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Stability-indicating chemometric methods for the determination of tazarotene

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Two multivariate calibration methods – principal component regression (PCR) and partial least square (PLS) – have been used to determine tazarotene in the presence of its degradation products. Both methods are useful in spectral analysis because of the simultaneous inclusion of many spectral wavelengths instead of the single wavelength used in derivative spectrophotometry. A great improvement in the precision and predictive abilities of these multivariate calibrations was observed. A calibration set was constructed for the mixture and the best model was used to predict the concentration of the selected drug. The proposed methods were applied successfully in the determination of tazarotene in laboratory-prepared mixtures and in commercial preparations. Tazarotene was analyzed with mean accuracies of 100.006 ± 0.695 and 100.007 ± 0.690 using the PCR and PLS methods, respectively. The validity of the proposed methods was assessed using the standard addition technique. The proposed methods were found to be rapid, simple and required no preliminary separation. They can therefore be used for the routine analysis of tazarotene in quality-control laboratories. Copyright © 2010 John Wiley & Sons, Ltd.

Introduction

Tazarotene 6-[(3,4-Dihydro-4,4-di-methyl-2H-1-benzothiopyran-6-yl)ethynyl]-3-pyridinecarboxylic acid ethyl ester, is a white, solid, third-generation retinoid approved for the treatment of psoriasis and acne vulgaris.[1] In mice, tazarotene (TZ) 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]

Chemometrics is the art of processing data with various numerical techniques in order to extract useful information.[3] It applies mathematical and statistical methods to design optimum procedures and to provide maximum chemical information through the analysis of chemical data.

Quantitative spectroscopy has been greatly improved by the use of a variety of multivariate statistical methods.[4–11] Multivariate calibrations are useful in spectral analysis because of the simultaneous inclusion of multiple spectral intensities which can greatly improve the precision and applicability of quantitative spectral analysis.[12]

Despite of the wide application of this dosage form in the treatment of psoriasis and acne, a literature survey reveals that there are few reported stability-indicating methods for the determination of TZ. The expensive and sophisticated High Performance Liquid Chromatography (HPLC)[13] was used for the determination of TZ in the presence of its hydrolytic and oxidative degradation product. The determination of TZ in the presence of its hydrolytic degradation product alone was carried out previously using derivative and derivative ratio spectrophotometry.[14] These methods depends on measuring the amplitude at one wavelength, which may be affected by several factors (for example, noise, scanning speed, $\Delta\lambda$ and smoothing function) in addition to the devisor in the derivative ratio method. All these factors were overcome by multivariate calibrations, which was the trigger for this work. As no method has been developed for the determination of TZ in the presence of its hydrolytic (HD) and hydrolytic oxidative (HOD) degradation products, this is the aim of this present work.

The adaptation of the proposed methods to the analysis of the available dosage form is also an important task in order to solve problems encountered in quality control and analysis of expired samples.

Experimental

Apparatus

SHIMADZU dual beam UV-visible spectrophotometer (Kyoto, Japan), model UV-1650 PC connected to IBM compatible and a HP1020 LaserJet printer. The bundled software, UV- Probe personal spectroscopy software version 2.21 (Shimadzu, Japan) was used. The spectral band was 2 nm and scanning speed is 2800 nm/min with 0.1 nm interval.

The absorption spectra of the reference and test solutions were carried out in 1-cm quartz cells over the range of 200–450 nm. PLS and PCR were modeled using PLS toolbox 2.0 software under MATLAB® 6.5. A Shimadzu (Japan) GC-MS-QP 1000 EX gas chromatography mass spectrometer (GC-MS) consisting of a gas chromatograph (GC-14A) and mass spectrometer was also used.

The GC-MS conditions

Column: a polyethylene glycol (At.wax) was used.

Carrier gas: helium.

Temperature program: started with initial temperature 120 °C at initial time: 1 min and program rate 10 °C/min until a final temperature 210 °C was reached.

