Asian Journal of Chemistry Vol. 21, No. 2 (2009), 1159-1170

Quantification of Chlordiazepoxide and Pipenzolate Bromide in Tablets and Comparison with Chromatographic Results

ABDIL ÖZDEMIR* and CAN SERKAN KESKIN
Department of Chemistry, Faculty of Arts and Sciences
Sakarya University, 54100 Serdivan, Sakarya, Turkey
Fax: (90)(264)3460371; Tel: (90)(264)2956046
E-mail: abdilo@sakarya.edu.tr

Multiple methods were developed for the quantitative analysis of binary mixture of chlordiazepoxide and pipenzolate bromide in a pharmaceutical preparation. These techniques are categorized into chemometric, graphical and chromatographic methods. Different group of methods were utilized in this study including partial least squares, principle component analysis and classical least squares as chemometric methods, first derivative (DER) as graphical methods and HPLC method as a chromatographic method. In chemometric calculations, UV absorption spectra are taken and without data pretreatment subjected methods were applied. In case of HPLC after proper method development and data treatment, calibration equations were obtained for each active compound. Chromatography was carried out on C-18 column with mobile phase comprising of acetonitrile-methanol (50:50, v/v) pumped at flow rate of 0.8 mL/min. For chromatographic runs, amitriptyline was used as an internal standard in all the samples. The absorbance data was obtained in the range of 200-300 nm for the spectrophotometric studies. Linearity range of all the methods was found to be 8-24 µg/mL for chlordiazepoxide and 4-12 µg/mL for pipenzolate bromide. Mean recoveries were found satisfactory in the range of 95-105 % for both drugs with limit of detection values in the range of 0.14-0.41 µg/mL for each drug. These studies indicated that the significant spectral overlap of the UV-spectra of the drugs showing the need for chemometrics or graphical modeling for simultaneous analysis of the mixtures.

Key Words: Chlordiazepoxide, Pipenzolate bromide, Partial least squares, Classical least squares, HPLC, First derivative.

INTRODUCTION

Chlordiazepoxide [7-chloro-2-(methylamino)-5-phenyl-3*H*-1,4-benzodiazepine 4-oxide hydrochloride] (CDP) is a sedative-hypnotic drug widely employed as a tranquilizer and antidepressant[1] and pipenzolate bromide (1-ethyl-3-hydroxy-1-methylpiperidinium bromide benzilate) (PZB) is an anticholinergic agent.

Libkol film coated tablets are a pharmaceutical formulation which contains both drugs. Its indications are spastic and irritable colon disease, acute and mucosa enterocolitis, dysmenorrheal, premenstrual symptoms. Individual drugs has been

studied by spectrophotometry and chromatography. Several analytical methods were described for the determination of chlordiazepoxide in pharmaceutical formulations and plasma samples. These include spectrophotometry[2-6], spectrofluorimetry[7,8], reversed-phase high-performance liquid chromatography[9-15], high-performance thin layer chromatography[16] and liquid chromatography[17-19]. There are a couple qualitative one reported spectrophotometric studies about pipenzolate bromide in the literature[20,21].

Chemometric calibration techniques in spectral analysis gained big importance in the quality control of drugs in mixtures and pharmaceutical formulations containing two or more drugs with overlapping spectra due to no need of any separation procedure before determination step. For the chemometric techniques, a calibration has to be constructed by the training set containing all the compounds that are represented by their absorbance values. The obtained calibrations are used to predict the concentration of the subjected compounds in tablets. The selected chemometric and graphical methods do not require any prior separation step as in HPLC method. For the spectrophotometric analysis, first derivative method has been selected as a graphical method[22,23].

Chromatographic method development requires some separation steps for the quantitative analysis of compounds in the mixtures. The separation step brings more time consumption and money cost for the analysis of subjected compounds. But this method can produced as good results as chemometric methods do for the mixture of multiple compounds.

