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4. AU: Please clarify the use of "the other" here versus the introductory part of the sentence that discusses two spots, which were the intact drug and the DEG. By this second "other," do you mean the third component of the mixture could not be identified because it did not generate a spot given its nature, ie, alcohol that evaporated, thus leaving no trace?
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DRUG FORMULATIONS AND CLINICAL METHODS

Novel Stability-Indicating Chemometric-Assisted Spectrophotometric Methods for the Determination of Chlordiazepoxide and Clidinium Bromide in the Presence of Clidinium Bromide's Alkali-Induced Degradation Product

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AU1 **Two simple and accurate chemometric-assisted spectrophotometric models were developed and validated for the simultaneous determination of** AU2 **chlordiazepoxide (CDZ) and clidinium bromide (CDB) in the presence of an alkali-induced degradation product of CDB in their pure and pharmaceutical formulation. Resolution was accomplished by using two multivariate calibration models, including principal component regression (PCR) and partial least-squares (PLS), applied to the UV spectra of the mixtures. Great improvement in the predictive abilities of these multivariate calibrations was observed. A calibration set was constructed and the best model used to predict the concentrations of the studied drugs. CDZ and CDB were analyzed with mean accuracies of 99.84 ± 1.41 and 99.81 ± 0.89% for CDZ and 99.56 ± 1.43 and 99.44 ± 1.41% for CDB using PLS and PCR models, respectively. The proposed models were validated and applied for the analysis of a commercial formulation and laboratory-prepared mixtures. The developed models were statistically compared with those of the official and reported methods with no significant differences observed. The models can be used for the routine analysis of both drugs in QC laboratories.**

Chlordiazepoxide (CDZ), 7-chloro-2-methylamino-5-phenyl-3*H*-1,4-benzodiazepine-4-oxide (Figure 1a), is the first benzodiazepine to have ever been synthesized. CDZ has amnestic, anticonvulsant, anxiolytic, hypnotic, and skeletal muscle-relaxant properties because it inhibits monosynaptic and polysynaptic reflexes by acting as an inhibitory neural transmitter or by blocking excitatory synaptic transmission (1). Clidinium bromide (CDB), 3-[2-hydroxy-2,2-diphenylacetyl]oxy-1-methyl-1-azabicyclo[2.2.2]octan-1-ium bromide (Figure 1b), is a quaternary ammonium anticholinergic drug with peripheral effects that may ease symptoms of cramping and abdominal stomach pain by decreasing stomach acid and slowing intestinal activity (2). It has antimuscarinic and antispasmodic effects. CDB is used in fixed combination with CDZ as an adjunctive therapy in the treatment of peptic ulcer disease and acute enterocolitis and to treat functional gastrointestinal motility disturbances (e.g., irritable bowel syndrome; 3, 4).

Many methods have been reported for the quantitative determination of CDZ based on spectrophotometry (5–7), electrochemical methods (8, 9), and chromatographic methods (10, 11).

A capillary electrophoresis method (12) and an electrochemical method (13) have been reported for the quantitative determination of CDB individually in formulation. Also, derivative spectrophotometry (14), several chromatographic methods (15–18), and spectrophotometry using multivariate calibration techniques (19) have been developed for the simultaneous determination of both drugs in combined dosage forms.

To the best of our knowledge, a survey of the literature shows that no published stability-indicating chemometric-assisted UV spectrophotometric models have been reported for the analysis of CDZ and CDB in the presence of CDB's main alkali-induced degradation product (DEG), so there is a need for chemometric models that permit the simple, accurate, and precise quantification of the proposed drugs in the presence of CDB's DEG using an International Conference on Harmonization (ICH) approach for stress testing (20).

Chemometric-assisted spectrophotometry can enhance S/N, improve the selectivity of determination, optimize experimental conditions, raise analytical operation efficiency, and provide more scientific information. Hence, it has been rapidly attracting analyst attention and been used for the simultaneous determination of multicomponents in recent years (21). Among the various chemometric approaches applied to multicomponent analysis, principal component regression (PCR) and partial least-squares (PLS) regression have been successfully adopted in many quantitative assays of pharmaceutical formulations.

