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Stability-indicating spectrophotometric methods for determination of tazarotene in the presence of its alkaline degradation product by derivative spectrophotometric techniques

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The stability of tazarotene (TZ) was investigated and two stability-indicating methods – namely, first derivative and a derivative ratio spectrophotometric method – were used to determine tazarotene in the presence of its alkaline degradation product (HD) using methanol as a solvent. A linear relationship was obtained in the range 1–10 μg ml^{-1} for both methods. By applying the proposed methods, it was possible to determine tazarotene in its pure powdered from with accuracy 99.35±1.410 (n = 10) for the first derivative method and 99.45 ± 1.053 (n = 10) for the derivative ratio method. First derivative and derivative ratio methods were used for the analysis of laboratory-prepared mixtures containing different ratios of tazarotene and its degradation product and they were valid in the presence of up to 70% and 80% degradation product, respectively. The proposed methods were validated and found to be suitable as stability-indicating assay methods for tazarotene in pharmaceutical formulations.

Keywords: first derivative; ratio derivative; stability-indicating methods; tazarotene

Introduction

Tazarotene 6-[(3,4-Dihydro-4,4-di-methyl-2H-1-benzothiopyran-6-yl)ethynyl]-3-pyridinecarboxylic acid ethyl ester, a white solid third-generation retinoid approved for the treatment of psoriasis and acne vulgaris.[1] In mice, tazarotene blocks ornithine decarboxylase enzyme activity, which is associated with cell proliferation and hyperplasia. In cell culture, it suppresses markers of epidermal inflammation and inhibits cornification of the keratinocytes.[2]

Tazarotene gel may be used as monotherapy or in combination with other medications, such as topical corticosteroids, for the treatment of localized plaque psoriasis. This is the first topical retinoid approved by the Food and Drug Administration (FDA) for the treatment of psoriasis. Side effects of burning, itching, and skin irritation are relatively common, and patients should avoid sun exposure.[2]

Despite of the wide application of this dosage form in the treatment of psoriasis and acne, a literature survey reveals that there is only one stability-indicating method reported for the determination of TZ applying HPLC technique.[3]

Derivative spectrophotometry is an analytical technique of good utility and offers background correction and better selectivity than normal spectrophotometry for resolving binary mixtures and some ternary mixtures.[4–9]

Another method for resolving binary mixtures without previous separation is the derivative ratio spectrophotometry, which was developed by Salinas *et al*.[10] In this method, the absorption spectrum of the mixture (absorbance at each wavelength) is divided by the absorption spectrum of a standard solution of one of the components, and the first derivative of the ratio spectrum is obtained. The concentration of the other component is then determined from a calibration graph. In this method, overlap of the spectra in a certain region is desirable, because on dividing of one spectrum by another, the error increases when one of the absorbances approaches zero.[10] This method permits the use of the wavelength of greatest sensitivity as the signal of measurements, either a maximum or a minimum. This method has been applied to the determination of binary mixtures.[10–13]

The aim of the present work is to develop feasible, sensitive and specific analytical procedures for the analysis of the investigated TZ in presence of its alkaline degradation product. Adaptation of the proposed procedures to the analysis of the available dosage form is also an important task.

Experimental

Apparatus

Spectrophotometer: SHIMADZU UV- 1601 PC, dual beam UV-visible spectrophotometer with two matched 1-cm quartz cells, connected to an IBM compatible personal computer (PC) and a HP-600 inkjet printer. Bundled UV-PC personal spectroscopy software version (3.7) was used to process the absorption and the derivative spectra. The spectral band width was 0.2 nm with wavelength scanning speed of 2800 nm.min^{-1}.

GC-MS: SHIMADZU GC-MS-QP 1000EX, composed of gas chromatographer (GC-14A) and mass spectrometer of electron voltage (70 ev). The GC-MS conditions: column; polyethylene

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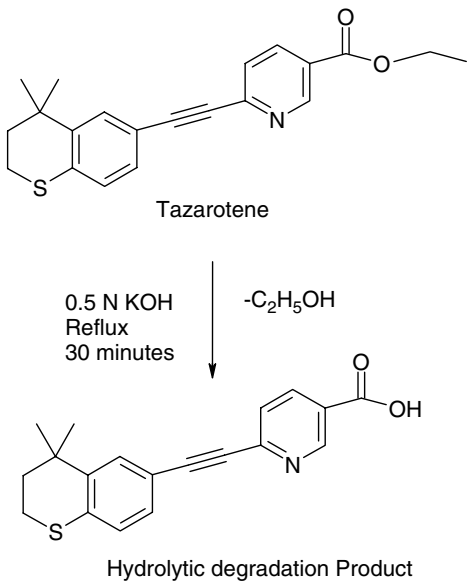

Figure 1. Reported pathway for the alkaline degradation of TZ.[3].

glycol(At.wax), mobile phase; helium gas. Temprature program: initial temp:120 °C, initial time: 1 min, program rate: 10 °C min^{-1}, final temp: 210 °C. Injector: 250 µl, Detector temp: 250 °C.

