-  $2 \times 10^{-4}$  M reaction product of Taz and Rbng at  $\lambda_{max} = 285$  nm, Time = 10 minutes

The optimum temp from the obtained results was found to be 50, 40 and 30°C, for the three represented Tret, Itret and Taz drugs respectively which gave maximum absorption.

#### Effects of pH on reaction product formation

The effect of pH on the drug-reagent complex

was studied by measuring absorbance of the reaction product of Tret, Itret, and Taz with reagents  $I_2$  or Rbng at  $\lambda_{max} = 295\text{nm}$ , or  $285\text{nm}$ . The highest absorbance value was observed at pH 6.0, 2.0 and 9.0 for the three represented drugs respectively as showed in Fig. 5.

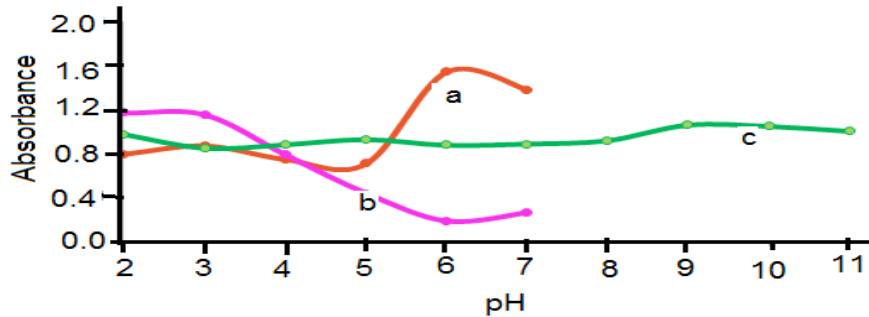


Fig. 5. Effect of pH on absorption spectrum of : a-  $1 \times 10^{-4}$  M Tret and  $I_2$  reaction product at 295 nm, time = 25 min and temperature = 50 °C, b-  $2 \times 10^{-4}$  M Itret and  $I_2$  reaction product at 295 nm, time = 45 minutes, c-  $0.8 \times 10^{-4}$  M Taz and Rbng reaction product at 285 nm, time =10 minutes and Temp = 30 °C.

#### Stoichiometric Relationship

The stoichiometric ratio between drugs and reagents was determined by the molar ratio method (MRM). For Tret a variable reagent concentration of  $I_2$  ( $0.4 - 1.2 \times 10^{-4}$  M), was added to constant drug concentration ( $1 \times 10^{-4}$  M) of Tret. For Itret method, a variable reagent concentration of

Itret ( $1-14 \times 10^{-4}$ M), was added to constant  $I_2$  concentration ( $5 \times 10^{-4}$  M). For Taz variable drug concentration was added to constant reagent conc  $2 \times 10^{-4}$ M. The spectrophotometric measurements of these solutions were recorded at  $\lambda_{max} = 295$  nm or 285 nm (Fig 6.).

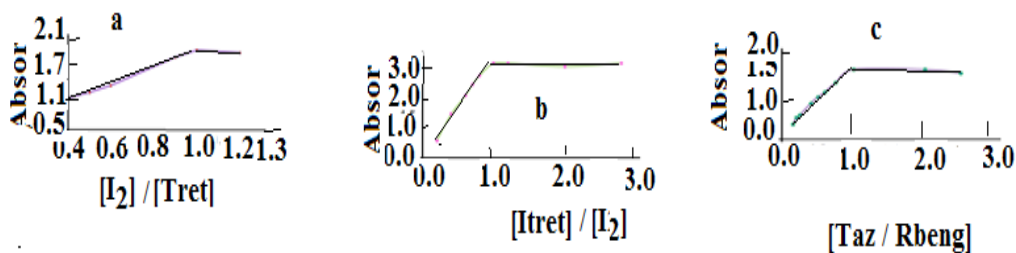


Fig. 6. (a) Molar ratio of Tret and  $I_2$  at Wavelength = 295 nm, time = 25 minutes and temp = 50 °C, (b). Molar ratio of Itret and  $I_2$  at wavelength = 295 nm, time = 45 minutes and temp = 40 °C, (C). Molar ratio of Taz and Rbng at wavelength = 285 nm, time = 10 minutes, temp = 30 °C and pH = 9.