In this study, different methods have been applied to improve the results of binary mixture analysis of chlordiazepoxide (CDP) and pipenzlate bromide (PZB). These methods can be categorized into two main analytical techniques as spectroscopic and chromatographic. Spectrophotometric study contains overlapped spectrum, thus it has to be resolved by chemometric or graphical methods. Two different chemometric techniques namely partial least squares regression (PLS) and classical least-squares (CLS) have been applied. As a graphical method, first derivative method developed and applied for the resolution of binary compounds. The results of chemometric and graphical methods were compared with the obtained HPLC method results. All these methods have been applied for the quantitative analysis of binary mixtures in synthetic mixtures and tablets and shown their superiority and shortcomings of them for the quantitative determination of chlordiazepoxide (CDP) and pipenzlate bromide (PZB).

EXPERIMENTAL

A Shimadzu UV-160 double beam UV-VIS spectrophotometer possessing a fixed slit width (1 nm) and loaded with a Shimadzu UVPC software was used for the absorption measurements. Data treatments, regressions and statistical analysis were performed by using EXCEL, PLS TOOLBOX 4.0 and Matlab software.

Chromatography was performed with an Agilent 1100 series HPLC system (Agilent Technologies, Inc., California and USA) provided with a binary pump, an

autosampler, a thermostatted column compartment and a multiwavelength diode array detector (DAD). Data were acquired and processed using an HP Chem Station for LC software from Hawlet-Packard. The column used was a Waters Symmetry C18 Column 5 µm, 4.6 mm × 250 mm. The flow rate was maintained at 0.8 mL/min and the injection volume was 20 µL. The mobile phase was prepared daily, filtered through a 0.45 µm membrane filter.

Standard solutions and commercial tablet formulation: Appropriate volume aliquots of the stock solutions were transferred to 25 mL volumetric flasks. The volumes were made up with a mixture of methanol-acetonitrile (50:50, v:v) to give a series of standard solutions in such a way that their final concentrations lay within desired range. Spectra of the mixtures were recorded between 200 and 400 nm taking absorbance data at 1 nm intervals. The readings were made at a constant time and within 1 h of preparation of the standard solutions to avoid any possible solvent effect on active compounds. The calibration procedure of chemometric methods was carried out by using 20 calibration standards prepared using different concentration of each compound. For the graphical methods 5 different concentrations were used. The linearity of the maximal signals was examined to select an adequate concentration range for spectrophotometric measurements. Thus, the chlordiazepoxide and pipenzlate bromide concentrations were varied between 8 to 24 and 4 to 12 µg/mL, respectively. The compositions of the 20 standard mixtures used in the calibration matrix for chemometric methods are shown in Table-1.

TABLE-1
CONCENTRATION SET OF TWO ACTIVE COMPOUNDS

Number	CDP	PZB	Number	CDP	PZB
1	8	8	11	12	0
2	12	8	12	16	10
3	16	8	13	16	12
4	20	8	14	8	6
5	24	8	15	12	4
6	16	4	16	8	8
7	16	6	17	16	10
8	16	8	18	24	8
9	16	0	19	8	12
10	0	8	20	12	8

CDP = Chlordiazepoxide; PZB = Pipenzlate bromide.

For the chromatographic procedure the same calibration set as in graphical methods was used. To eliminate any instrumental effects and fluctuations, amitriptyline was used as an internal standard in all the chromatographic runs. Calibration graphs were obtained by using ratio of peak areas of active compounds to the internal standard. For the tablet analysis, same spectrophotometric procedures were followed. The chromatography was carried out at room temperature and the injection volume was 20 µL for all experiments. The flow-rate was 0.8 mL/min and the mobile phase consisted of methanol-acetonitrile (50:50, v:v).

As a pharmaceutical preparation, Libkol film coated tablets produced by Saba pharmaceutical firm was used in this study. It contains 5 mg of CDP and 2.5 mg of PZB in each coated tablet. For the calibrations, pure active compounds of pharmaceutical preparation were donated by Turkish Pharmaceutical Industrial Firms.

Tablet analysis: For the tablet analysis, 10 tablets were weighed and powdered in a mortar. From the average of those 10 tablets, two tablets amount was weighed and dissolved in 25 mL of solvent system. The prepared solutions were filtered with 0.2 µm disposable membrane filter. The final solution was diluted to the working concentration range for application of the graphical, mathematical and chromatographic methods.

