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Stability-indicating chemometric methods for the determination of pyritinol dihydrochloride

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Three multivariate calibration methods, including classical least square with nonzero intercept (CLS), principal component regression (PCR) and partial least square (PLS), have been used for the determination of pyritinol dihydrochloride in the presence of its degradation product. The CLS, PCR and PLS techniques are useful in spectral analysis because the simultaneous inclusion of many spectral wavelengths instead of the single wavelength used in derivative spectrophotometry has greatly improved the precision and predictive abilities of these multivariate calibrations. A training set was constructed for the mixture and the best model was used for the prediction of the concentration of the selected drug. The proposed procedures were applied successfully in the determination of pyritinol dihydrochloride in laboratory-prepared mixtures and in commercial preparations. Pyritinol dihydrochloride was analysed with mean accuracies 99.99 ± 0.905, 99.91 ± 0.966 and 99.92 ± 0.962 using the CLS, PCR and PLS methods respectively. The validity of the proposed methods was assessed using the standard addition technique. The proposed procedures were found to be rapid and simple and required no preliminary separation. They can therefore be used for the routine analysis of pyritinol dihydrochloride in quality-control laboratories.

Keywords: classical least square; principal component regression; partial least square; pyritinol dihydrochloride

Introduction

Pyritinol dihydrohloride (Figure 1)(CAS number 1098-07-1) has the IUPAC name [5,5- dihydroxy- 6,6-dimethyl- 3,3- dithio dimethylene bis (4-pyridyl methanol) dihydrochloride monohydrate].[1] It has been described as a nootropic drug, which has no vitamin B_6 activity.[2]

The determination of pyritinol dihydrochloride in tablets was studied by several colorimetric, [3–5] spectrophotometric,[6,7] electrochemical[8] and high-performance liquid chromatographic methods.[9]

For drugs containing no ester or amide group that can be hydrolysed by acids, bases, moisture or heat, the only pathway for degradation is through oxidation. Drug oxidation often limits the stability of the drug product. Alkyl hydroperoxides, hydrogen peroxide and peroxy acids accelerate compound oxidation by a free-radical mechanism that resembles photodegradation. A trace concentration of peroxide in pharmaceutical formulations can significantly reduce their shelf lives.[10] Oxidation reactions of organosulfur compounds have received considerable attention in the literature. Numerous individual contributions[11–15] and several review articles[16,17] discuss the sulphide reactions with hydroperoxy compounds.

Chemometrics is the art of processing data with various numerical techniques in order to extract useful information.[18] It is the application of 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.[19–26] Multivariate calibrations are useful in spectral analysis because the simultaneous inclusion of multiple spectral intensities can greatly improve the precision and applicability of quantitative spectral analysis.[27]

The determination of the presence of pyritinol dihydrochloride in its degradation product was carried out previously using derivative spectrophotometry.[7] This method 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). All these factors were overcome by the multivariate calibrations, which was the trigger for this work.

The CLS method requires that all components in the calibration samples to be known. For the PCR and PLS methods, unlike CLS all overlapping spectral components are not required to be known.[28] The use of the CLS model with nonzero intercept will remove any noise or baseline drift. This was carried out by adding a raw of ones to our original training set concentration matrix.[19] Any absorbance that is not related to a component in the mixture will be attributed to the excess component (raw of ones), which will be ignored as it is not present.

The present work aims to develop feasible, sensitive and specific analytical procedures for the analysis of pyritinol dihydrochloride in the presence of its degradation product. Adaptation of the proposed procedures 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.

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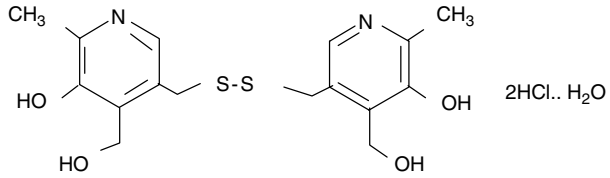

Figure 1. The structure of pyritinol dihydrochloride.

Experimental

Apparatus

A Shimadzu (Japan) 1601 PC double-beam UV-visible spectrophotometer was used. The software was UVPC personal spectroscopy software version 3.7 (Shimadzu). The absorption spectra of the solutions were carried out in 1 cm quartz cells over the range 200–400 nm. The data points were collected at 1 nm intervals. The data were saved in ASCII data-file format. PCR and PLS were modelled using PLS-Toolbox 2.0 under MATLAB™ version 6.5.