#### Validity of Beer's law

Standard calibration curves for Tret, Itret, and Taz with reagents were constructed by plotting absorbance versus concentration at optimum described experimental conditions. Beer's law was valid over the concentration range 9.041 - 29.71, 35.57 - 119.6 and 10.48-71.11 respectively

using  $I_2$  or Rbng. Table 1 shows the analytical parameters obtained such as slope, intercept, correlation coefficient, Sandell sensitivity, molar absorptivity, standard deviation, and relative standard deviation, limit of quantification and limit of detection.

**TABLE 1. Analytical parameters for spectrophotometric determination of the standard retinoids by the proposed methods at the selected proper condition.**

Parameters	Tret	Itret	Taz
Drug	Tret	Itret	Taz
Reagent	I <sub>2</sub>	I <sub>2</sub>	Rbng
Time (minutes)	20 – 60	40 - 60	10 - 60
Temperature (°C)	50	40	30
λ <sub>max</sub> (nm)	295	295	285
pH	6	2	9
Beer's law (μg mL <sup>-1</sup> )	9.041 – 29.71	- 119.635.57	71.1110.48 –
LOD (μg mL <sup>-1</sup> )	4.347	5.757	4.825
LOQ (μg mL <sup>-1</sup> )	13.17	17.45	14.62
R <sup>2</sup>	0.9974	0.9997	0.9993
Regression equation	y = 0.0437x + 0.0628	y = 0.0197x - 0.2472	Y = 0.0209x + 0.1875
Molar absorptivity × 10 <sup>4</sup> (L mol <sup>-1</sup> cm <sup>-1</sup> ) × 10 <sup>4</sup>	1.312	0.5920	0.7339
SD	0.4738-0.1312	1.021-0.4560	0.1498 - 1.100
RSD %	1.946-0.8794	1.862-0.8108	1.874-0.5556
Sandell sensitivity (μg cm <sup>-2</sup> )	0.0229	0.0508	0.0479
Recovery %	97.84 – 102.8	98.67 – 101.8	98.32 – 102.0

This table proves the high sensitivity of the proposed methods in the determination of the drugs under investigation. The assay of Tret, Itret, and Taz were validated with respect to linearity, limit of detection, and quantification, repeatability and reproducibility. The linearity of calibration graphs was proved by the high values of the correlation coefficient (R<sup>2</sup>). The limits of detection (LOD) and limit of quantification (LOQ) for the proposed methods were calculated in Table (1) and their values confirm the sensitivity of the proposed method.

#### Accuracy and precision

The accuracy and precision of the proposed methods were established by measuring the content of Tret, Itret and Taz in pure form at different concentration levels of five replicate. The results of standard deviation (SD), relative standard deviation (RSD) and recoveries by the proposed methods are presented in Tables 2,3. The inter-day precision of the proposed methods is performed by carrying out five replicate experiments at each concentration level within 6 hours (Table 2).

#### Analytical applications

The applicability of the proposed methods for the determination of Tret, Itret and Taz has been tested on commercially available pharmaceutical

formulations. The results of the proposed methods were compared with those obtained by the official methods[5, 8, 16] (Table 4).

These results were compared with those obtained from the reference spectrophotometric methods[5,8,16] for Tret, Itret and Taz dosage forms by statistical analysis with respect to the accuracy (by student's t-test) and precision (by F-test)[38]. No significant differences were found between the calculated and theoretical values of t- and F-tests at 95% confidence level proving similar accuracy and precision in the determination of the studied drugs by the proposed and references methods.

#### Conclusion

The data given above reveal that the proposed methods are simple, accurate and sensitive with good precision and accuracy. Also, the reagents utilized in the proposed methods are cheaper, readily available and the procedures do not involve any critical reaction conditions or tedious sample preparation. Thus, these proposed spectrophotometric methods can be successfully applied for the determination of Tret, Itret and Taz in the pure form and in pharmaceutical preparations.

TABLE.2. Within – day precision of the determination of Tret and Itret using I<sub>2</sub> and Taz with Rbng reagent