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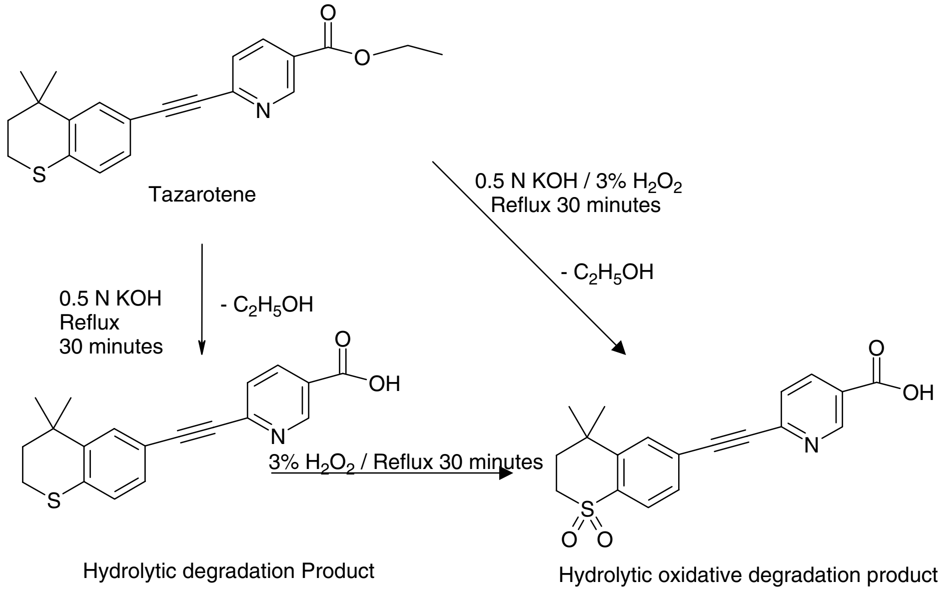

Figure 1. Suggested and reported pathways for TZ degradation.

Injector: 250 ul,
Detector temperature: 250 °C.

Reagents and chemicals

All chemicals used were of analytical grade and solvents were of spectroscopic grade.

1-Methanol (Adwic)
2-Potassium hydroxide, 1 N methanolic solution, (Adwic).
3-30% H_2O_2 (Adwic)

Pure TZ certified to contain 99.89%, was kindly provided by the Marcyrl Pharmaceuticals and Chemical Industries Company (Cairo, Egypt).

Acnitaz gel was bought from the market (Batch No. 85332) and was labeled to contain 0.1 g% TZ.

Procedure

Degradation of TZ

The suggested and reported pathways for degradation are shown in Figure 1.

Preparation of hydrolytic degradation (HD) product. The drug (50 mg) was weighed in a conical flask and dissolved in 25 ml methanol; 25 ml 1 N KOH was added and the solution was subjected to reflux at 100 °C for 30 min. The degradation product was separated on preparative TLC plates using a mixture benzene: chloroform: ammonia (5: 5: 0.01 by volume) as a developing solvent.[14]

Preparation of oxidized hydrolytic degradation product. The drug (50 mg) was weighed in a conical flask, dissolved in 20 ml methanol; 25 ml 1 N KOH and 5 ml hydrogen peroxide 30% (v/v) were added and the solution was subjected to reflux at 100 °C for 30 min. The degradation product was separated on preparative TLC plates using a mixture benzene: chloroform: ammonia (5: 5: 0.01 by volume) as a developing solvent.

Standard stock solutions

a. TZ standard stock solution; 50.0 µg/ml in methanol
b. HD standard stock solution; 50.0 µg/ml in methanol
c. HOD standard stock solution; 50.0 µg/ml in methanol

PCR and PLS chemometric models

Construction of the calibration set. Different mixtures of TZ and its degradation product were prepared by transferring different volumes of their stock solutions (50.0 µg/ml) into 25 ml measuring flasks as shown in Table 1. The volume was completed with methanol and the absorbance of these mixtures was recorded between 200 and 450 nm at 1 nm intervals (Figure 2).

