13

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Spectrophotometric Microdetermination of Tretinoin, Isotretinoinusing Iodine and Tazarotene Microdetermination Via Reaction with Rose-Bengal Reagent

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THE 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 μg cm^{-2}, LOQ = 13.17 – 17.45 μg mL^{-1}, LOD = 4.347 – 5.757 μg 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 μg 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 chemicalstructures of these retinoid drugs are shown in Fig.1.

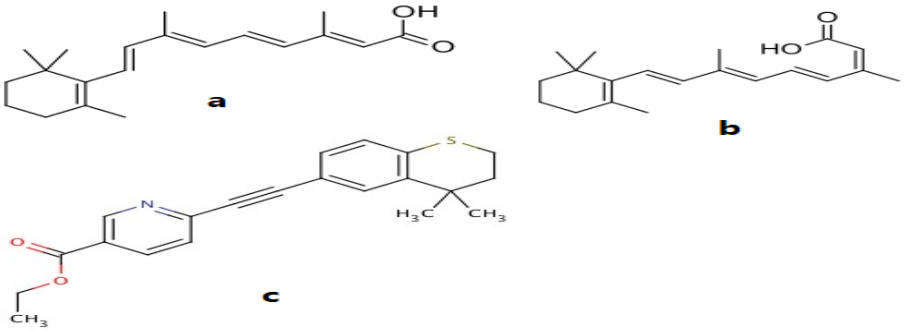

Fig.1. the chemical structures of (a) Tret, (b) Itret and (c) Taz.

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^{-1}) and Itret (M.wt = 300.44 g mol^{-1}) were provided by Medzen pharmaceutical industries Company, Cairo, Egypt. The standard Taz(M.wt = 351.436 g mol^{-1}) was provided by Delta pharma quality assurance department, Egypt. The reagents used were Rbng disodium salt and Iodine (I_2) (Mw.t 253.81 g mol^{-1}) 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. Acretin 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 labeledto contain (20 mg Itret $capsule^{-1}$).

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: VELP-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 x 10^{-3} M I_2 (M. Wt. = 253.81 g mol^{-1}), was prepared by dissolving accurate weight (0.0126 g) and complete the volume by ethanol 95% in 100 mL volumetric flask. Solution of 1x10^{-3} M Rbng reagent (M. Wt. = 1017.64 g mol-1), was prepared by dissolving weight of (0.0.0508 g) in appropriate volume of distilled water and the volume completed to 50 mL. Solutions of 1 X 10^{-3} M (300.44µg ml^{-1}) 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 X 10^{-3} M (351.46 µg mL^{-1}) 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 x 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 (2x10^{-4}M); while that of the drug within the range (0.3 - 2 x10^{-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 λ_{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 resultingTHF layer was transferred to 25 ml measuring flask and the volume completed to the mark with ethanol 95 % leading to 0.5x10^{-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- 1.95mL of 0.5 x 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 mLof 2 x 10^{-3} M taken from this solution. Then 2ml buffer of pH = 2 was added. The appeared turbidity was overcome byaddition 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 x 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 x10^{-3} M was added to variable conc of Taz 0.5 – 1.6 x10^{-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

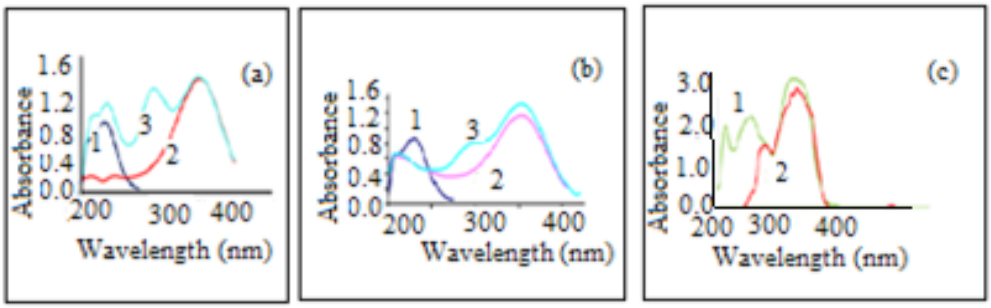

Fig.2. UV Absorption spectra of (a) 1- 1 x 10^{-4} M I_2, 2 – 1 x 10^{-4} M Tret, 3-Product of 1 x 10^{-4} M Tret and I_2 using 1 x 10^{-4} M I_2 as a blank (b) Absorption spectra of: 1- 1 x 10^{-4} M I_2, 2 – 1 x 10^{-4} M Itret, 3-Product of 1 x 10^{-4} M Itret and I_2 using 1 x 10^{-4} M I_2 as a blank. (c) Absorption spectra of: 1- 1.3 x 10^{-4} M Taz, 2- 1.3 x 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 be20, 40 and 10 minutes for Tret, Itret and Taz respectively.