RESULTS AND DISCUSSION

Fig. 1a shows the electronic absorption spectra of CDP and PZB in the 200-400 nm regions evidencing their structural differences. Each analyte exhibits only one maximum in their electronic absorption spectra: λ_{max} of CDP lies at 262 nm and λ_{max} of PZB lies at 202 nm. The strong signal overlapping of the absorption spectra of compounds give some difficulties to construct calibration equations. Sometimes, it is not possible to resolve the strong overlapped absorption spectra of compounds using graphical methods. The chemometric methods become method of selection to resolve the overlapped spectra. For instance, the overlap of the first derivative spectra did not produce good results to be resolved by the zero-crossing strategy.

For the simultaneous determination of mixtures of analytes employing chemometric methods, appropriate training set designs are required. In this study this combination was made for a binary mixture that covers all the possible tablet combinations and cross combinations of two drugs. Thus, a training set of 20 samples with a central composite design was prepared, by appropriate dilution of the working solutions which is shown in Table-1. A recovery set of 15 binary samples were concomitantly prepared and the electronic spectra of both sets were recorded between 200 and 400 nm. For the graphical methods 5 different calibration data were used. Fig. 1a shows the zero-order absorption spectra of subjected compounds at 5 different concentrations. The spectra of compounds were collected in the same range (200-400 nm). Recovery studies were achieved on a 15 set of compounds that represent the tablet combination of those drugs. Chromatographic studies were carried out using same experimental conditions as in graphical method. Fig. 2 shows the chromatogram of both drugs and internal standard recorded at 206 nm.

Data processing and model building: For each group of methods, different data matrix was constructed including training, recovery and standard addition and tablet data matrix. Chemometric methods (PLS and CLS) require a training set forthe calibration step. The absorption of training set samples was collected in the wavelength range of 200 to 400 nm and without a data treatment; training data matrix was formed in 20 × 1000 dimensions. In same way validation set was collected in 15 × 1000 data matrix. At the final step standard addition and tablet sample results were combined in another data matrix.