Pure samples

Pure Tazarotene was kindly supplied by Marcyrl Pharmaceuticals and Chemical Industries Company (Cairo, Egypt). Its purity was certified to be 99.89 ± 0.691.

Market samples

Acnitaz® gel, labelLed to contain 0.1 g % Tazarotene, batch number 85332, manufactured by Marcyrl Pharmaceuticals and Chemical Industries Company (Cairo, Egypt). All chemicals used were of analytical grade and solvent are of spectroscopic grade.

1- Methanol (Adwic).
2- Potassium hydroxide (1 M methanolic solution) (Adwic).

Procedures

(*D*) *Preparation of the degradation product of tazarotene*

Fifty mg of pure TZ was accurately weighed and dissolved in 25 ml methanol and 25 ml 1 M KOH, then reflux the solution at 100 °C for

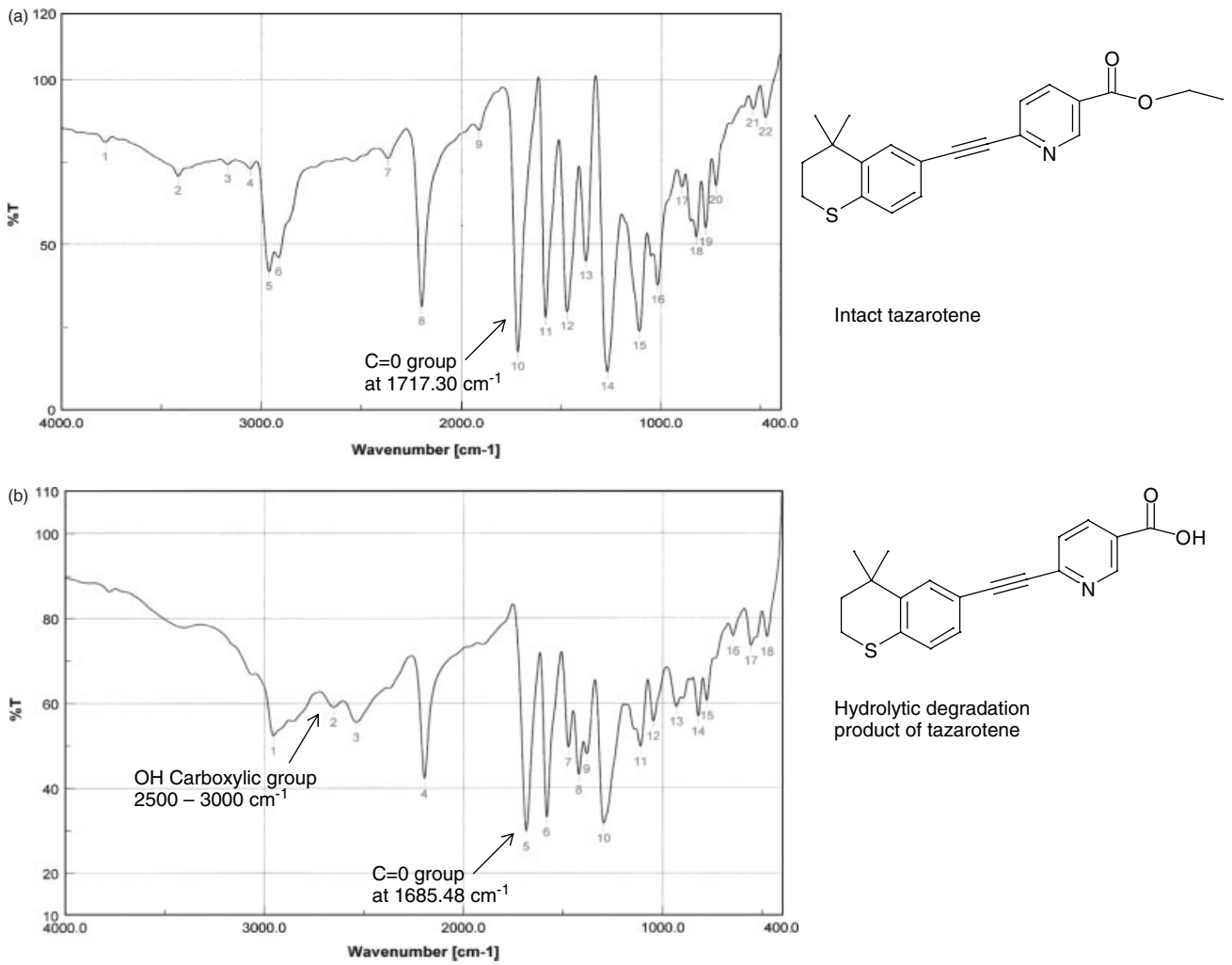

Figure 2. IR-spectra of (A) intact TZ and (B) its Hydrolytic degradation product (HD).

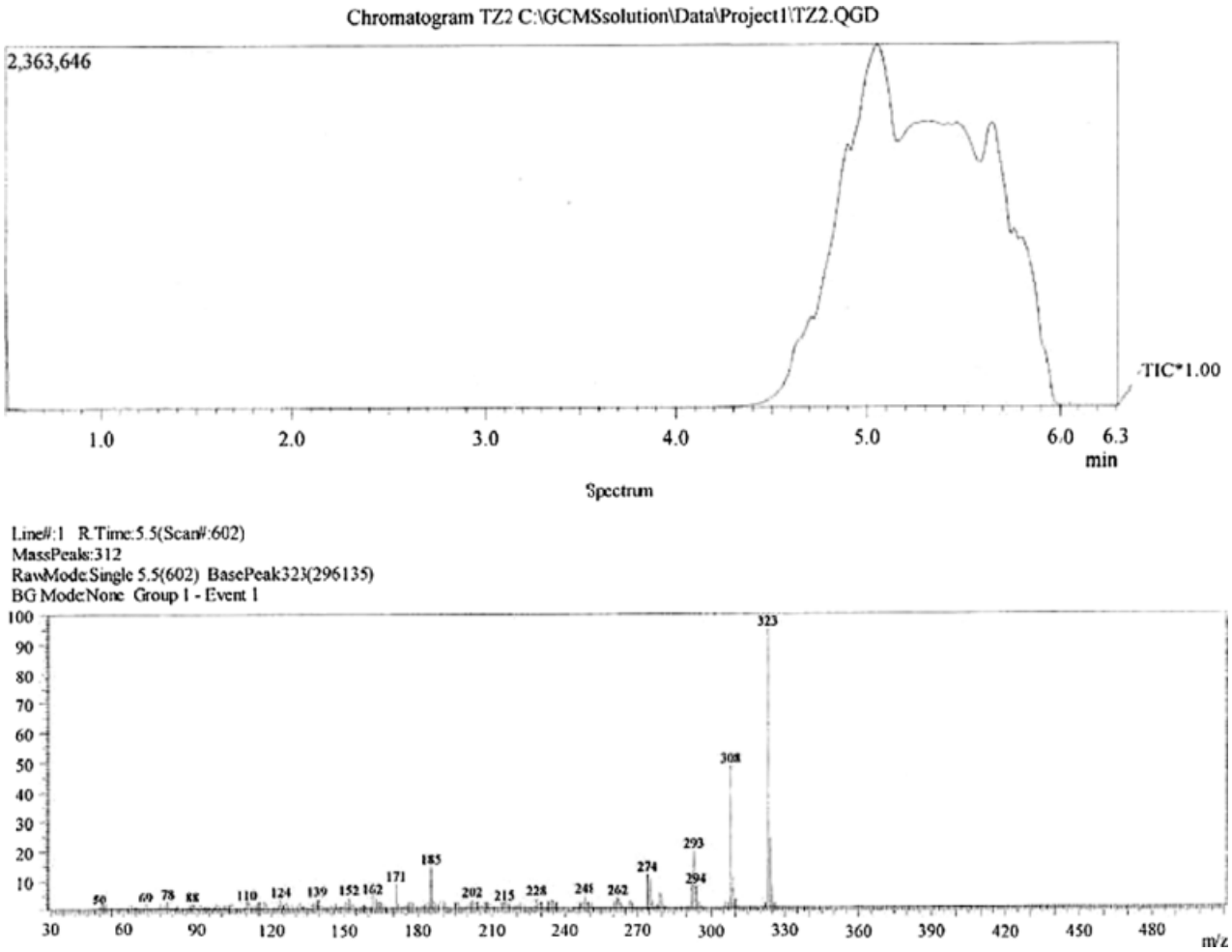

Figure 3. Mass spectrum of hydrolytic degradation product showing a peak at m/z = 323 corresponding to the molecular weight of HD.