Pre-processing the data. The regions from 200–220 nm and above 400 nm were rejected.

Constructing the models. The calibration set absorbance was used to build the PCR and PLS models; concentration matrices together with PLS-Toolbox 2.0 software were used for the calculations.

Selection of the optimum number of factors to build the PCR and PLS models. The cross validation method, leaving out one sample at a time, was used to select the optimum number of factors.[3] Given a set of 15 calibration samples, the PCR and PLS calibrations were performed on 14 samples. By using this calibration, the concentration of the sample left out was predicted. This process was repeated a total of 15 times until each sample had been left out once. The predicted concentrations were then compared with the known concentrations. The root mean square error of calibration (RMSEC) was calculated in the same manner each time a new factor was added to the model. The maximum number of factors used to calculate the optimum RMSEC was selected to be eight. The method described by Haland and Thomas[5,15] was used for selecting the optimum number of factors.

Table 1. The concentration of different mixtures of TZ, HD and HOD used in the calibration set

				PLS		PCR	
Mixture No.	TZ µg/ml	HD µg/ml	HOD µg/ml	Found* TZ µg/ml	Recovery % of TZ	Found* TZ µg/ml	Recovery % of TZ
1	7.00	7.00	7.00	6.94	99.19	6.94	99.17
2	7.00	5.00	9.00	7.00	99.97	7.00	99.96
3	5.00	5.00	6.00	5.01	100.10	5.00	100.09
4	5.00	9.00	9.00	5.01	100.16	5.01	100.17
5	9.00	6.00	7.00	9.01	100.06	9.01	100.06
6	6.00	9.00	6.00	9.02	100.21	9.02	100.21
7	7.00	7.00	6.00	7.01	100.09	7.01	100.10
8	7.00	6.00	8.00	6.07	101.10	6.07	101.11
9	6.00	6.00	9.00	5.93	98.76	5.93	98.76
10	6.00	8.00	8.00	7.93	99.08	7.93	99.07
11	8.00	9.00	7.00	9.05	100.54	9.05	100.54
12	9.00	8.00	9.00	7.99	99.93	7.99	99.92
13	8.00	7.00	9.00	7.10	101.36	7.10	101.37
14	7.00	9.00	5.00	9.00	99.97	9.00	99.97
15	9.00	9.00	8.00	8.96	99.59	8.96	99.59
Mean					**100.007**		**100.006**
SD					**0.690**		**0.695**
RSD%					**0.689**		**0.695**

* Average of three determinations.

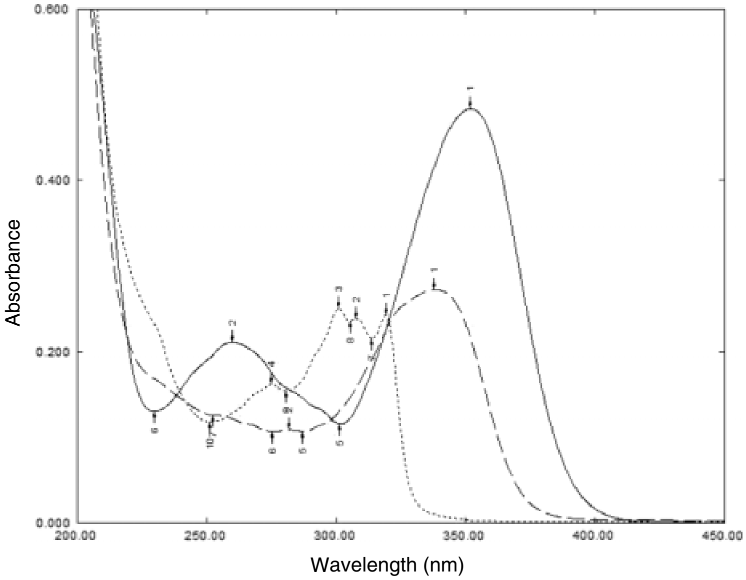

Figure 2. Absorption spectra for TZ (———), HD (- - - -) and HOD (· · · ·) 5 µg/ml each.