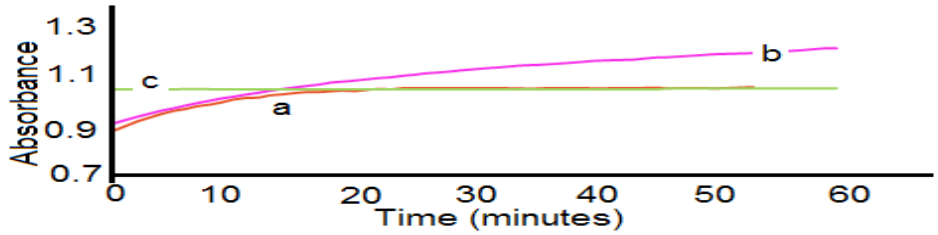

Fig. 3. Effect of Time on reaction product of : a- 1x10^{-4} M Tret and I_2 at Wavelength = 295 nm, b- 2x10^{-4} M Itret and I_2 at Wavelength = 295 nm, c- 2x10^{-4} M Taz and Rbng at Wavelength = 285 nm.

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).

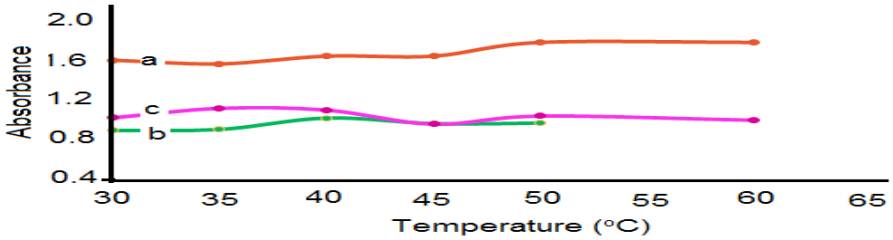

Fig.4. Effect of Temperature on reaction product of: a- 2x10^{-4} M Tret and I_2 at λ_{max} = 295 nm, Time = 25 min, b- 2x10^{-4} M reaction product of Itret and I_2 at λ_{max} = 295 nm, Time = 45 minutes, c- 2x10^{-4} M reaction product of Taz and Rbng at λ_{max} = 285 nm, Time = 10 minutes

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 λ_{max} = 295nm, or 285nm . 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.

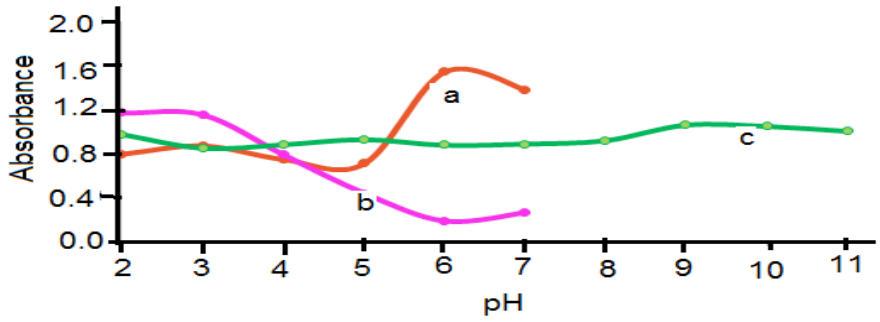

Fig. 5. Effect of pH on absorption spectrum of : a- 1x10^{-4} M Tret and I_2 reaction product at 295 nm, time = 25 min and temperature = 50 °C, b- 2x10^{-4} M Itret and I_2 reaction product at 295 nm, time = 45 minutes, c- 0.8x10^{-4} M Taz and Rbng reaction product at 285 nm, time =10 minutes and Temp = 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 X 10^{-4} M), was added to constant drug concentration (1 X 10^{-4}) M of Tret. For Itret method, avariable reagent concentration of Itret (1-14 X 10^{-4}M), was added to constant I_2 concentration (5 X 10^{-4}) M. For Taz variable drug concentration was added to constant reagent conc 2x10^{-4}M. The spectrophotometric measurements of these solutions were recorded at λ_{max} = 295 nm or 285 nm (Fig 6.).