A Shinadzu (Japan) GC-MS-QP 1000 EX gas chromatography mass spectrometer (GC-MS) was also used consisting of a gas chromatograph (GC-14A) and mass spectrometer.

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^{-1} until a final temperature 210 °C was reached.

Injector: 250 µL.

Detector temperature: 250 °C.

Reagents and chemicals

All chemicals were of analytical grade.

- Hydrochloric acid (Prolabo, France).
- Pyritinol dihydrochloride monohydrate powder was kindly supplied by E. Merck, Darmstadt, Germany. Its purity was checked in our laboratory and was found to be 99.92 ± 1.172 according to the reported method.[7]
- Encephabol® tablets batch numbers 13 476 and 13 168 were purchased from the Egyptian market. Each tablet is claimed to contain 200 mg of pyritinol dihydrochloride. Encephabol tablets are manufactured by El Nile pharmaceutical company under license of E. Merck, Darmstadt, Germany.

Standard solutions

I- Pyritinol dihydrochloride stock standard solution (0.25 mg mL^{-1}) in 0.1 M hydrochloric acid.

II- Degradation product stock solution (0.25 mg mL^{-1}) in 0.1 M hydrochloric acid.

Procedures

Degradation of pyritinol dihydrochloride[7]

The drug (200 mg) was weighed in a stoppered test tube and 10 mL hydrogen peroxide 30% (v/v) was added. The tube was left for 1 h then heated to 100 °C to evaporate excess oxygen. The residue was then dissolved in 5 mL methanol and the degradation product was separated on preparative TLC plates using a mixture of n-butanol, acetic acid and water (4: 1: 1 v/v/v) as a developing system.[29]

Table 1. The concentration of different mixtures of pyritinol dihydrochloride and its degradation product used in the training set

Sample number	Pyritinol dihydrochloride (µg/mL)	Degradation product (µg/mL)
1	15.0	10.0
2	13.5	14.0
3	18.0	6.0
4	13.5	10.0
5	18.0	6.0
6	16.5	10.0
7	12.0	10.0
8	16.5	18.0
9	16.5	0.0
10	15.0	0.0

Construction of the training set

Different mixtures of pyritinol dihydrochloride and its degradation product were prepared by transferring different volumes of their stock solutions (0.25 mg mL^{-1}) into 25 mL measuring flasks as shown in Table 1. The volume was completed with 0.1 M hydrochloric acid and the absorbance of these mixtures was recorded between 200 and 400 nm at 1 nm intervals (Figure 2).

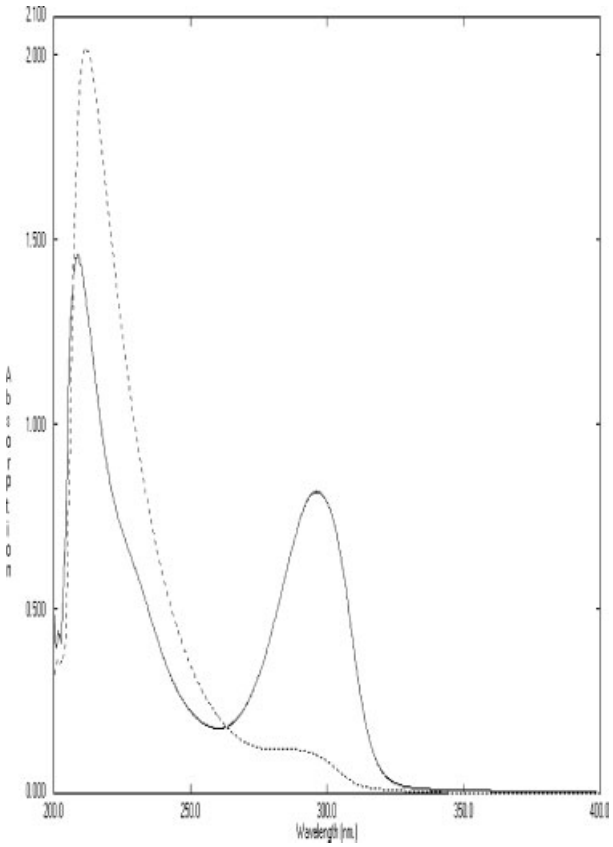

Figure 2. Absorption spectra of pyritinol dihydrochloride 20 µg ml^{-1} (———), and degradation product 6 µg ml^{-1} (- - - -) using 0.1 M hydrochloric acid as a solvent.

Pre-processing the data

The regions from 200–220 nm and above 330 nm were rejected. For CLS method, CLS model with nonzero intercept was constructed.