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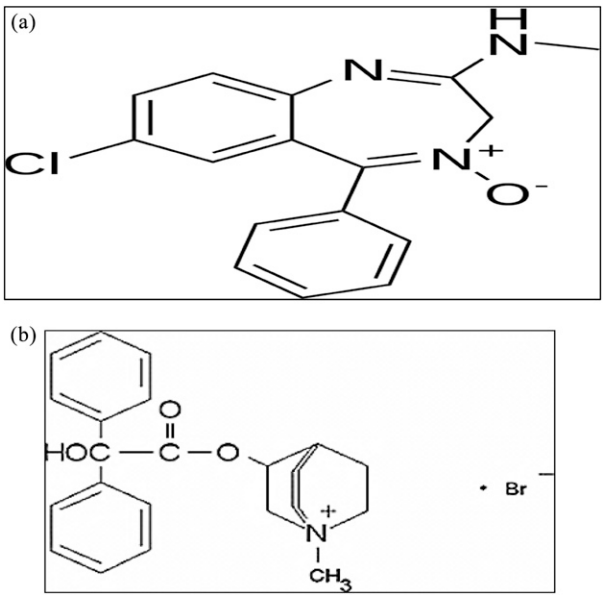


Figure 1. (a) Chemical structure of chlordiazepoxide. (b) Chemical structure of clidinium bromide.

According to the reported method (16), a full stress study on CDB was conducted, and it was found that this drug is more highly labile to alkaline hydrolysis than acidic ones, in addition to having increased stability under oxidative conditions. So we focused on the complete degradation of the drug under alkaline conditions.

The aim of the present study was to develop and validate two novel, simple, sensitive, selective, and precise stability-indicating chemometric-assisted spectrophotometric models, as well as investigate the ability of these chemometric methods to quantify CDZ and CDB in the presence of the alkali-induced DEG of CDB without any prior separation and with highly overlapping spectra and apply these optimized models to a pharmaceutical preparation.

Experimental

Apparatus and Software

(a) *IR spectrometer.*—IR spectra were measured with a JASCO 460 plus FTIR spectrometer (Japan).

(b) *MS.*—MS spectra were measured with a Shimadzu Qp-2010 plus MS (Shimadzu, Kyoto, Japan).

(c) *UV-Vis spectrophotometer.*—Components were measured at a wavelength range of 200–400 nm with a JASCO double-beam UV-Vis spectrophotometer (Model No. V-760; Japan) connected to a compatible Acer computer with Microsoft Excel 2010 software.

(d) *Spectra manager software.*—PLS and PCR were modeled using PLS-Toolbox 2.0 software (JASCO) with Matlab (Version 7.9).

(e) *UV lamp.*—Spots were visualized with a short-wavelength UV lamp (254 nm; United States). AU3

(f) *TLC.*—TLC was performed with a Camag TLC Scanner 3 densitometer (Model No. 35/N130319) equipped with winCATS software.

Reagents

(a) *Methanol.*—HPLC grade (99.5%; Sigma-Aldrich Chemie GmbH, Munich, Germany).

(b) *Ethyl acetate.*—Analytical grade [99%; El-Nasr Pharmaceutical Chemicals Co. (ADWIC), Cairo, Egypt].

(c) *Ammonia.*—Analytical grade [El-Nasr Pharmaceutical Chemicals Co. (ADWIC)].

(d) *Sodium hydroxide (NaOH).*—Analytical grade [El-Nasr Pharmaceutical Chemicals Co. (ADWIC)].

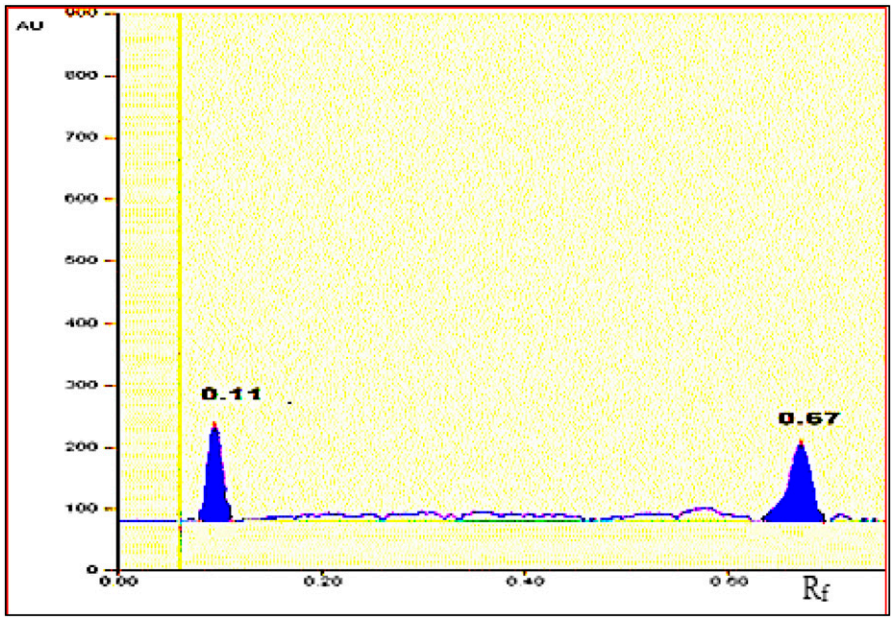
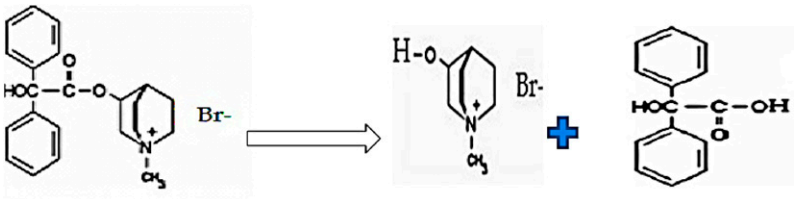