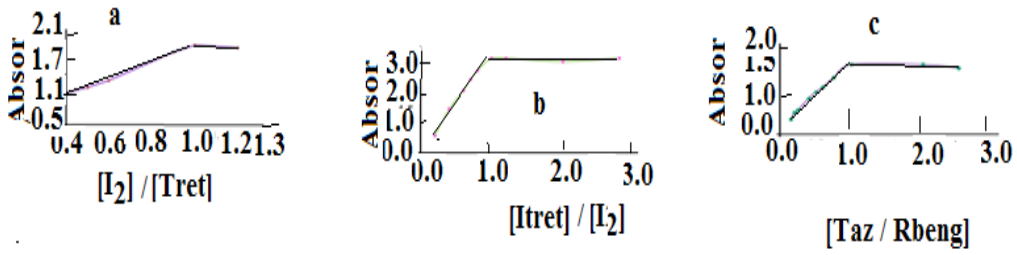

Fig. 6. (a) Molar ratio of Tret and I2 at Wavelength = 295 nm, time = 25 minutes and temp = 50 °C, (b). Molar ratio of Itret and I_2 at wavelength = 295 nm, time = 45 minutes and temp = 40 °C, (C). Molar ratio of Taz and Rbng at wavelength = 285 nm, time = 10 minutes, temp = 30 °C and pH = 9.

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 prper condition.

Parameters			
Drug	Tret	Itret	Taz
Reagent	I_2	I_2	Rbng
Time (minutes)	20 – 60	40 - 60	10 - 60
Temperature (°C)	50	40	30
λmax (nm)	295	295	285
pH	6	2	9
Beer's law (µg mL^{-1})	9.041 – 29.71	- 119.635.57	71.1110.48 –
LOD (µg mL^{-1})	4.347	5.757	4.825
LOQ (µg mL^{-1})	13.17	17.45	14.62
R^2	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 x 10^4 ($L\ mol^{-1}cm^{-1}$) X 10^4	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^{-2})	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^2). 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 methodsare 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_2 and Taz with Rbng reagent

Compound	Reagent	[Conc. taken] µg mL^{-1}	[Conc. found] µg mL^{-1}	Recovery %	SD*	RSD %*
Tret	I_2	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_2	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_2 and Taz with Rbng reagent.

RSD %*	SD*	recovery %	[Conc. found] µg mL^{-1}	[Conc. taken] µg mL^{-1}	Reagent	Compound
1.990	0.2867	99.97	14.42 ± 0.2867	14.42	I_2	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_2	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 [5]	101.9 ± 1.160	X ± SD[a]	Tret
	2.367 (2.447) **	t-Value[b]	Acretin cream (30 g $cream^{-1}$)
	2.392 (6.94) **	F-Value[b]	
101.5 ± 1.09 [8]	100.3 ± 2.6	X ± SD[a]	Itret
	1.658 (1.833) **	t-Value[b]	Isotretinoin capsule
	4.368 (5.19) **	F-Value[b]	
100.28 ± 2.43 [16]	101.0 ± 3.351	X ± SD[a]	Taz
	0.3206 (2.447) **	t-Value[b]	Acnitaz gel (15 g gel^{-1})
	1.905 (19.2) **	F-Value[b]	

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.

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التقدير الطيفي الدقيق لادوية التريتنوين و الايزوتريتونوين بتفاعلها مع اليود والتازروتين مع الروزبنجال

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تم دراسة التفاعل بين كاشف اليود او الروزبنجال مع تلاثة ادوية من عائلة الرتينويد (التريتنوين والايزوترنينوين والتازروتين) لاقتراح طرق قياس طيفى تتسم بالسرعة والبساطة والحساسية للتقدير الدقيق لتلك الادوية, تعتمد هذه الطرق على تكوين نواتج للمتفاعلات 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 (٢٠و ٤٠ و ١٠) واس هيدروجينى(٦ و ٢ و ٩) على الترتيب وكذلك حساب المعاملات التحليليـــة مثـــل الانحراف المعيارى (١,١٠١ - ٠,١٣١٢) والانحـــراف المعيارى النسبى (٪ ١,٩٤٦ - ٠,٥٥٥٦) ومعامل حساسية ساندل٠,٠٢٢٩ - ٠,٠٥٠٨ -ميكرو جرام لكل سنتيمتر مربع وحد الاكتشاف٥,٧٥٧ – ٤,٣٤٧ وحد التقدير ١٧,٤٥ – ١٣,١٧ ميكروجرام لكل مليمتر وذلك لتقدير دقة وحساسية تلك الطرق .

وجد ان قانون بير صالح فى نطاق ٢٩,٧١ – ٩,٠٤١و١١٩,٦ – ٣٥,٧٥ , ٧١,١١ – ١٠,٤٨ مجم لكل مليمتر بنسبة استرداد(٩٧,٨٤)-(١٠٢,٨ و٩٨,٦٧)-(١٠١,٨ و ٩٨,٣٢- ١٠٢,٠) على الترتيب.

تنشئ اهمية هذا البحث من وجود العديد من التطبيقات لتك الأدوية كأدوية لعلاج الأمراض الجلدية , نجحت الطرق االمقدمة فى تحليل الرتينات المقترحة فى حالتها الخام ومستحضراتها الصيدلانية ووجد انها تتفق احصائياً مع الطرق المنشورة (بواسطة اختبارىT و F).