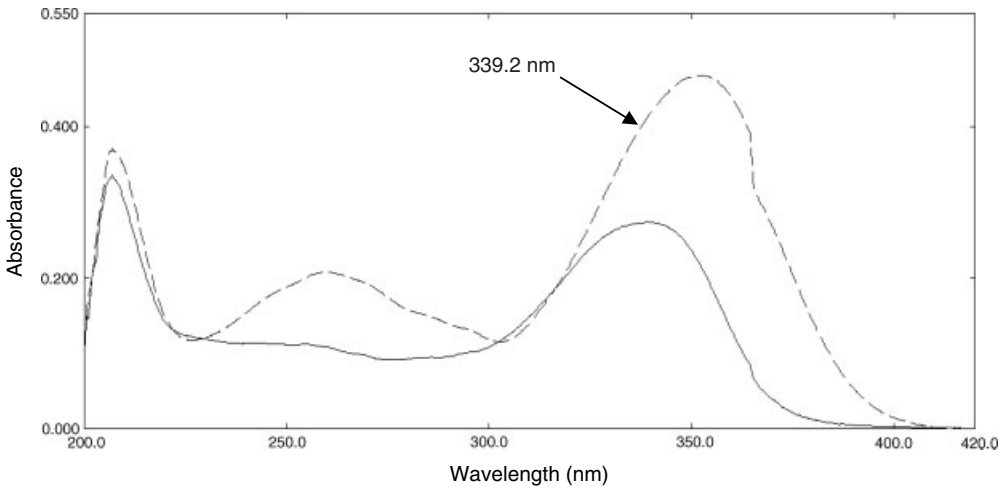

Figure 4. Zero order absorption spectrum of TZ 5 μg/ml (- - - -) and 5 μg/ml HD (———) using methanol as blank.

30 min. The time required for complete degradation was followed by spotting on TLC plates at 5-min intervals for 30 min. The plates was developed using benzene: chloroform: ammonia (5 : 5 : 0.01 by volume) till complete degradation.

The degraded solution was applied as a band onto several preparative TLC plates. The plates were developed using the aforementioned solvent system in chromatographic tank previously saturated for 30 min with the developing solvents and then dried in air.

The bands were visualized under UV light at 254 nm and then scraped and the silica suspended in the least amount of methanol. It was then filtered and the filtrate was left to dry at room

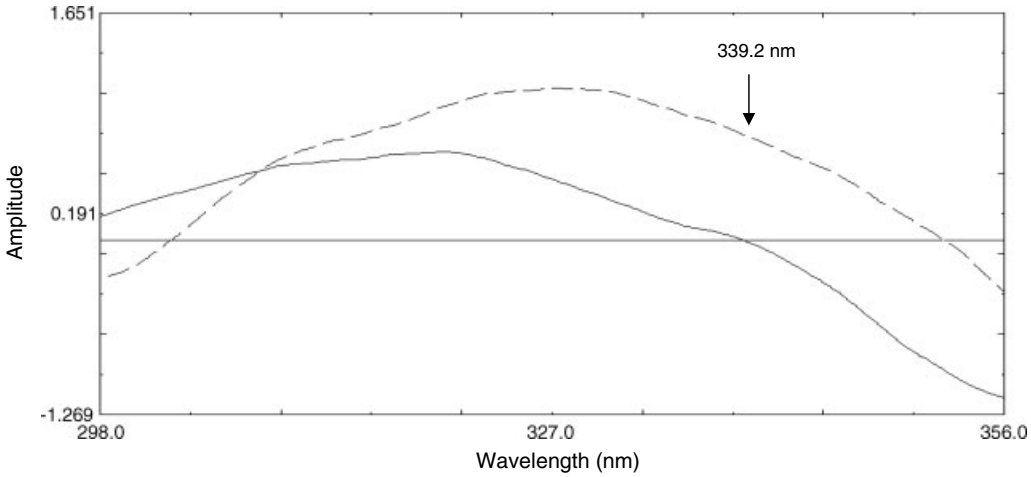

Figure 5. First derivative absorption spectra of TZ 5 μg/ml (- - - -) and 5 μg/ml HD (———) using methanol as blank.