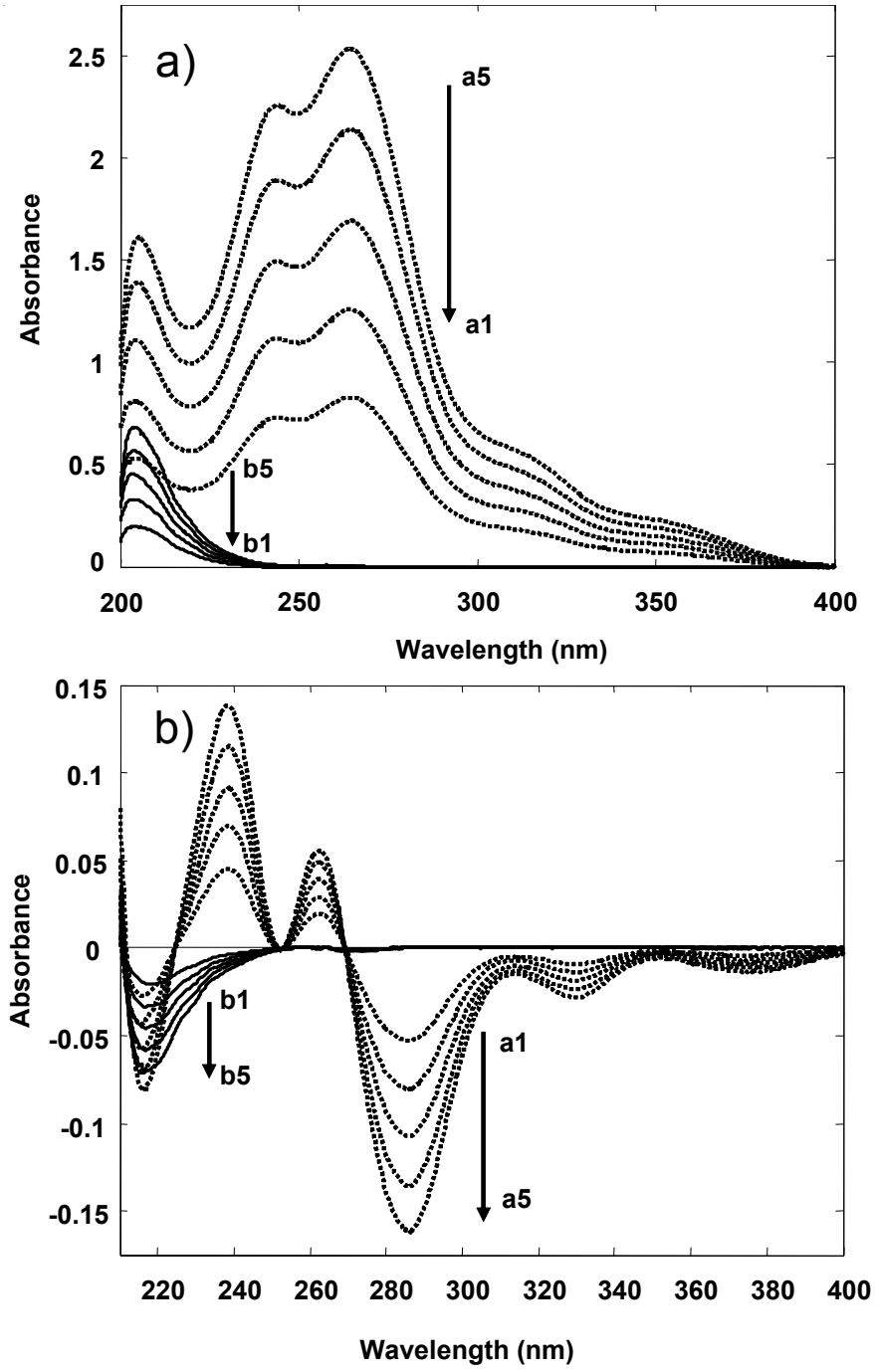

Fig. 1. Absorption spectra of CDP (⋯⋯⋯) and PZB (——) (a), first derivative spectra of CDP (⋯⋯⋯) and PZB (——) (b) in the concentration range of (a1 to a5) 8-24 µg/mL and b1 to b5 4-12 µg/mL

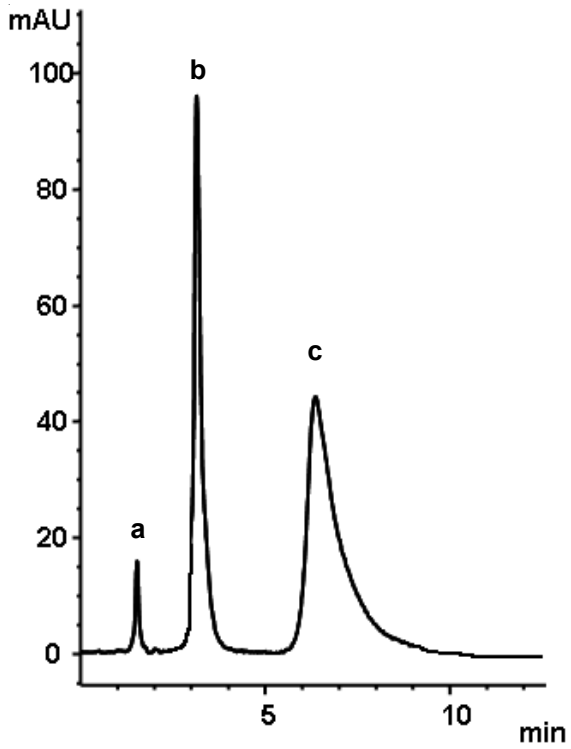

Fig. 2. HPLC chromatogram of subjected compounds containing 8 µg/mL PZB (a) 16 µg/mL CDP (b) and 16 µg/mL amitriptyline internal standard (c) at 206 nm

PLS method was run on the training data set of absorption UV spectra and optimum number of factor was selected. The selection of the number of factor used in the calibration with this method is very important for achieving for the best model development. The number of factors was estimated by cross-validation method. For the calculations, PLS TOOLBOX 4.0 was used. The program allowed to select the best factor by cross-validation method. In the method development step different factor numbers were tested and factor number 5 was found to be best for PLS method for the quantitative studies of experimental active compounds. Depending on the selected factor, calculated calibration data, prediction residuals sum for squares (PRESS) and standard error of calibration (SEC) were calculated and shown in Table-2.