Constructing the models

To build the CLS model, the computer was fed with the absorbance and concentration matrices for the training set. Calculations were carried out to obtain the 'K' matrix. For the PCR and PLS models, the training set absorbance was used and concentration matrices together with PLS-Toolbox 2.0 software for the calculations.

Selection of the optimum number of factors to build the PCR and PLS models

The crossvalidation method, leaving out one sample at a time, was used to select the optimum number of factors.[18] Given a set of ten calibration samples, the PCR and PLS calibrations were performed on nine samples. By using this calibration, the concentration of the sample left out was predicted. This process was repeated a total of ten times until each sample had been left out once. The predicted concentrations were then compared with the known concentrations and the root mean square error of calibration (RMSEC) was calculated. The 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 six (half the number of samples plus one).[19] The method described by Haland and Thomas[20,30] was used for selecting the optimum number of factors.

Construction of the validation set

Different mixtures of pyritinol dihydrochloride and its degradation product were prepared by transferring different volumes from their stock solutions into 25 mL measuring flasks as shown in Table 2.[2] The suggested models were applied to predict the concentrations of pyritinol dihydrochloride.

Validation

Several diagnostic tools were used to evaluate the predictive abilities of the suggested chemometric methods.

- The predicted concentrations of the validation samples were plotted against the actual concentration values.
- Concentration residuals versus actual concentration plot (model and sample diagnostic). The residuals (the difference between the actual and the predicted concentration) were plotted against the actual concentrations for the validation samples.
- Root mean square error of prediction (RMSEP) (model diagnostic). The RMSEP for the predicted concentrations of the validation samples was calculated.
- The Q^2 (model diagnostic) parameter was calculated to determine the variation in the sample predictions.

Application of the proposed methods for the analysis of pyritinol dihydrochloride in Encephabol® tablets

Ten Encephabol® tablets were accurately weighed, ground and thoroughly mixed. Powdered tablets equivalent to 25 mg of pyritinol dihydrochloride were weighed into a 250 mL beaker and then 50 mL of 0.1 M hydrochloric acid was added. The suspension was stirred for 15 minutes using a magnetic stirrer then filtered into a 100 mL volumetric flask. The residue was washed three times each with 10 mL 0.1 M hydrochloric acid and completed to volume with 0.1 M hydrochloric acid. Then 1.8 ml of the extracted solution was transferred accurately into 25 ml measuring flask, completed to volume with 0.1 M hydrochloric acid. The spectra of the prepared solutions were measured then the developed multivariate models, CLS, PCR and PLS were applied for calculation of pyritinol dihydrochloride concentration.

Results and Discussion

Three different chemometric methods were applied for the determination of pyritinol dihydrochloride in presence of its degradation product including CLS, PCR and PLS.

The oxidation of the disulphide linkage occurs via sulfoxide to sulfonate. This oxidation pathway is considered as a method of degradation according to the International Conference of Harmonization (ICH).

In the case of pyritinol dihydrochloride, applying the ICH guidelines for oxidative degradation in tablets showed no degradation. Hence forced degradation under stressed conditions was followed.

The proposed scheme for preparing the degradation product is shown in Figure 3.

Using the NMR spectra to identify the structure of the degradation product was useless as no change in the type and number of proton signal occurs. Moreover, in the IR spectra, no change in the functional groups occurs except for the new S=O group in the degradation product. The S=O band will appear in the double bond region (1600–1800 cm^{-1}) and thus it will be masked by the C=C of the aromatic pyridine ring therefore the IR spectra will be unvalued.[31]

In the GC-MS spectrum, a parent molecular ion peak was identified at $m/z = 233$, which was in accordance with the molecular weight of the suggested degradation product. Thus, the GC-MS could verify the structure of the degradation product. The appearance of one peak in the GC-MS chromatogram indicated

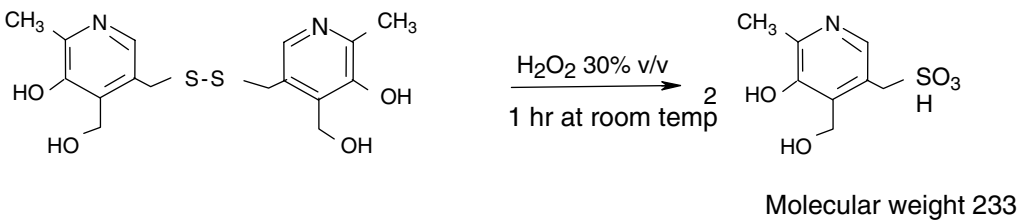

Figure 3. The proposed scheme for the oxidative degradation of pyritinol dihydrochloride.