Construction of the validation set. Different mixtures of TZ and its degradation products were prepared by transferring different volumes from their stock solutions into 25 ml measuring flasks as shown in Table 2. The suggested models were applied to predict the concentrations of TZ. The predicted concentrations of the validation samples were plotted against the actual concentration values to evaluate the predictive abilities of the suggested chemometric methods.

Application of the proposed methods for the analysis of TZ in Acnitaz gel. 2.5 g gel was accurately weighed into a 100-ml beaker and sonicated in 15 ml methanol for 10 min, 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. 2 ml of the extracted solution were accurately transferred into a 25-ml measuring flask and completed to the mark using the same solvent. The spectra of the prepared solutions were measured. Then the developed multivariate models, PCR and PLS, were applied to calculate TZ concentration.

Results and Discussion

Two chemometric methods – PCR and PLS – were applied for the determination of TZ in the presence of its degradation products.

Table 2. Percent recoveries of TZ in the validation set using PCR and PLS methods

Mixture No.	Mixture composition (µg/ml) TZ	HD	HOD	Recovery* % of TZ PLS method TZ	PCR method TZ
1	5.00	8.00	7.00	102.08	102.13
2	8.00	5.00	8.00	99.45	99.46
3	5.00	7.00	8.00	99.57	99.61
4	7.00	8.00	6.00	99.91	99.92
5	8.00	8.00	5.00	101.07	101.09
6	8.00	6.00	6.00	100.31	100.33
7	6.00	5.00	7.00	98.75	98.79
8	5.00	6.00	5.00	100.03	100.07
9	6.00	7.00	5.00	100.64	100.68
Mean				**100.201**	**100.231**
SD				**0.979**	**0.983**
RSD%				**0.977**	**0.981**

* Average of three determinations.

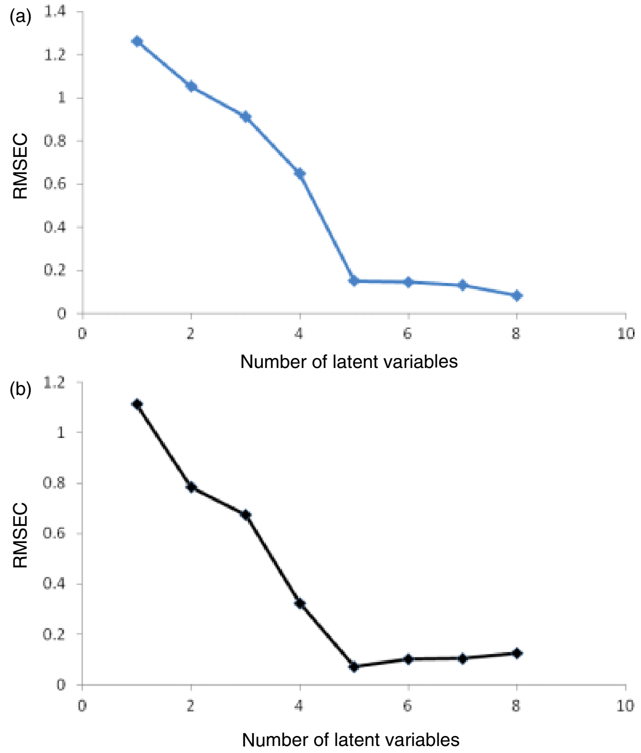

Figure 3. RMSEC plot of the cross validation results of the calibration set as a function of the number of latent variables used to construct the (a) PLS and (b) PCR models.