Figure 2. Densitometeric chromatogram clidinium bromide (2 µg/band) at R_f = 0.11) in the presence of the alkali–induced degradation product at (R_f = 0.67) using an ethyl acetate–methanol–ammonia mixture (8 + 3 + 1, v/v/v) as the mobile phase at 222 nm.



Clidinium Bromide C22-H26-Br-N-O3
M.W. 432 g/mol

Alkali-induced degradation products of clidinium bromide
M.W. 228 g/mol

Figure 3. The suggested degradation pathway of clidinium bromide with 0.1 M NaOH for 60 min at 90°C.

(e) *Concentrated hydrochloric acid (HCl).*—Analytical grade [El-Nasr Pharmaceutical Chemicals Co. (ADWIC)].

(f) *Standards.*—CDZ and CDB were supplied by EVA Pharmaceutical Co. (Egypt). The purity of CDZ was found to be 99.45 ± 1.14% according to a *British Pharmacopoeia* method (8), and the purity of CDB was found to be 99.52 ± 0.93% according to another reported method (16).

(g) *Sample.*—Commercial sugar-coated tablets were purchased from a local Egyptian pharmacy. Each tablet contained 5 mg CDZ and 2.5 mg CDB (Batch No. 1310047).

(h) *Stock standard solutions of 250 µg/mL CDZ and CDB in methanol.*—The solutions were freshly prepared by dissolving 25 mg of each pure compound in methanol in 100 mL volumetric flasks diluted to volume with the same solvent. All solutions were stored in a refrigerator at 4°C.

(i) *Working standard solutions of 100 µg/mL CDZ and CDB in methanol.*—The corresponding stock standard solutions were diluted using methanol and stored in a refrigerator until use.

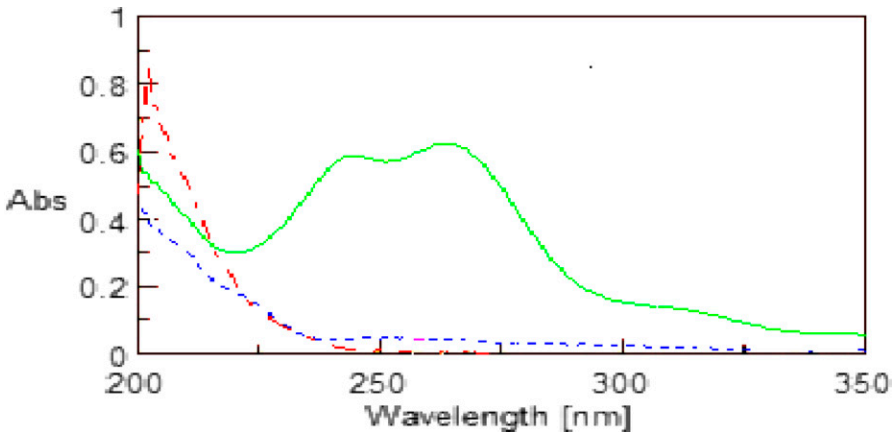
(j) *Stock solution of 250 µg/mL alkali-induced DEG.*—Accurately weighed 25 mg pure CDB powder were transferred into a 100 mL flask; 0.1 M NaOH and 0.1 M HCl were prepared by dilution using distilled water; and 20 mL of 0.1 M NaOH were added and heated at 90°C for 60 min, the solution neutralized with 20 mL of 0.1 M HCl, and the solution diluted to volume with methanol.

(k) *Working solution of 100 µg/mL alkali-induced DEG.*—The corresponding stock solution was diluted using methanol and stored in a refrigerator until use.