For CLS calculations simple algorithm were written and computed in MATLAP 7.0 software. The calculated PRESS and SEC values were shown in same Table-2. Full spectral region was used for the calibrations as in PLS method. The selected region covers the maximum absorption spectrum of both active compound that gives comparative results with PLS. Summarized calibration information including SEC and PRESS is shown in Table-2.

TABLE-2
STATISTICAL PARAMETERS IN THE PLS AND CLS CALIBRATION STEPS

Parameters	PLS		CLS	
	CDP	PZB	CDP	PZB
PRESS	0.0741	0.0220	0.0879	0.0834
SEC	0.2723	0.1482	0.2965	0.2888
SEP	0.1692	0.0598	0.2778	0.2601
r	0.9984	0.9970	0.9992	0.9915
n	0.2743	0.0604	0.3943	0.3909
m	0.9889	0.9930	0.9631	0.9636

PRESS = Predicted residual sum of squares; SEC = Standard error of calibration; SEP = Standard error of prediction.

The PRESS values provide a measure of how well the calibration set is predicting the concentration for each number of factors. In the comparison of two methods PLS method shows the lower values of PRESS and SEC indicates the power of method for the quantitative calculations. Table-2 shows all the statistical parameters of calibration step including PRESS, SEC, standard error of prediction (SEP), correlation coefficient (r) standard error of intercept ($SE_{(n)}$) and standard error of slope ($SE_{(m)}$) for the chemometric methods. These parameters indicate the accuracy of method performance. Correlation values better than 0.99 were obtained in most of the cases, indicating excellent linear relationships between calculated and actual concentration values. The statistical results of the applied methods showed that CLS algorithm applied to absorption data evidence some difficulties of this model to resolve the less absorbing species.

First derivative of the spectra was calculated by $\Delta\lambda = 5$ nm intervals and resulting spectra were shown in Fig. 1b. After derivation of absorption spectra of compounds, zero crossing points were determined to establish two different calibration equations. For each compound a graph was plotted by using concentration *versus* absorption values. The obtained straight lines can be used for concentration calculation in synthetic mixtures and tablets. The amount of CDP and PZB in the binary mixture was found to be proportional to the signals and statistical parameters of their calibration equations summarized in Table-3.

The single point calibrations bring some problems for the resolution of overlapped spectra. The main reason of this particular case is that the subjected compound does not give maximum absorption at the zero-crossing point of other compound which will not be possible to get good calibration equations. The other reason could be the increased noise level after derivation especially for the higher order derivatives. This problem becomes important when the low absorbing species are in question as in present case. Obtained noise level in the derivatives is high especially in the long wavelength region. By using a classical smoothing procedures noise could be removed but it brings some other problems including deformation of the derivative curves, which is stronger in the short wavelength and weaker in the long wavelength region.

TABLE-3
STATISTICAL RESULTS OF CALIBRATION GRAPHS OBTAINED BY GRAPHICAL AND CHROMATOGRAPHIC METHODS

Method		λ (nm)	Regression equation	r	SE(m)	SE(n)	SE(r)
DER	CDP	284.4	$A = -0.0067C_{CDP} + 0.0010$	0.9997	0.0005	0.0009	0.0007
	PZP	224.6	$A = -0.0040C_{PZB} + 0.0010$	0.9982	0.0019	0.0002	0.0002
HPLC	CDP	206.0	$A = 0.0371C_{CDP} + 0.0711$	0.9992	0.0009	0.0149	0.0111
	PZB	206.0	$A = 0.0684C_{PZB} + 0.0017$	0.9993	0.0015	0.0128	0.0095

C = Concentration (µg/mL); A = Amplitudes at selected wavelength for CDP and PZB; r = Regression coefficient; SE(r) = Standard error of linear regression; SE(m) = Standard error of slope; SE(n) = Standard error of intercept.

The given chromatogram in Fig. 2 corresponds to the concentration of 8-24 µg/mL CDP and 4-12 µg/mL PZB. The detector responses were measured in terms of peak area. An internal standard amitriptyline was used during the studies. The regression equations are calculated from the calibration graphs, along with the standard deviations of the slope (Sb) and the intercept (Sa). The linearity of calibration graphs and conformity of the absorption measurements to Beer's law were proved by the high values of the correlation coefficients (r) of the regression equations. The obtained calibration equations are represented in Table-3. In the comparison of statistical parameters of calibrations, there is no significant difference between the methods.