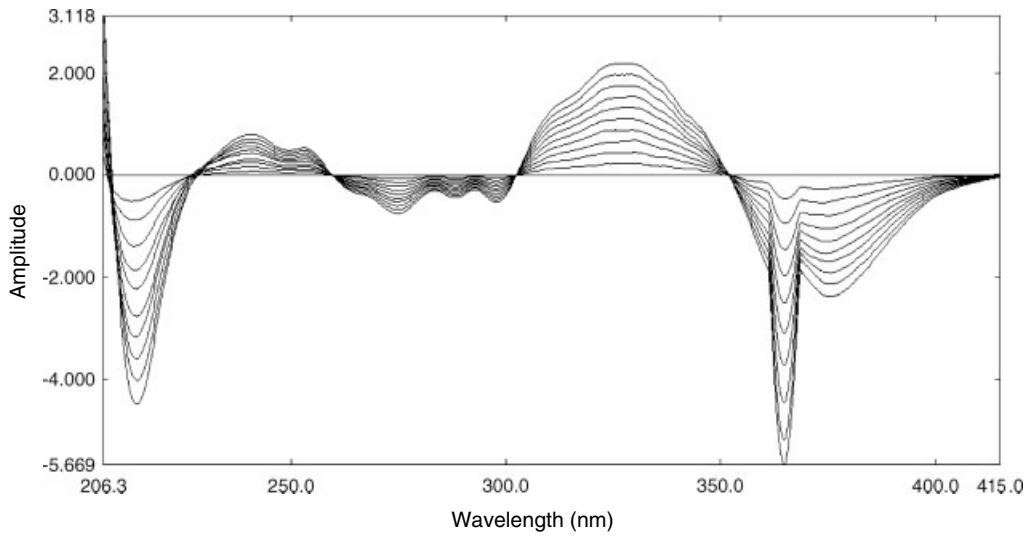

Figure 6. First derivative absorption spectra of TZ 1 – 10 μg/ml using methanol as a blank.

temperature (25 °C) to obtain the degradation product. The purity of the degradation product obtained was tested by dissolving a small portion in methanol, applying to TLC plates and developing using the previously mentioned solvent system. The structure of the isolated degradation product was elucidated using IR and mass spectrometry.

Standard stock solutions

1. TZ standard stock solution; 20.0 μg/ml in methanol
2. HD standard stock solution; 20.0 μg/ml in methanol

Spectral characteristics of TZ and its alkaline degradation product

Into two separate 10-ml volumetric flasks, aliquots containing 50 μg of TZ and 50 μg of HD were separately transferred from their respective stock solutions (20.0 μg/ml), and the volume was completed to mark with methanol. The zero-order (D_0) absorption spectrum of each solution was recorded against methanol as a blank.

Construction of calibration curve

For (^1D): Accurately aliquots of 0.5, 1, 1.5, 2.0, 2.5, 3, 3.5, 4.0, 4.5 and 5.0 ml of TZ stock solution (20 μg ml $^{-1}$) were transferred into 10 ml volumetric flasks then completed to volume with methanol. The peak amplitude of the obtained first derivative spectra was measured at 339.2 nm ($\Delta\lambda = 4$ nm). A calibration curve relating the amplitude of the first derivative curve at 339.2 nm to the corresponding concentrations of TZ was constructed.

For (^1DD): Accurately aliquots of 0.5, 1, 1.5, 2.0, 2.5, 3, 3.5, 4.0, 4.5 and 5.0 ml of TZ stock solution (20 μg ml^{-1}) were transferred into 10 ml volumetric flasks then completed to volume with methanol.

Into a 10-ml volumetric flask, 2.0 ml of the alkaline degradate (HD) stock solution (20 μg ml^{-1}) were transferred accurately then completed to volume with methanol. The spectra of the prepared standard solutions were scanned from 200 to 450 nm and stored in the computer.

For the determination of TZ in presence of its degradation product, the stored spectra of TZ (absorbance at each wavelength) were divided by the spectrum of 6 μg ml^{-1} HD, then the first derivative of the ratio spectra (^{1}DD) with $\Delta\lambda = 4$ nm was obtained. The amplitude of the first derivative peak of (TZ/HD) was measured