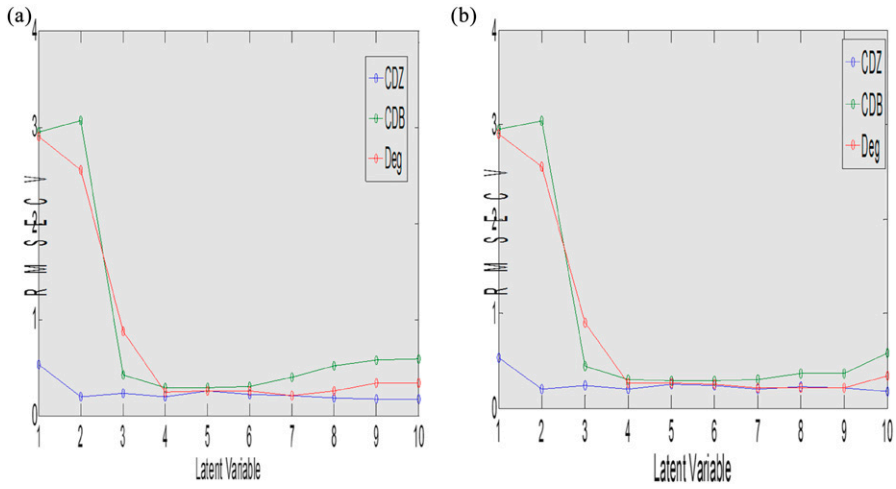
Procedure

Preparation of the alkali-induced degradation product.—Systematic studies were performed focusing on CDB degradation using different concentrations of NaOH at different temperatures for different time intervals. It was found that heating with 0.1 M NaOH in a water bath at 90°C for 60 min was sufficient for complete degradation. Degradation was monitored by TLC using an ethyl acetate–methanol–ammonia mixture (8 + 3 + 1, v/v/v) as the developing system, and spots were visualized with the UV lamp at 254 nm.

Precoated TLC aluminum plates (20 × 20 cm, 0.25 mm Alugram; NanoSIL Silica Gel G/UV254; Macherey Nagel, Germany) were used as the stationary phase in a chromatographic glass tank with a lid (22 × 25 × 10 cm). The samples of CDB and its alkali-induced DEG were applied to TLC plates in bands (a band width of 6 mm, with bands spaced 1 cm apart from each other and 1 cm from the bottom edge of the plate). The applied volume per band was 10 µL using an autosampler with a Camag microsyringe (100 µL). The plate was developed up to a distance of approximately 10 cm from the lower edge using a mobile phase consisting of a ethyl acetate–methanol–ammonia mixture (8 + 3 + 1, v/v/v). Linear ascending development was carried out in a chromatographic tank previously saturated with the developing system for 1 h at room temperature. The developed plates were air-dried and scanned at 222 nm. Detection was carried out using the Camag TLC Scanner 3 densitometer equipped with winCATS



AU13 **Figure 4. Zero–order spectra of chlordiazepoxide (5 µg/mL; solid line), clidinium bromide (10 µg/mL; dot-dashed line) and the alkali–induced degradation product (5 µg/mL; dashed line) separately in methanol.**



AU14 **Figure 5. Root mean square error of cross-validation of the calibration set of chlordiazepoxide, clidinium bromide, and degradation product as a function of latent variables used to construct (a) partial least-squares and (b) principal component regression calibration models.**

software and operated in absorbance mode, with a deuterium lamp as the source of radiation, slit dimension kept at 3 × 0.45 mm, and 20 mm/s scanning speed. By examining the TLC plates, only two different spots were obtained: one for the intact drug at R_f = 0.11 and the other for the main DEG at R_f = 0.67, whereas the other was alcohol and thus volatile during the process (Figure 2). AU4

Degradation was accomplished. The suggested degradation pathway (22) is shown in Figure 3. The degradate solution was evaporated on a rotary evaporator until dryness and the residue collected and dissolved in dichloromethane for IR analysis. The degradate powder was also elucidated using MS.

Principal Component Regression and Partial Least-Squares Chemometric Models

(a) *Construction of the calibration set.*—Different aliquots of the working solutions of CDZ, CDB, and DEG were separately transferred and mixed together into a series of 10 mL volumetric flasks that were diluted to volume with methanol to prepare concentrations of the 12 mixtures for the calibration set in concentration ranges of 1–12, 3–12, and 3–12 µg/mL for CDZ, CDB, and DEG, respectively. The absorbance values of these mixtures were recorded at 200–400 nm in 1 nm intervals against methanol as the blank. The composition of the samples was designed randomly according to a five-level calibration design (23) in order to obtain uncorrelated or orthogonal concentration profiles that obey Beer's law.