HPLC is a powerful method for the quantitative determination of drugs in pharmaceutical preparations and it is the method of selection in pharmaceutical area for the analysis. Although it provides good results, it requires some extra work for method development that includes proper column, mobile phase, temperature and solvent selection. These requirements also bring extra time and money cost for the analysis of compounds. After method development, HPLC data also needs some treatment before the construction of calibration equations. In this method, peak area readings are also important case for a better calibration data and sometimes it is not possible to make correct peak area readings of low absorbing species in the mixtures. Existence of small noise and other instrument fluctuations will bring peak area reading errors that affects the calibration equations.

Method validation: The validity of the calibration models was tested evaluating precision (relative standard deviation), accuracy (% recovery) and linearity (regression equations). Thus, 5 different models were employed to predict the concentrations of the two analytes in the 15 samples of the validation set, with the results collected in Table-4, in terms of per cent recovery values varied between 95-105 %. The recovery error of each component in the mixture was calculated as the relative standard error (RSD) of the recovery per cent. All the results for the five methods are satisfactory with a % RSD < 3.0 and % recovery value 100 ± 5.

The chemometric methods were evaluated using statistical comparison of subjected methods on binary mixture of CDP and PZB. From the recovery results, subject error of prediction (SEP) values of each component by application of chemometric

TABLE-4
RECOVERIES OF CDP AND PZB IN VARIOUS SYNTHETIC MIXTURES BY SELECTED METHODS

No.	Added (µg/mL)		Found (µg/mL)								Recovery (%)							
	Method		PLS		CLS		DER		HPLC		PLS		CLS		DER		HPLC	
	CDP	PZB	CDP	PZB	CDP	PZB	CDP	PZB	CDP	PZB	CDP	PZB	CDP	PZB	CDP	PZB	CDP	PZB
1	8	8	7.97	7.98	8.08	8.25	7.66	8.20	7.96	8.21	99.7	99.7	100.9	103.2	95.7	102.5	99.5	102.7
2	12	8	12.02	8.03	12.35	8.29	11.93	8.14	11.74	7.84	100.2	100.4	103.0	103.7	99.4	101.8	97.9	98.0
3	16	8	16.04	8.00	15.59	8.08	15.46	8.27	15.95	8.16	100.3	100.0	97.4	100.9	96.6	103.4	99.7	102.0
4	20	8	20.04	8.03	19.80	7.71	19.21	8.21	20.05	7.78	100.2	100.3	99.0	96.4	96.0	102.6	100.3	97.3
5	24	8	23.96	7.99	23.64	7.83	23.86	8.18	24.33	7.98	99.8	99.9	98.5	97.8	99.4	102.2	101.4	99.8
6	16	4	15.91	4.00	15.39	4.12	15.92	4.06	15.88	4.09	99.5	100.0	96.2	103.0	99.5	101.6	99.2	102.3
7	16	6	16.00	6.02	15.54	6.11	15.82	6.28	15.76	6.10	100.0	100.3	97.1	101.8	98.9	104.6	98.5	101.6
8	16	8	15.97	7.99	15.86	8.32	15.81	7.85	15.54	8.17	99.8	99.9	99.1	103.9	98.8	98.1	97.1	102.1
9	16	10	16.03	10.00	15.73	9.96	15.63	10.36	15.87	9.92	100.2	100.0	98.3	99.6	97.7	103.6	99.2	99.2
10	16	12	16.02	11.98	15.77	11.72	15.50	12.24	15.72	12.18	100.1	99.8	98.5	97.7	96.9	102.0	98.3	101.5
11	8	6	8.23	5.84	7.97	6.21	7.60	6.20	8.07	6.03	102.9	97.3	99.6	103.5	95.0	103.4	100.9	100.6
12	12	4	12.31	4.11	12.23	4.14	11.95	3.92	11.87	4.13	102.5	102.9	101.9	103.5	99.6	98.1	98.9	103.2
13	8	8	8.19	7.89	8.15	8.15	7.62	8.25	8.07	8.20	102.4	98.6	101.9	101.9	95.3	103.1	100.9	102.4
14	16	10	16.36	9.98	15.79	10.12	15.71	9.78	15.81	10.07	102.2	99.8	98.7	101.2	98.2	97.8	98.8	100.7
15	24	8	24.47	7.89	23.63	7.74	23.86	8.24	24.29	8.14	102.0	98.6	98.4	96.8	99.4	103.0	101.2	101.7
										Mean	100.8	99.8	99.2	101.0	97.8	101.8	99.5	101.0
										SD	1.22	1.18	1.91	2.68	1.69	2.14	1.28	1.73
										RSD	1.21	1.18	1.93	2.66	1.73	2.10	1.29	1.71