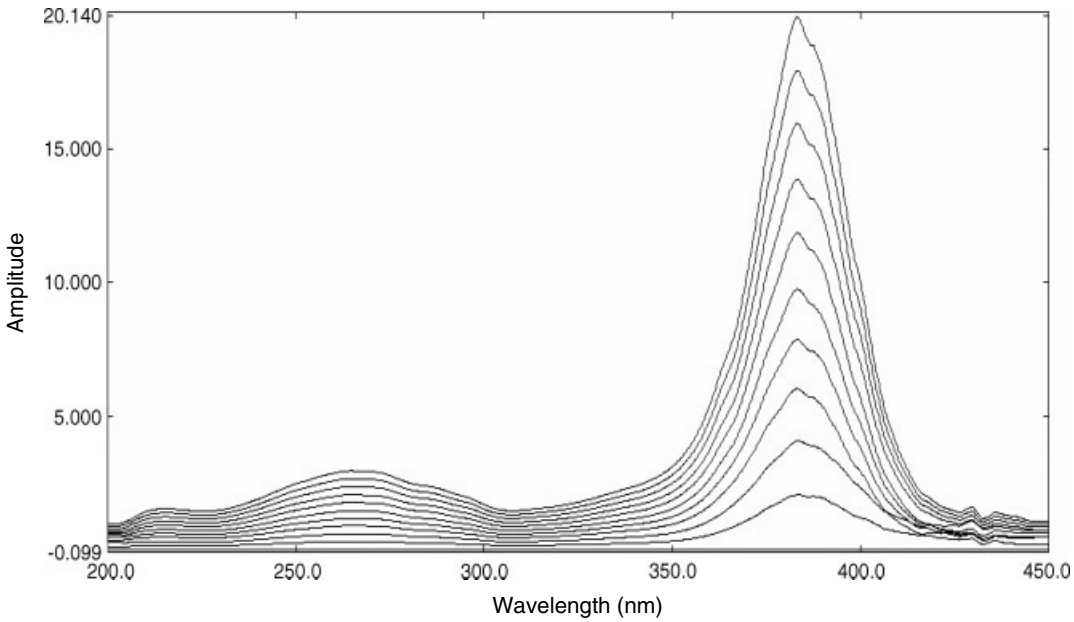

Figure 7. Ratio spectra of TZ (1–10µg/ml) using 4 µg/ml of HD as a divisor and methanol as blank.

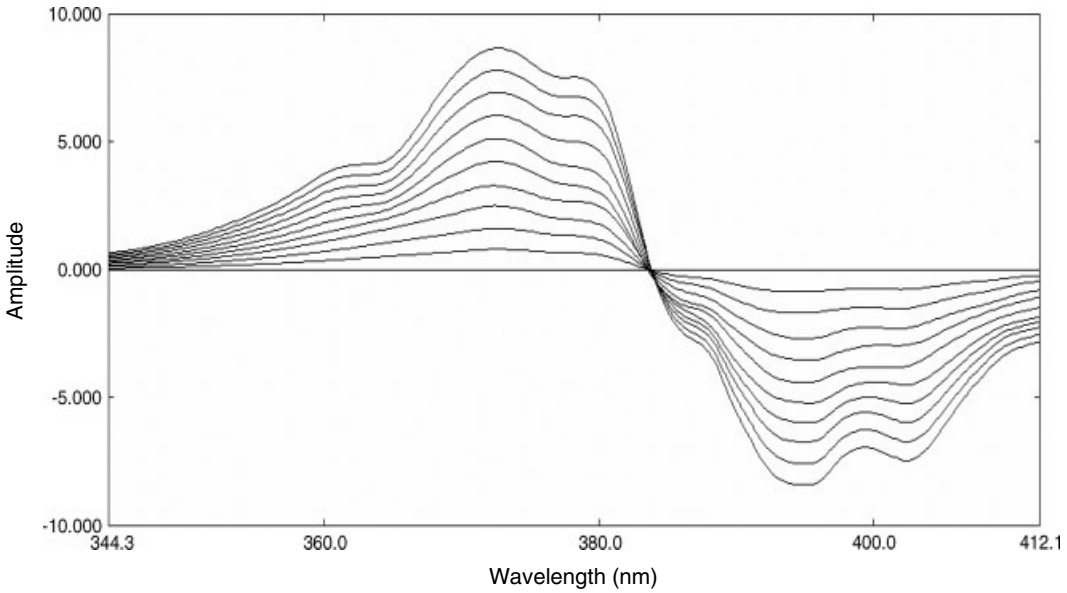

Figure 8. First derivative of ratio spectra of TZ (1–10 µg/ml) using 4 µg/ml of HD as a divisor and methanol as blank.

at 395.0 nm [the λ of zero crossing with the first derivative peak of degradation product].

A calibration graph relating the peak amplitude at 395.0 nm to the corresponding concentrations in µg ml^{-1} of TZ was constructed.

Application of the D_1 and DD_1 methods for the determination of TZ in Acnitaz gel

0.5 gm gel was accurately weighed into a 100-ml beaker and sonicated in 15 ml methanol for 10 min and then filtered into 50-ml volumetric flask. The residue was washed three times using 10 ml methanol and completed to the mark with the same solvent. 4 ml of the extracted solution was accurately transfered into a 10-ml measuring flask. The process was completed as described under the section: *Results and Discussion*.