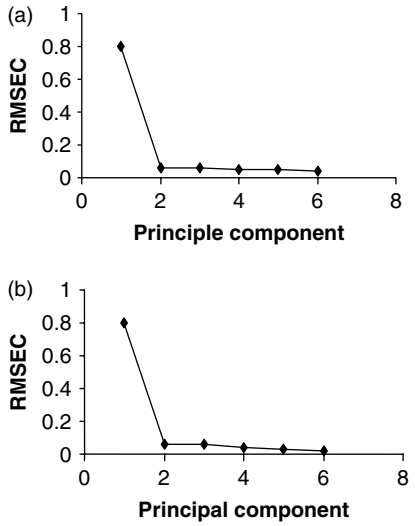

Figure 4. (a) Root mean square error of calibration plot of the cross validation results of the training set as a function of the number of principal components used to construct the PCR calibration for pyritinol dihydrochloride in presence of its degradation product. (b) Root mean square error of calibration plot of the cross validation results of the training set as a function of the number of principal components used to construct the PLS calibration for pyritinol dihydrochloride in presence of its degradation product.

the purity because the presence of impurities will increase the number of peaks in the GC chromatogram.

Mixtures with different concentrations of pyritinol dihydrochloride and its degradation product were used as calibration samples to construct the model (Table 1). The spectra of these mixtures were collected and examined, the noisy region from 200–219 nm and the near zero absorbance after 330 nm accounted for the rejection of these parts from the spectra.

For the CLS method, the training set was used for constructing CLS model or (K) matrix (i.e. absorptivity at different wavelengths) but poor predictions were obtained. The results were greatly improved by using the CLS model with nonzero intercept.

The selection of the optimum number of factors for the PCR and PLS techniques was a very important step before constructing the models because if the number of factors retained was more than required more noise would be added to the data. On the other hand, if the number retained was too small meaningful data that could be necessary for the calibration might be discarded. Different methods could be used to determine the optimum number of factors.[18,32] In this study, the leave-one-out crossvalidation method was used and the RMSEC values of different developed models were compared. The model selected was that with the smallest number of factors such that RMSEC for that model was not significantly greater than RMSEC from the model with additional factor. As the difference between the minimum RMSEC and other RMSEC values became smaller, the probability that each additional factor was significant became smaller.[19] Two factors were found suitable for both PCR and PLS methods as in Figures 4a and b.

To validate the predictive ability of the suggested models, they were used to predict the concentration of pyritinol dihydrochloride in laboratory prepared mixtures containing different ratios, where satisfactory results were obtained (Table 2).

The suggested CLS, PCR and PLS were validated using several diagnostic tools. These tools were grouped into two categories: the model diagnostic tools used to determine the quality of the model and the sample diagnostic tools used to study the relationship between the samples and to identify unusual samples.

The predicted concentrations of the validation samples were plotted against the known concentration values 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. Pyritinol dihydrochloride, in all samples, lay on a straight line and the equations of these lines were $y = 0.984, x + 0.2573$ ($r^2 = 0.9975$) for CLS, $y = 0.9825, x + 0.2779$ ($r^2 = 0.9980$), for PCR and PLS. All plots had a slope of nearly 1 and an intercept close to zero.

The concentration residuals were also plotted against the actual concentrations for the validation set samples (Figure 5a, b). This tool was used to determine whether the model accounted for the concentration variation in the validation set and it also provided information about how well the method would predict future samples. The residuals for all samples appeared to be randomly distributed around zero. These figures showed that the error in prediction was about ± 0.097 for pyritinol dihydrochloride in CLS and ± 0.082 in PCR and PLS models.

The RMSEP was another diagnostic tool for examining the errors in the predicted concentrations. It indicated both the precision and accuracy of predictions as it played the same role as standard deviation in indicating the spread of the concentration errors.[32]

Table 2. Results of the analysis of the mixtures of the validation set of pyritinol dihydrochloride and its degradation product by the proposed methods

	Concentration (μg/mL)		Pyritinol dihydrochloride recovery (%)		
Sample no.	Pyritinol dihydrochloride	Deg. Product	CLS non zero	PCR	PLS
1	18.0	14.0	100.71	100.56	100.57
2	12.0	6.0	100.38	100.47	100.47
3	16.5	18.0	99.97	99.93	99.93
4	15.0	14.0	98.84	98.90	98.90
5	15.0	2.0	100.74	100.64	100.64
6	13.5	14.0	100.94	100.90	100.90
7	18.0	18.0	99.45	99.47	99.47
8	18.0	0.0	99.44	99.50	99.50
	Mean		100.06	100.05	100.05
	S.D		0.757	0.706	0.707