Compound	Reagent	[Conc. taken] µg mL <sup>-1</sup>	[Conc. found] µg mL <sup>-1</sup>	Recovery %	SD*	RSD %*
Tret	I <sub>2</sub>	9.61	9.428 ± 0.1847	98.06	0.1847	1.965
		12.62	12.90 ± 0.1829	102.2	0.1829	1.420
		17.43	17.08 ± 0.2007	98.01	0.2007	1.173
		22.53	22.45 ± 0.4435	99.63	0.4435	1.976
		28.54	28.65 ± 0.2253	100.4	0.2253	0.7880
Itret	I <sub>2</sub>	90.13	91.00 ± 1.354	101.0	1.354	1.488
		108.2	110.9 ± 1.667	102.5	1.667	1.502
		114.2	112.3 ± 1.336	98.31	1.336	1.193
		117.2	114.8 ± 2.255	98.01	2.255	1.963
		120.2	122.4 ± 1.405	101.8	1.405	1.153
Taz	Rbng	33.39	32.89 ± 0.5517	98.52	0.5517	1.678
		38.66	38.65 ± 0.6820	99.98	0.6820	1.770
		56.23	55.38 ± 0.9722	98.49	0.9722	1.754
		61.51	60.49 ± 1.039	98.34	1.039	1.723
		70.29	70.14 ± 1.301	99.79	1.301	1.860

a: mean value of five replicates

**TABLE 3. Between – day precision of the determination of Tret and Itret using I<sub>2</sub> and Taz with Rbng reagent.**

RSD %*	SD*	recovery %	[Conc. found] µg mL <sup>-1</sup>	[Conc. taken] µg mL <sup>-1</sup>	Reagent	Compound
1.990	0.2867	99.97	14.42 ± 0.2867	14.42	I <sub>2</sub>	Tret
1.909	0.3286	99.07	17.26 ± 0.3286	17.43		
1.346	0.2968	97.54	21.98 ± 0.2968	22.53		
1.895	0.4890	97.51	25.78 ± 0.4890	26.44		
1.629	0.4663	100.0	28.56 ± 0.4663	28.54		
1.144	1.11	103.4	93.21 ± 1.11	90.13	I <sub>2</sub>	Itret
0.7465	0.7886	100.6	105.8 ± 0.7886	105.2		
1.265	1.398	99.98	111.1 ± 1.398	111.2		
1.500	1.731	100.1	115.4 ± 1.731	115.4		
1.253	1.473	98.90	118.9 ± 1.473	120.2		
1.862	0.5247	100.5	28.27 ± 0.5247	28.12	Rbng	Taz
1.427	0.5117	102.1	35.87 ± 0.5117	35.15		
1.516	0.7868	98.41	51.88 ± 0.7868	52.72		
1.789	1.056	98.82	59.04 ± 1.056	59.75		
1.725	1.128	97.95	65.41 ± 1.128	66.78		

a: mean value of five replicates.

**TABLE 4. Application of the proposed method to the determination of the studied drugs in its pharmaceutical preparations.**

Official method	Proposed method		Sample
100.11 ± 0.75 <sup>(5)</sup>	101.9 ± 1.160	X ± SD <sup>a</sup>	Tret
	2.367 (2.447) **	t-Value <sup>b</sup>	Acetin cream (30 g cream <sup>-1</sup> )
	2.392 (6.94) **	F-Value <sup>b</sup>	
101.5 ± 1.09 <sup>(8)</sup>	100.3 ± 2.6	X ± SD <sup>a</sup>	Itret
	1.658 (1.833) **	t-Value <sup>b</sup>	Isotretinoin capsule
	4.368 (5.19) **	F-Value <sup>b</sup>	
100.28 ± 2.43 <sup>(16)</sup>	101.0 ± 3.351	X ± SD <sup>a</sup>	Taz
	0.3206 (2.447) **	t-Value <sup>b</sup>	Acnitaz gel (15 g gel <sup>-1</sup> )
	1.905 (19.2) **	F-Value <sup>b</sup>	

a: Mean values for five replicates, b: mean value of 3, 6 and 3 for the three official methods respectively \*\*the values between brackets are the tabulated F- and t-values at P = 0.05.

### References

- Siddiqui, M.R., Alothmana, Z.A. and Rahman, N., Analytical techniques in pharmaceutical analysis: A review. *Arabian Journal of Chemistry* **10** (1), 1409–21 (2017)
- Tehrani, M. B., Namadchian, M., Vatan, S. F. and Souri, E., Derivative spectrophotometric method for simultaneous determination of clindamycin phosphate and tretinoin in pharmaceutical dosage forms. *DARU J. of Pharmaceutical Sciences*, 21-29 (2013).