GC-MS conditions

The alkaline degradation product was analyzed by the GC-MS adopting the conditions described under the section: *Apparatus*.

Results and Discussion

Recently TZ has been approved as a prescription topical retinoid sold as a gel for treatment of psoriasis, acne, and sun-damaged skin (photodamage).

Degradation was not observed in TZ during stress conditions like UV light (254 nm), heat (60 °C) and in an acid (0.5 N HCl) while

Table 1. Determination of TZ in laboratory prepared mixtures by the proposed spectrophotometric methods

	Concentration (μg ml^{-1})		D_1 method	DD1 method
Degradation product %	TZ	HD	Recovery %	Recovery %
10	9	1	100.32	100.05
20	8	2	100.54	100.12
30	7	3	99.12	100.09
40	6	4	99.93	99.95
50	5	5	100.75	100.32
60	4	6	100.57	100.37
70	3	7	101.04	100.41
80	2	8	105.78*	100.79
90	1	9	111.35*	103.77*
Mean			**100.32**	**100.26**
S.D.			**0.633**	**0.269**
RSD%			**0.631**	**0.269**

* Rejected.

TZ was degraded into HD during alkaline hydrolysis with 0.5 N NaOH (3).

In this study, TZ was degraded by refluxing in 0.5 N KOH (methanolic solution) and the degradation process was monitored by spotting on TLC plates at 5-min intervals and developing using chloroform : benzene : ammonia (5 : 5:0.01 by volume) as a developing solvent.

It was found that complete degradation of TZ occurs after 30 min of reflux with 0.5 N KOH and the reported pathway for degradation is represented in Figure 1. The degradation product was separated and its structure elucidated by IR spectrometry. The IR spectra of intact TZ and its alkaline degradate (Figures 2A and 2B) show that the characteristic broad band at 2500 to 3000 cm^{-1} corresponding to OH carboxylic group which is not present in the spectrum of intact TZ (Figure 2A) appeared in the IR spectrum of its alkaline degradation product (Figure 2B). The proposed structures of HD was further confirmed by the mass spectrometry where the GC-MS chart shows the parent peak at m/z = 323 corresponding to the molecular weight of HD, (Figure 3), thus confirming the previously reported mechanism of degradation [3].

Potassium hydroxide was chosen for alkaline hydrolysis instead of NaOH owing to its higher solubility in methanol, since the aqueous solutions cause TZ to precepitate due to its relatively high lipophilicity and low water solubility. Different concentrations of potassium hydroxide (methanolic solution) and different time periods of heat were tested; thin layer chromatography was applied and the optimum conditions were found to be as mentioned under preparation of the alkaline dgradation product.

The absorption spectra of TZ and HD (Figure 4) show severe overlap that does not allow the use of direct spectrophotometric analysis of TZ in the presence of its alkaline degradation product. Upon examining their first derivative spectra (Figure 5) it was noticed that TZ can be determined at wavelength about 339.2 nm, where HD has no contribution (zero crossing). A linear relationship was obtained in the range 1–10 μg ml^{-1} for TZ (Figure 6). The corresponding regression equation was computed and found to be:

$$P = 0.1513\,C + 0.0053 \quad r = 0.9998 \text{ at } \lambda 339.2 \text{ nm}$$

where C is the concentration of TZ in μg ml^{-1}, P is the amplitude of the first derivative curve at 339.2 nm for TZ and r is the correlation coefficient. The proposed method was found to be valid in the range 1–10 μg ml^{-1} for TZ, as shown by the small intercept and correlation coefficient approaching unity.

The derivative ratio method permits the determination of components in mixtures at wavelengths corresponding to a maximum or minimum. The values at these points sometimes permit better sensitivity and better accuracy.[14] The main parameters that affect the shape of the derivative ratio spectra are wavelength, scanning speed, the concentration of the standard solution used as a devisor, wavelength increment over which the derivative is obtained ($\Delta\lambda$), and the smoothing function. The ratio spectra presented in Figure 7 and the first derivative of the ratio spectra presented in Figure 8 may provide good proof for this understanding. The effect of wavelength scanning speed was studied. It was found that at a high speed, noisy spectra were obtained while at low scanning speed, the noise was

Table 2. Determination of TZ in Acnitaz® gel by the proposed spectrophotometric methods and the reported[3] method and application of standard addition technique