(b) *Preprocessing the data.*—CDZ, CDB, and DEG were UV-scanned separately in methanol at a wavelength range of 200–400 nm. The zero-order absorption spectra of CDZ and CDB showed two maxima peaks (264 and 243 nm) for CDZ and one maxima peak at 202 nm for CDB (Figure 4). The PCR and PLS models were tested to determine their ability to evaluate these drugs in synthetic mixtures and in their pharmaceutical preparation; however, results obtained at all ranges of wavelengths tested at 200–400 nm were erroneous when comparing true and predicted concentrations of the drugs in the validation set at ranges above 275 nm because that region showed no spectra and, consequently, very high noise. Afterward, the two multivariate calibration methods, PLS and PCR, were performed on the experimental data obtained from the UV spectra of the mixtures of in the region range of 200–275 nm and resolved the overlapping peaks of CDZ and CDB and the alkali-induced DEG in the synthetic mixtures and in the pharmaceutical preparation, producing satisfactory results.

(c) *Constructing the models.*—For both PCR and PLS models, the absorption spectra of the prepared mixtures were recorded

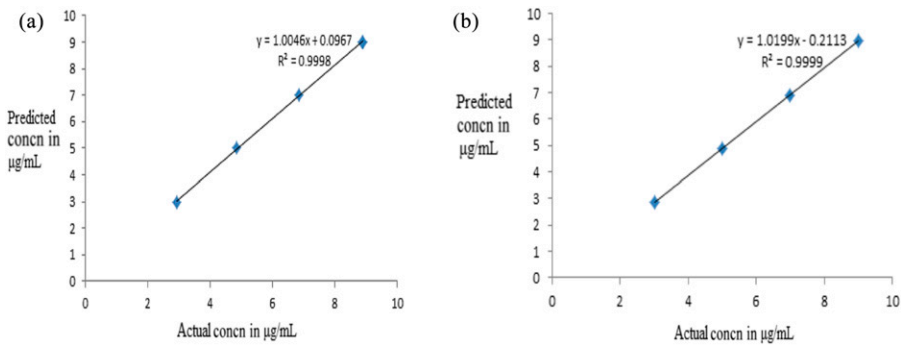


Figure 6. Predicted concentrations versus actual concentrations of chlordiazepoxide in the validation set using (a) partial least-squares and (b) principal component regression models.

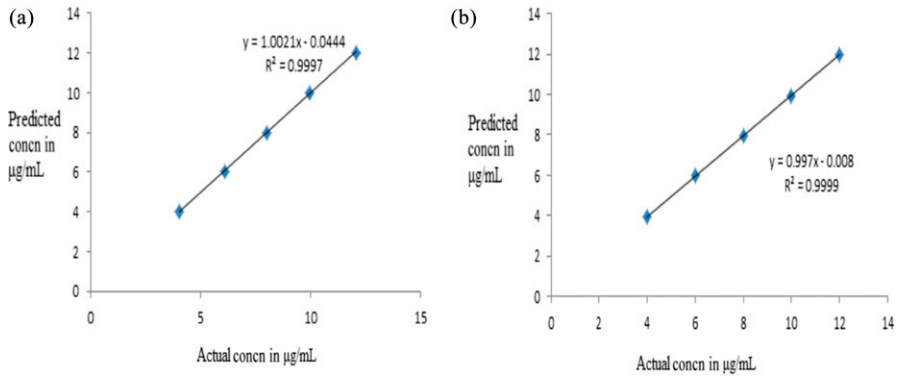


Figure 7. Predicted concentrations versus actual concentrations of clidinium bromide in the validation set using (a) partial least-squares and (b) principal component regression models.

and imported into Matlab (Version 7.9) with PLS-Toolbox 2.0 software for subsequent data manipulation.

(d) *Selection of the optimum number of factors to build the models.*—A cross-validation method was used leaving out one sample at a time to select the optimum number of factors. Given a set of 12 calibration samples, PCR and PLS calibrations were performed, and, using these calibrations, the concentration of the sample left out was predicted.

The predicted concentrations were then compared with the actual concentrations and the root mean square error of cross-validation (RMSECV) calculated. RMSECV was calculated for each model as follows:

$$\mathrm{RMSECV} = \sqrt{(\mathrm{PRESS}/n)}$$

where n = number of calibration set samples.

$$\mathrm{PRESS} = \sum \left(\mathrm{Y_{pred}} - \mathrm{Y_{true}}\right)^2$$

where $\mathrm{Y_{pred}}$ and $\mathrm{Y_{true}}$ = predicted and true concentrations in micrograms per milliliter, respectively. The maximum number of factors used to calculate the optimum RMSECV was selected as 3 (Figure 5).

(e) *Construction of the validation set.*—Different aliquots of the working solutions of CDZ, CDB, and DEG were separately transferred and mixed together into a series of 10 mL volumetric flasks that were diluted to volume with methanol to prepare the concentrations of the eight mixtures of the validation set. The absorbance values of these mixtures were recorded at 200–400 nm at 1 nm intervals against methanol as the blank. The suggested models were applied to these mixtures to predict the concentrations of CDZ, CDB, and DEG.