3. Gupta, A., Gulati, M. and Pandey, N. K., A validated UV spectrophotometric method for simultaneous estimation of tretinoin and benzoyl peroxide in bulk and semi-solid dosage form. *RJC*, **2** (3), 649-54 (2009).
4. Sheliya, K., Shah, K. and Kapupara, P., Development and validation of analytical method for simultaneous estimation of mometasone furoate, hydroquinone and tretinoin in topical formulation by RP-HPLC. *Journal of Chemical and Pharmaceutical Research*, **6** (4), 934-940 (2014).
5. Elzanfaly, E. S., Saad, A. S. and Abd-Elaleem, A. B., Simultaneous determination of retinoic acid and hydroquinone in skin ointment using spectrophotometric technique (ratio difference method). *Saudi Pharmaceutical Journal*, **20**, 249-53, (2012).
6. Pankti D., Kusum, M. and Mehul, P., Development and validation of UV-Visible spectrophotometric method for simultaneous estimation of Mometasone Furoate, Hydroquinone and Tretinoin from their pharmaceutical dosage form. *Int. J. Pharm. Sci. Rev. Res.*, **21**(1), 296-30 (2013).
7. Bordbar, M., Yeganeh-Faal, A., Ghasemi, J., Ahari-Mostafavi, M.M., Sarlak, N. and Baharifard, M.T., Simultaneous spectrophotometric determination of minoxidil and tretinoin by the H-point standard addition method and partial least squares. *Chemical Papers*. **63** (3) 336-44 (2009).
8. Patel, P., Kabra, P., Kimbahune, R. and GH, U. Quantitative estimation of isotretinoin (13-cis retinoic Acid) in bulk and formulation by UV - visible spectrophotometry. *RJPBCS*, **2** (1), 167-172 (2011).
9. Waghmare, N., Waghmare, P., Wani, S. and Yerawar, A., Development of Isotretinoin Gel for the Treatment of Acne Vulgaris. *RJPBCS*, **2**(1),220-230 (2011).
10. Kane, M. A., Folias, A.E. and Napoli, J. L., HPLC/UV quantitation of retinal, retinol, and retinyl esters in serum and tissues. *Anal. Biochem* **378**, 71-79 (2008).
11. Diniz, D. G. A., Alves, C. P. I., Castro, N. C., Rodovalho, L. F. F., Benfca, P. L., Valadares, M.C. and Lima, E. M., Isotretinoin-Containing Liposomes: Obtention, Characterization and In Vitro Cytotoxicity on Leukemia Cells. *Applied Cancer Research* , **28** (3):106-12 (2008).
12. Patel, M. R., Patel, R. B., Parikh, J. R., and Patel, B. G., Improving the Isotretinoin Photostability by Incorporating in Microemulsion Matrix. *ISRN Pharmaceutics*, 1-6 (2011).
13. Vasanthi, R., Rajitha, N., Raja, M. A., Shrish, V., Banji, D., and Kumar, D. S., Analytical method development and validation of Isotretinoin in tablet dosage formulation. *Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry*. **3**(3), 145 – 53 (2015).
14. Jogarami, R., Jain, P. and Sharma, S., Validated UV spectrophotometric method development for simultaneous estimation of Tazarotene and Hydroquinone in Gel Preparation. *Journal of Pharmacy Research*, **5** (4), 2273-75, (2012).
15. Rawat, D., Gunjan, A., Gupta, M., Singh, S., and Pathak, A. Spectrophotometric and validated rp – hplc method for the estimation of retinoid drug tazarotene in gel Formulation. *AJPER*, **2** (2), 72-89, (2013).
16. Elzanfaly, E.S., Saad, A. and Abd-Elaleem A.B., A novel simple method for resolving overlapped spectral data and Its application as a stability indicating method for Determination of Tazarotene. *Pharmaceut Anal Acta*, **3** (3), (2012).
17. Badawy, A. M., Abd El-Alim-A. B. and Saad, A. S. Stability-indicating spectrophotometric methods for determination of tazarotene in the presence of its alkaline degradation product by derivative spectrophotometric techniques. *Drug Test. Analysis*, **2**, 130-36, (2010).
18. Tashtoush B. M., Jacobson, E. L. and Jacobson, M. K., A rapid HPLC method for simultaneous determination of tretinoin and isotretinoin in dermatological formulations. *J of Pharmaceutical and Biomedical Analysis*, **43**, 859-64, (2007).
19. Schmidt, C. K., Brouwer, A. and Nau, H., Chromatographic analysis of endogenous retinoids in tissues and serum. *Analytical Biochemistry*, **315**, 36-48, (2003).
20. Wu, L., Wu, J., Zhou, K., Cheng, F. and Chen, Y., Determination of isotretinoin in human plasma by high performance liquid chromatography-electrospray ionization mass spectrometry. *J of Pharmaceutical and Biomedical Analysis*, **56**, 324-329, (2011).
21. Guimarães, C. A., Mena, F., Mena, B., Lebrun, I., Quenca-Guillen, J. S., Auada, A.V. V., Mercuri, L. P., Ferreira, P. and Santoro, M. I. R. M.; Determination of isotretinoin in pharmaceutical formulations by reversed-phase HPLC. *jbise*, **3**, 454-58, (2010).
22. Gamble, M. V., Shang, E., Zot, R. P., Mertz Debra, J. R., Wolgemuth, D. J. and Blaner, W. S., Biochemical properties, tissue expression, and gene structure of a short chain dehydrogenase reductase able to catalyze cis-retinol oxidation. *JLR*, **40**, 2279-2292, (1999).
23. Lima, E. M., Diniz, D. G. A. and Antoniosi-Filho, N. R., Development of a gas chromatography method for the determination of Isotretinoin and *Egypt. J. Chem.* **61**, No.1 (2018)