			Standard addition					
					D_1 Method		DD_1 Method	
Product		Reported* method[3]	Taken μg ml^{-1}	Added μg ml^{-1}	Found μg ml^{-1}	Recovery** %	Found μg ml^{-1}	Recovery** %
TZ in Acnitaz® gel	**D_1 Method**	100.37 ± 1.191	4	2	1.98	99.00	1.97	98.50
0.1 g% Tazarotene	100.23 ± 0.973							
B.No 85332	**DD_1 Method**							
	100.124 ± 0.98							
				4	3.97	99.25	3.99	99.75
				6	6.07	101.17	6.02	100.33
				Mean		99.81		99.53
				SD		1.187		0.935
				RSD%		1.190		0.940

* Reversed phase high performance liquid chromatography method based on using C18 (250 mm × 4.6 mm 5 μm) column using water pH 2.5 with orthophosphoric acid : acetonitrile (15 : 85, *v*/*v*) as a mobile phase.
** Average of 3 determinations.

Table 3. Statistical comparison for the results obtained by the proposed spectrophotometric methods and the reported method for the analysis of TZ in pure powder form

Value	D_1 method	DD_1 method	Reported[3] method*
Mean	99.35	99.45	99.76
SD	1.410	1.053	1.181
RSD%	1.411	1.059	1.184
n	10	10	5
Variance	1.988	1.109	1.391
Student's t test	0.557 (2.160)	0.516 (2.160)	–
F value	1.430 (5.999)	1.254 (3.633)	–

* Reversed phase high performance liquid chromatography method based on using C18 (250 mm × 4.6 mm 5 µm) column using water pH 2.5 with orthophosphoric acid : acetonitrile (15 : 85, *v*/*v*) as a mobile phase.
The values in the parenthesis are the corresponding theoretical values of *t* and F at (P=0.05).

Table 4. Assay validation sheet of the proposed spectrophotometric methods for the determination of TZ

Parameter	D_1 method	DD_1 method
Accuracy (mean ± SD)	99.35 ± 1.410	99.45 ± 1.053
Specificity	100.32 ± 0.633	100.26 ± 0.269
Precision		
Repeatability*	100.27 ± 1.132	100.26 ± 1.054
Intermediate precision**	100.72 ± 0.961	100.35 ± 1.122
Linearity		
Slope	0.151	0.9033
Intercept	0.005	0.0904
Correlation coefficient (r)	0.9998	0.9994
Range	1–10 µg/ml	1–10 µg/ml

* The intra-day (n = 3), average of 3 concentrations (2,4,6 µg/ml) for TZ repeated 3 times within the day.
** The inter-day (n = 3), average of 3 concentrations (2,4,6 µg/ml) for TZ repeated 3 times in 3 successive days.

decreased but a longer time was needed for the measurements, so medium scanning speed was chosen to perform measurements. The concentration of the devisor was also studied and it was found that on dividing over the normalized spectrum, very high amplitude values were obtained, and on dividing by 10 µg ml^{-1} the sensitivity of the method was decreased. Dividing by the spectrum of 4 µg ml^{-1} alkaline degradation product gave the best compromise in terms of sensitivity, repeatability. and signal-to-noise ratio. A linear relationship was obtained in the range 1–10 µg ml^{-1} for TZ. The corresponding regression equation was computed and found to be:

$$P = 0.9033\,C + 0.0904 \quad r = 0.9997 \quad \text{at } \lambda 395.0\ \text{nm}$$

where C is the concentration of TZ in µg ml^{-1}, P is the peak amplitude of the first derivative of the ratio spectrum curve at 395.0 nm for TZ and r is the correlation coefficient. The selectivity of the proposed procedures was assessed by the analysis of laboratory-prepared mixtures containing different ratios of the drug and alkaline degradation product, where satisfactory results were obtained over the calibration ranges as shown in Table 1. The proposed procedures were also applied for the determination of TZ in Acnitaz gel. The validity of the proposed procedures was further assessed by applying the standard addition technique. The results obtained are shown in Table 2.

The proposed derivative spectrophotmetric methods are applications to reported derivative techniques that could be found suitable to determine TZ in the presence of its alkaline degradation product. Accordingly, the methods were then considered as stability indicating. Meanwhile, the only stability-indicating method reported for the determination of TZ applying HPLC technique and there are no spectrophotometric stability-indicating methods available for this drug.

Results obtained by the proposed procedures for the determination of pure samples of TZ were statistically compared to those obtained by the reported method[3], no significant differences between the results were obtained as presented in Table 3. A validation sheet is also presented in Table 4.

Conclusion

From the previous discussion, it can be concluded that the proposed procedures are simple and do not require sophisticated techniques or instruments. They are also sensitive and selective and can be used for the routine analysis of TZ in its available dosage forms. The method is also suitable and valid for application in laboratories lacking liquid chromatographic instruments.

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