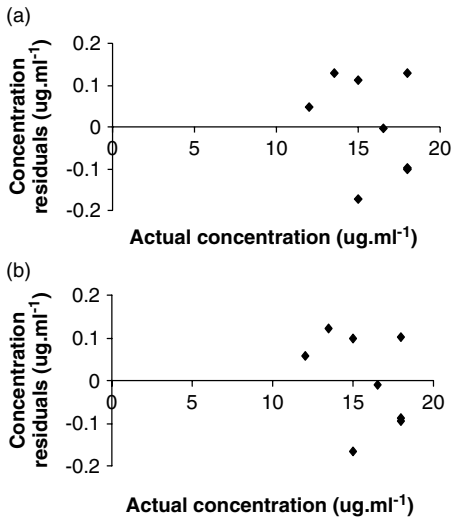

Figure 5. (a) Concentration residuals versus actual concentration of pyritinol dihydrochloride in the validation set using CLS method. (b) Concentration residuals versus actual concentration of pyritinol dihydrochloride in the validation set using PCR and PLS methods..

The RMSEP values were calculated and found to be 0.11027, 0.10139 and 0.10143 for pyritinol dihydrochloride in the CLS, PCR and PLS methods respectively.

The Q^2 values of these methods were also calculated to determine the variation in the samples prediction. So long as its value was closer to 1 this indicated good prediction. They were found to be 0.9972 for pyritinol dihydrochloride in the CLS method and 0.9976 in the PCR and PLS methods.

The chemometric methods (CLS, PCR and PLS) were applied successfully to the analysis of pyritinol dihydrochloride in Encephabol tablets. The results are shown in Table 3. To assess the accuracy of the method, the standard addition technique was carried out. The recoveries were found to be satisfactory indicating that the additives in the tablets formulation did not interfere (Table 4).

Statistical analysis of the results obtained by the suggested methods and the reported method of analysis was carried out. Table 5 showed that the calculated *t* and *F* values were less than the theoretical ones, indicating no significant differences between the proposed methods and the reported one.

Table 5. Statistical comparison for the results obtained by the proposed methods and the reported method for the analysis of pyritinol dihydrochloride in pure powder form

	Proposed methods			
Item	CLS	PCR	PLS	*Reported method*[a]
Mean	99.99	99.91	99.92	99.92
S.D.	0.905	0.966	0.962	1.172
Variance	0.819	0.933	0.925	1.375
n	10	10	10	6
F test	1.678 (3.48)	1.473 (3.48)	1.485 (3.48)	
Student's *t* test	0.134 (2.145)	0.018(2.145)	0.000 (2.145)	

The figures in parentheses are the corresponding tabulated values at p = 0.05.[33]
[a] Stability indicating spectrophotometric method.[7]

Table 3. Quantitative determination of pyritinol dihydrochloride in Encephabol tablets by the proposed methods

	Found % ± S.D.[a]			
Encephabol tablets claimed to contain 200 mg pyritinol.2HCl Batch number	CLS	PCR	PLS	Reported method[b]
13476	99.45 ± 0.957	100.11 ± 1.201	100.12 ± 1.199	99.43 ± 0.854
13168	99.89 ± 0.990	99.00 ± 1.271	99.00 ± 1.270	98.85 ± 0.911

[a] Each result is the average of the percentage recoveries of 6 different concentrations within the calibration range (each concentration is determined in triplicate).
[b] Stability indicating spectrophotometric method [7].
(Measuring th e amplitude of the first derivative curve of pyritinol dihydrochloride at λ284 nm (Δλ = 4 nm) using 0.1 M hydrochloric acid as a solvent.)

Table 4. Results of the standard addition technique for the determination of pyritinol dihydrochloride by the proposed methods

	Standard added(mg)	Recovery % of added		
Batch number	Pyritinol dihydrochloride	CLS	PCR	PLS
13 476	25.00	101.54	100.96	100.96
	37.50	100.98	101.20	101.20
	50.00	100.06	99.03	99.03
Mean ± S.D.[a]		100.86 ± 0.747	100.39 ± 1.189	100.39 ± 1.189

[a] Each result is the average of the percentage recoveries of six different concentrations within the calibration range (each concentration is determined in triplicate).