(f) *Application to the analysis of the pharmaceutical preparation.*—Twenty tablets of Librax were accurately weighted and ground to a fine powder to determine average tablet weight. An amount equivalent to one tablet (containing 5 mg CDZ and 2.5 mg CDB) was accurately transferred into a 100 mL volumetric flask. The powder was extracted with 30 mL methanol for 20 min using a vortex shaker and diluted to volume with methanol to obtain a concentration of 50 µg/mL CDZ and 25 µg/mL CDB. Subsequently, the solution was filtered with Whatman No. 10 filter paper (pore size = 11 µm), 2 mL filtrate accurately transferred into a 10 mL volumetric flask, and the solution diluted to volume with methanol to obtain a final concentration claimed to be 10 µg/mL CDZ and 5 µg/mL CDB.

Predictive Abilities of the Developed Models

Diagnostic tools were applied to evaluate the predictive abilities of the suggested models.

(a) *Predicted versus actual concentration plot (model and sample diagnostic).*—The predicted concentrations of the validation set were plotted against the actual concentration values of the validation set (Figures 6 and 7).

Table 1. Concentrations of chlordiazepoxide, clidinium bromide, and degradation product in the calibration and validation sets using PCR and PLS models

				Concn, µg/mL		
				CDZ	CDB	DEG
	Levels			Linearity range, µg/mL		
Mix No.	CDZ	CDB	DEG	1–12	3–12	3–12
1[a]	0	0	0	7	8	8
2[a]	2	–1	2	11	6	12
3[b]	–1	2	0	5	12	8
4[a]	2	0	–1	11	8	6
5[a]	0	–1	–1	7	6	6
6[a]	–1	–1	1	5	6	10
7[b]	–1	1	2	5	10	12
8[b]	1	2	1	9	12	10
9[a]	2	1	0	11	10	8
10[b]	1	0	2	9	8	12
11[b]	0	2	2	7	12	12
12[a]	2	2	–2	11	12	4
13[a]	2	–2	1	11	4	10
14[a]	–2	1	–2	3	10	4
15[b]	1	–2	0	9	4	8
16[b]	0	1	1	7	10	10
17[b]	1	1	–1	9	10	6
18[a]	1	–1	–2	9	6	4
19[a]	–1	–2	–1	5	4	6
20[a]	–1	0	–2	5	8	4

[a] Calibration set.

[b] Validation set.

Table 2. Recoveries of chlordiazepoxide, clidinium bromide, and degradation product in the validation set with partial least-squares and principal component regression models

CDZ			CDB			DEG		
	Recovery, %			Recovery, %			Recovery, %	
True concn, µg/mL	PLS	PCR	True concn, µg/mL	PLS	PCR	True concn, µg/mL	PLS	PCR
5	98.89	97.00	12	100.77	102.79	8	100.14	99.64
5	99.40	98.10	10	100.89	102.81	12	99.98	100.84
9	99.89	99.92	12	98.03	97.93	10	98.48	97.44
9	99.02	98.39	8	98.43	97.07	12	101.57	101.11
7	98.34	98.51	12	98.68	98.50	12	101.08	100.59
9	98.97	98.84	4	98.31	98.33	8	101.79	101.63
7	98.43	97.92	10	99.82	99.67	10	102.85	102.51
9	99.12	99.85	10	101.91	102.01	6	100.43	101.79
Mean ± SD	99.01 ± 0.50	98.57 ± 0.98		99.61 ± 1.45	99.89 ± 2.32		100.79 ± 1.34	100.69 ± 1.57

(b) *Root mean square error of prediction (model diagnostic).*—The root mean square error of prediction (RMSEP) was calculated for the predicted concentration of the validation set as follows:

$$\mathrm{RMSEP} = \sqrt{\sum \left(Y_{\mathrm{pred}} - Y_{\mathrm{true}}\right)^2}$$

Results and Discussion

The parent drug stability test guideline issued by the ICH (20) suggests that stress studies like hydrolysis should be carried out on a drug to establish the drug's inherent stability characteristics to identify DEGs and thus support the suitability of the proposed analytical procedures. It also requires that analytical procedures for testing the stability of samples be stability-indicating and fully validated.