TZ was subjected to hydrolytic degradation within 30 min upon reflux in 0.5N methanolic potassium hydroxide solution.[14] Hydrolysis and oxidation of TZ were found to occur after 30 min upon reflux in methanolic solution of 0.5N KOH and 3% H_2O_2. The proposed scheme for degradation is shown in Figure 2.

The GC-MS was able to verify the structures of the degradation products, where the parent molecular ion peaks for HD and HOD were identified at $m/z = 323$ and $m/z = 355$, respectively, in accordance with the molecular weights of the suggested degradation products.

Mixtures with different concentrations of TZ and its degradation products were used as calibration samples to construct the models (Table 1). The spectra of these mixtures were collected and examined; the noisy region from 200–220 nm and the near zero absorbance after 400 nm accounted for the rejection of these parts from the spectra.

The selection of the optimum number of factors for the PCR and PLS methods was a very important pre-construction step: if the number of factors retained was more than required, more noise would be added to the data; if the number retained was too small, meaningful data that could be necessary for the calibration might be discarded. Different methods can be used to determine the optimum number of factors.[3,16] In this study, the leave-one-out cross-validation method was used and the RMSEC values of different developed models were compared. Five factors were found suitable for both PCR and PLS methods (Figures 3A and 3B).

To validate the predictive ability of the suggested models, PCR and PLS were employed to predict the concentration of TZ in nine laboratory-prepared mixtures containing different ratios, where satisfactory results were obtained Table 2.

Table 3. Determination of TZ in Acnitaz® gel by the proposed chemometric methods and the established[13] method and application of standard addition technique

Product	PLS	PCR	Established* method(13)	Standard addition Taken µg/ml	Added µg/ml	Found µg/ml PLS	PCR	Recovery** % PLS	PCR
TZ in Acnitaz® gel 0.1 g% **Tazarotene B.No 85332**	100.09 ± 0.55	100.11 ± 0.49	100.37 ± 1.19	4.00	3.00	2.98	2.98	99.33	99.33
					4.00	3.99	3.99	99.75	99.75
					5.00	5.02	5.01	100.40	100.20
					Mean			99.827	99.760
					SD			0.539	0.435
					RSD%			0.540	0.436

* Reversed phase HPLC 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 three determinations.

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Table 4. Statistical analysis of the results obtained by applying the proposed chemometric methods and the established[14] method for the determination of TZ in pure bulk powder

	PLS	PCR	Established (14) method*
Value	TZ		TZ
Mean	100.007	100.006	99.760
SD	0.690	0.695	1.179
RSD%	0.689	0.695	1.179
n	9	9	5
Variance	0.476	0.483	1.390
Student's *t*-test (2.179)	0.501	0.498	–
F value (3.84)	2.920	2.878	–

* Reversed phase HPLC 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 ($P = 0.05$).

The predicted concentrations of the validation samples were plotted against the known concentrations to determine whether the model accounted for the concentration variation in the validation set. Plots were expected to fall on a straight line with a slope of 1 and zero intercept. TZ, in all samples, lay on a straight line and the equations of these lines were $y = 0.9988\ x - 0.006$ ($r = 0.9990$) for PCR and $y = 0.9996\ x + 0.0143$ ($r = 0.9991$) for PLS. Both plots had a slope of almost 1 and an intercept close to zero.

The proposed PCR and PLS methods were successfully used for the determination of TZ in Acnitaz gel (Table 3). Each value indicated is the mean of three determinations of the same commercial batch. The validity of the proposed methods was further assessed by applying the standard addition technique.

Statistical analysis of the results obtained by the suggested methods and the reported method of analysis was carried out. Table 4 shows that the calculated *t* and F values were less than the theoretical ones, indicating no significant differences between the proposed methods and the reported method.

Conclusion

We can conclude that the proposed methods are simple and do not require complicated techniques or instruments. They are sensitive and selective, and thus can be applied for the routine analysis of TZ in pure form and in its available dosage forms.

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