- its degradation products in pharmaceuticals. *J. Jpba*, **38**, 678–85, (2005).
24. Patel, P., Kimbahune, R., Kabra, P., Delvadiya K. and Nargund L.V.G., Development and validation of reverse phase liquid chromatography method for estimation of isotretinoin (13 -Cis Retinoic Acid) in pharmaceutical dosage form. *Rasayan J.Chem.*, **4**(1), 153-58, (2011).
  25. Guimarães, C.A., Mena, F., Mena, B., Lebrun, J., Quenca-Guillen, J. S., Auada, A.V. V., Mercuri, L. P., Ferreira, P. and Santor M. I. R. M., Determination of isotretinoin in pharmaceutical formulations by reversed-phase HPLC. *J. BiSE*, **3**, 454-58, (2010).
  26. Moghimi, H., Noorani, N. and Zarghi, A., Stereoselective Permeation of Tretinoin and Isotretinoin through Enhancer-Treated Rat Skin. I. Effect of Ethanol and Sodium Dodecyl Sulfate. *IJPR*, **2**, 127-33, (2003).
  27. Moghimi, H., Noorani, N. and Zarghi, A., Stereoselective Permeation of Tretinoin and Isotretinoin through Enhancer-Treated Rat Skin. II. Effects of Lipophilic Penetration Enhancers. *IJPR*, **3**, 17-22, (2004).
  28. Tashtoush, B. M., Jacobson, E. L. and Jacobson M. K., A rapid HPLC method for simultaneous determination of tretinoin and isotretinoin in dermatological formulations. *Journal of Pharmaceutical and Biomedical Analysis*, **43**, 859–64, (2007).
  29. Satyanarayana, P.V.V. and Murali, M., Development and validation of LC method for the estimation of tretinoin in pharmaceutical dosage form. *International journal of research and reviews in pharmacy and applied science*, **1**(1), 2249-1236, (2011).
  30. Roy, C., Chakrabarty, J., Rao, S., Modi, P. B. and Vairale, A., Residue determination of Clindamycin phosphate and Tretinoin on the surface of manufacturing equipment by RP-HPLC. *J. of Pharmacy Research*, **5**(7), 3665-69, (2012).
  31. Roy, C. and Chakrabarty, J., Stability indicating RP-HPLC method development and validation for determination of potential degradation impurities of tretinoin in tretinoin topical pharmaceutical formulation. *Der Pharmacia Sinica*, **4**(4), 6-14, (2013).
  32. Pathare, D. B., Jadhav, A. S. and Shingare, M. S. A Validated Stability Indicating RPLC Method for Tazarotene, *Chromatographia*, **66**(3/4), 247-250, (2007).
  33. Roy, C. and Chakrabarty, J., Development and validation of a Stability-indicating rp-hplc method for the Simultaneous determination of phenoxyethanol Methylparaben, propylparaben, mometasone Furoate, and tazarotene in topical Pharmaceutical dosage formulation. *Sci Pharm*, **81**, 951–67, (2013).
  34. Patel, M. R., Patel, R. B., Parikh, J. R. and Patel, B. G., HPTLC method for estimation of tazarotene in topical gel formulations and in vitro study. *Anal. Methods*, **2**, 275–81, (2010).
  35. Hasani, M. and Mafakheri, N., A Spectrophotometric and Thermodynamic Study of the Charge-Transfer Complexes of Iodine with Nortriptyline and Imipramine Drugs in Chloroform and Dichloromethane Solutions, *Russian J. of physical chemistry*, **10** (6), 929–934, (2016).
  36. Shi, L., Qian, Y., Lin, S., Yang, N., Li, N. and Liu, J., Sodium chlorite-iodine-methyl acetoacetate oscillatory reaction investigated by UV-vis spectrophotometric method, *J Iran chemsoc*, **10**, 21–28, (2013).
  37. S. I. M. Zayed, Two charge-transfer complex spectrophotometric methods for the determination of sulphiride in pharmaceutical formulations, *Cent. Eur. J. Chem.*, **7**(4), 870–875, (2009).
  38. Ramadan, A., Mandil, H. and Alshelhawi, N., Spectrophotometric determination of Rosuvastatin pure form and pharmaceutical formulation by the oxidation using iodine and formation triiodide complex in acetonitrile, *Int J Pharm Sci*, **6** (5), 579-585, (2014).
  39. Kakhki, R.M., Nejati-Yazdinejad, M. and Kakeh, F., Extraction and determination of Rose Bengal in water samples by dispersive liquid-liquid microextraction coupled to UV-Vis spectrophotometry, *Arabian Journal of Chemistry*, **10** (2), 2518–2522, (2017).
  40. El-didamony, A.M. and Hafeez, S.M., Extractive spectrophotometric determination of some Anti histamine drugs from pharmaceutical formulation using Rose Bengal, *International J. Of Pharmacy And Pharmaceuticals Science*, **7** (6), (2015).
  41. Sayed, R.A., Hassan, W.S., El-Mamml, M.Y. and Shalaby, A., New spectrophotometric and conductometric methods for Macrolide antibiotics determination in Pure and Pharmaceutical dosage forms Using Rose Bengal, *Journal of Spectroscopy*, 1-13, (2013).
  42. Britton, H. and Robinson, R., Universal buffer solutions and the dissociation constant of veronal, *J. Chem. Soc.*, 1456–1462, (1931).