Elucidation of the Intact and Main Alkali-Induced Degradation Product Can Be Accomplished by IR Spectroscopy and MS

Determination of molecular structure is especially important for either new reaction products or new materials isolated from the degradation of a drug subjected to a stress study and can be achieved via IR and MS. In IR spectroscopy, certain atomic vibrations give rise to bands; these bands are characteristic of the presence of certain functional groups. In addition, the mass spectrum of a compound typically shows a number of signals, and the peak at the highest *m/z* (molecular ion) usually corresponds to the mass of the whole molecule. Signals with lower *m/z* are fragment ions and can provide some structural information. By examining these key regions in an IR chart and by interpretation of the mass spectrum, a chemist can determine which functional groups are present or absent in a product and their MW. Thus, the reaction pathway and the chemical formula of the products can be suggested and accomplished by IR and MS (24–26).

IR spectrometry.—The IR spectrum of CDB showed a sharp peak at 3224.98 cm^{-1} and a peak at 1728.22 cm^{-1} due to the C=O in the ester group, whereas the IR spectrum of DEG showed a broad peak for the OH group in COOH at 3448.72 cm^{-1} and a peak at 1620.21 cm^{-1} due to the C=O in the acid, which confirmed the hydrolysis reaction of the ester group in the intact drug.

MS.—The structure of the main DEG was confirmed by MS. The MS chart of CDB showed a peak at *m/z* 431, whereas the MS chart of DEG showed an evident peak at *m/z* 227, $(M-1)^+$. AU6

Chemometrics is the art of processing data with various numerical techniques in order to extract useful information. It is the application of mathematical and statistical methods to design optimum procedures and provide maximum chemical information through the analysis of chemical data (27).

In this work, novel stability-indicating chemometric-assisted spectrophotometry approaches were applied for the simultaneous determination of CDZ, CDB, and DEG, these namely being PCR and PLS. Both models are useful in spectral analysis because of the simultaneous inclusion of many spectral wavelengths instead of the single wavelength used in derivative spectrophotometry.

Table 3. Summary of results obtained by applying diagnostic tools for model validation of the chemometric-assisted spectrophotometric methods

	CDZ		CDB		DEG	
Validation parameter	PLS	PCR	PLS	PCR	PLS	PCR
Predicted versus known concn plot						
Slope	1.0046	1.0199	1.0021	0.997	1.0066	0.9739
Intercept	0.0967	–0.2113	–0.0444	–0.008	–0.0495	0.2609
Correlation coefficient	0.9999	0.9999	0.9998	0.9999	0.9998	0.9999
Root mean square error of prediction	0.2912	0.2318	0.1732	0.2319	0.2748	0.2849

Table 4. Statistical analysis of the results obtained by applying the proposed chemometric methods compared with the official and reported methods for the determination of chlordiazepoxide and clidinium bromide, respectively, in pure powdered form

	PLS		PCR		Official BP method[a,b]	Reported HPLC method[c]
Parameters	CDZ	CDB	CDZ	CDB	CDZ	CDB
Mean ± SD, %	99.84 ± 1.41	99.56 ± 1.43	99.81 ± 0.89	99.44 ± 1.41	99.45 ± 1.14	99.52 ± 0.93
Variance	1.9881	2.0449	0.7921	1.9881	1.2996	0.8649
n	12	12	12	12	6	6
Student's *t*-test (2.119)[d]	0.568	0.898	0.469	0.949		
F-test (4.704)[d]	1.530	2.364	1.641	2.299		

[a] BP = *British Pharmacopoeia*.

[b] The official method for CDZ is a nonaqueous potentiometric titrimetric method (8).

[c] Reported HPLC method using a C18 column and 0.05 M potassium dihydrogen phosphate buffer (pH 4.0) adjusted with 0.5% orthophosphoric acid–methanol–acetonitrile (40 + 40 + 20, v/v/v) at 220 nm (16).

[d] Numbers in parentheses represent the corresponding tabulated *t*- and *F*-values at $P = 0.05$.

PLS and PCR are factor analysis models which allow the establishment of a relationship between matrixes of chemical data (27). One of the clearest explanations of these models was given by Haaland and Thomas (28). PLS was related to PCR in that spectral decomposition was performed. PCR decomposition was significantly influenced by variations that had no relevance to the analyte concentrations, whereas in PLS, the spectral decomposition was weighted to the concentration (29).

Multivariate calibrations are useful in spectral analysis because of the simultaneous inclusion of multiple spectral intensities that can greatly improve the precision and predictive abilities of quantitative spectral analysis. These factors (noise, scanning speed, and smoothing function) were overcome by multivariate calibrations, which were what prompted this current work.

PCR and PLS are based on factor analysis. Their advantage is the transformation of numerous orthogonal variables into latent vectors. The first latent vectors contain information carried in the manifest variables, whereas the later ones represent noise that can be filtered out and not considered during modeling.

The first step in the determination of the cited drugs by multivariate calibration models involved constructing the calibration matrix for the mixture. The calibration set was obtained by using the absorption spectra set of 12 mixtures of CDZ, CDB, and DEG with different ratios of each component, as listed in Table 1.