Conclusion

From the above discussion we can conclude that the proposed methods are simple, do not require complicated techniques or instruments, are sensitive and selective and can be used for the routine analysis of pyritinol dihydrochloride in pure form and in its available dosage forms.

References

[1] J. O. Neil Maryadele, *The Merck Index*, 14 edn, Merck Research Laboratories: White House Station, **2006**.
[2] C. S. Seam, *Martindale, The Complete Drug Reference*, 34 edn, Pharmaceutical Press: London, **2005**.
[3] R. T. Sane, U. A. Ghorpade, A. D. NadKarni, V. J. Banavalikar, *J. Indian Drugs* **1986**, *23*, 306.
[4] R. T. Sane, R. S. Samant, V. G. Nayak, *J. Indian Pharm. Sci.* **1988**, *50*, 161.
[5] C. S. P. Sastry, D. Murali Krishna, *Analytical Let.* **1995**, *28*, 1197.
[6] S. M. Hassan, S. M. El Ashry, M. M. El Kedrawy, *Spectroscopic Let.*, **1990**, *23*, 1273.
[7] M. A. Shehatta, M. A. El-Sayed, M. G. El-Bardicy, M. F. El-Tarras, *AOAC*, **2005**, *88*, 80.
[8] F. Belal, *J. Acta. Pharm.* **1991**, *41*, 129.
[9] F. Belal, *Analytical Let.* **1989**, *22*, 1897.
[10] L. Bateman, K. Hargave, *Proc. Roy. Soc. London.* **1954**, *A224*, 389.
[11] R. Curci, G. Modena, *Tetrahedron* **1966**, *22*, 1227.
[12] G. Modena, P. Todesco, *Chem. Soc.* **1962**, 4920.
[13] C. G. Overberger, R. W. Cummins, *J. Amer. Chem. Soc.* **1953**, *75*, 4250.
[14] C. G. Overberger, R. W. Cummins, *J. Amer. Chem. Soc.* **1953**, *75*, 4783.
[15] D. Swern, *Chem. Rev.* **1949**, *45*, 1.
[16] D. Barnard, L. Bateman, J. Cunneen, *Organic Sulphur Compounds*, Pergamon: New York, **1961**, pp. 229.
[17] E. J. Berhman, J. O. Edwards, *Prog. Phys. Org. Chem.* **1967**, *4*, 93.
[18] R. Kramer, *Chemometric Techniques for Quantitative Analysis*, Marcel Dekker Inc.: New York, **1998**.
[19] A. Espinosa-Mansilla, A. Munoz de la Pena, F. Salinas, *Anal. Chim. Acta* **1993**, *276*, 141.
[20] D. M. Haland, E. V. Thomas, *Analytical Chem.* **1988**, *60*, 1193.
[21] W. Lindberg, J. A. Persson, S. Wold, *Analytical Chem.* **1983**, *55*, 643.
[22] D. M. Haland, *Analytical Chem.* **1988**, *60*, 1208.
[23] C. W. Brown, P. F. Lynch, R. J. Obremski, D. S. Lavery, *Analytical Chem.* **1982**, *54*, 1472.
[24] M. Donahue, C. W. Brown, B. Caputo and M. D. Modell, *Analytical Chem.* **1988**, *60*, 1873.
[25] A. Espinosa-Mansilla, F. Salinas, I. D. Orbepaya, *Anal. Chim. Acta* **1995**, *313*, 103.
[26] H. C. Goicoechea, A. C. Olivieri, *J. Pharm. and Biomed. Anal.* **1999**, *20*, 255.
[27] Y. Ni, X. Gong, *Anal. Chem. Acta* **1997**, *354*, 163.
[28] D. M. Haland, E. V. Thomas, *Analytical Chem.* **1990**, *62*, 1091.
[29] M. Abdel-kawy, *J. Bull. Fac. Pharm. Cairo. Univ.*, **1993**, *31*(1), 11.
[30] D. M. Haland, E. V. Thomas, *Analytical Chem.* **1988**, *60*, 1202.
[31] T. W. Graham Solomons, *Organic Chemistry*, John Wiley & Sons, Inc.: New York, **1980**.
[32] R. B. Kenneth, J. P. Randy, M. B. Seasholtz, *Chemometrics: A Practical Guide*, John Wiley & Sons, Inc.: New York, **1988**.
[33] Loyd V. Allen, *Remington, The Science and Practice of Pharmacy*, 19 edn, Easton: Pennsylvania, **1995**, pp. 116.