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## التقدير الطيفي الدقيق لادوية التريتنوين و الايزوتريتونوين بتفاعلها مع اليود والتازروتين مع الـروزبنجال

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تم دراسة التفاعل بين كاشف اليود او الـروزبنجال مع ثلاثة ادوية من عائلة الـترينويد ( التريتنوين و الايزوترينوين و التازروتين) لاقتراح طرق قياس طيفي تتسم بالسرعة والبساطة والحساسية للتقدير الدقيق لتلك الادوية. تعتمد هذه الطرق على تكوين نواتج للمفاعلات بين تلك الادوية والكواشف.

تم قياس الطيف لنواتج التفاعلات تحت الظروف القياسية المختارة من درجة حرارة (٥٠ و ٤٠ و ٣٠) وزمن اكبر من او يساوى (٢٠ و ٤٠ و ١٠) واس هيدروجيني (٦ و ٢ و ٩) على الترتيب وكذلك حساب المعاملات التحليلية مثل الانحراف المعياري (١,١٠١ - ١,٣١٢) والانحراف المعياري النسبي (% ١,٩٤٦ - ٠,٥٥٥٦) ومعامل حساسية ساندل (٠,٠٢٢٩ - ٠,٠٥٠٨) -ميكرو جرام لكل سنتيمتر مربع وحد الاكتشاف ٥,٧٥٧ - ٤,٣٤٧ وحد التقدير ١٧,٤٥ - ١٣,١٧ ميكرو جرام لكل ملليمتر وذلك لتقدير دقة وحساسية تلك الطرق.

وجد ان قانون بير صالح في نطاق ٢٩,٧١ - ٩٠,٤١ و ١١٩,٦٥ - ٣٥,٧٥ , ٧١,١١ - ١٠,٤٨ مجم لكل ملليمتر بنسبة استرداد (٩٧,٨٤) - (١٠٢,٨) و (٩٨,٦٧) - (١٠١,٨) و (٩٨,٣٢ - ١٠٢,٠) على الترتيب.

تنشئ اهمية هذا البحث من وجود العديد من التطبيقات لتلك الادوية كأدوية لعلاج الأمراض الجلدية , نجحت الطرق المقدمة في تحليل الرتيندات المقترحة في حالتها الخام ومستحضراتها الصيدلانية ووجد انها تتفق احصائياً مع الطرق المنشورة (بواسطة اختبارى T و F).