Selection of the optimum number of factors was very important 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 calibration would be discarded. So, the number of factors should account, as much as possible, for the experimental data without resulting in overfitting.

In this study, leave-one-out cross-validation was used, and the RMSECV values were used as diagnostic tests to examine errors in the predicted concentrations. RMSECV values indicated both the precision and accuracy of the predictions. They were recalculated upon addition of each new factor to the PLS and PCR models. The selected model was the one with the smallest number of factors such that the RMSECV for that model was not significantly greater than RMSECV of the model with the additional factor. Three factors were found suitable for both PCR and PLS (Figure 5).

To validate the prediction ability of the suggested models, they were applied to predict the concentrations of both drugs in the validation set. Satisfactory results were obtained and are presented in Table 2.

Validation

The validation of the suggested models was done using diagnostic tools. These tools were divided into two categories, namely model diagnostic tools, which were used to determine the

Table 5. One-way ANOVA testing for the different proposed methods compared with the official/reported methods used for the determination of CDZ and CDB in pure powdered form[a]

Source of variation	DF	Sum of squares	Mean square	*F*-value	*F*-critical
		CDZ			
Between groups	2	0.676	0.338	0.246	3.354
Within groups	27	37.105	1.374		
Total	29	37.781			
		CDB			
Between groups	2	0.095	0.047	0.026	3.354
Within groups	27	48.406	1.792		
Total	29	48.501			

[a] At the 0.05 level, the population means are not significantly different.

Table 6. One-way ANOVA testing for the different proposed methods compared with the official/reported methods used for the determination of CDZ and CDB in their pharmaceutical dosage form[a]

Source of variation	DF	Sum of squares	Mean square	*F*-value	*F*-critical
		CDZ			
Between group	2	9.394	4.697	2.717	5.143
Within group	6	10.371	1.729		
Total	8	19.765			
		CDB			
Between-group	2	14.680	7.340	4.015	5.143
Within-group	6	10.968	1.828		
Total	8	25.648			

AU18 [a] At the 0.05 level, the population means are not significantly different.

quality of the model, and sample diagnostic tools, which were used to study the relationship between the samples and identify unusual samples.

The predicted concentrations of the validation set were plotted against the actual concentration values of the validation set (Figures 6 and 7). This was used to determine whether the model accounted for the concentration variation in the validation set. All plots had a slope and correlation coefficient of nearly 1 and an intercept close to zero (Table 3).

RMSEP was another diagnostic tool to examine errors in the predicted concentrations. It indicated both the accuracy and precision of the predictions, as it played the same role of SD in indicating the spread of errors (Table 3).

Statistical analysis of the results obtained by applying the proposed chemometric models compared with the official and reported methods for the determination of CDZ and CDB, respectively, in pure powdered form are shown in Table 4. One-way analysis of variance (ANOVA) testing for the different proposed models compared with the official and reported methods used for the determination of CDZ and CDB in pure powdered form are shown in Table 5. One-way ANOVA testing for the different proposed models compared with the official and reported methods used for the determination of CDZ and CDB in their pharmaceutical dosage form are shown in Table 6.

Conclusions

The proposed models are simple and rapid, require no preliminary separation or complicated techniques or instruments, and are sensitive and selective, thus applicable for the routine analysis of drugs in their pure form and in their available dosage form. Nowadays, multivariate calibration has become an indispensable part of modern analytical chemistry. A goal of multicomponent spectral analysis is to construct a calibration model relating the outputs of a multivariate spectrophotometer to the composition or properties of analytical samples. Among the different regression methods that exist for multivariate calibration, factor analysis-based models, including PCR and PLS regression, have received considerable attention in the chemometric-assisted spectrophotometry literature. These techniques are powerful multivariate statistical tools that have been successfully and widely applied to the quantitative analysis of spectroscopic data because of their ability to overcome problems common to this data, such as colinearity, band overlap, and interactions.

PCR does not require selection of certain wavelengths to be included in the model, working with a wide range of the spectrum that gives an averaging effect of the error so that the obtained model will be less susceptible to spectral noise. PCR can be used for very complex mixtures because only knowledge of the constituents of interest is required. However, PCR has some disadvantages, such as being slower at calculation, having more complex models to understand and interpret, and requiring large numbers of samples for accurate calibration.

PCR assumes that error exists only in instrumental response and that the concentration matrix is error-free, whereas PLS assumes that error is equally distributed between concentration matrix and instrumental response (spectral) matrix. However, since its introduction, PLS appears, by most accounts, to have become the model of choice among chemists, as it not only has the advantages of PCR, but it also produces more robust models by removing noise from both absorbance and concentration data.

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