DER = First derivative; SD = Standard deviation; RSD = Relative standard deviation.

methods were calculated and shown in Table-2. By considering these results, it is obvious that the predictive ability of PLS is better than CLS method.

The interference of excipients in the pharmaceutical tablet was studied in detail by PLS, CLS, first derivative and HPLC methods, therefore standard addition technique was applied to commercial tablets containing these two compounds. In application of standard addition method, standard deviations, standard errors and relative standard deviations for the proposed methods for 6 replicates were calculated and shown in Table-5. According to the obtained results a good precision and accuracy were observed for these methods. In the comparison of applied methods, PLS method produced the lowest RSD values for both drugs and obtained results are comparable with the HPLC method results. In general no interference was observed from the tablet excipients of two compounds.

TABLE-5
RESULTS OF STANDARD ADDITION METHOD APPLIED TO COMMERCIAL TABLET PREPARATION BY INVESTIGATED METHODS

	PLS		CLS		DER		HPLC	
	CDP	PZB	CDP	PZB	CDP	PZB	CDP	PZB
Mean	4.96	2.53	4.91	2.54	4.90	2.58	4.97	2.49
SD	0.01	0.01	0.04	0.02	0.05	0.03	0.02	0.04
RSD	0.24	0.52	0.78	0.87	1.00	1.21	0.48	1.71
Se	0.00	0.01	0.02	0.01	0.02	0.01	0.01	0.02
CL (p = 0.05)	0.01	0.01	0.03	0.02	0.04	0.02	0.02	0.03

SD = Standard deviation; RSD = Relative standard deviation; CL = Confidential limit; DER = First derivative.

The limit of detection (LOD) and the limit of quantitation (LOQ) for the 5 procedures were calculated from the calibration data and shown in Table-6. Both characteristic properties show the sensitivity of methods. While LOD is the lowest analyte concentration that instrument can detect, LOQ is the minimum quantifiable concentration. The developed methods are sensitive enough to determine as low as 0.14 µg/mL and as high as 0.41 µg/mL for LOD.

The selectivity of the method was determined by testing pharmaceutical preparation. These results showed that both the detection and the quantification limits were in acceptable range. Table-6 shows detailed information about LOD and LOQ for CDP and PZB for five methods. PLS and HPLC methods produced the best results for both LOD and LOQ.

The proposed methods were evaluated in the assay of commercial tablets. Six replicate determinations were made. The obtained results are satisfactory for the recovery of both drugs and were in good agreement with the label claims (Table-6). To evaluate the subjected methods, the results were compared with those obtained by an HPLC method.

In order to select the most appropriate procedures for this multiple determination, their performances were evaluated and a method comparison was carried out by

TABLE-6
RESULTS OF THE COMMERCIAL LIBKOL TABLET PREPARATION BY INVESTIGATED METHODS

	PLS		CLS		DER		HPLC	
	CDP	PZB	CDP	PZB	CDP	PZB	CDP	PZB
Mean:	4.96	2.54	4.92	2.57	4.90	2.54	5.05	2.55
SD	0.02	0.02	0.05	0.02	0.05	0.04	0.03	0.04
RSD	0.43	0.59	0.98	0.89	1.05	1.50	0.69	1.74
SE	0.01	0.01	0.02	0.01	0.02	0.02	0.01	0.02
CL (P=0.05)	0.02	0.01	0.04	0.02	0.04	0.03	0.03	0.04
ANOVA	2.5731	2.2283	2.5731	2.2283	2.5731	2.2283	2.5731	2.2283
$F_{theoretical}$	2.7587		2.7587		2.7587		2.7587	
$F_{calculated}$	0.1964	0.1938	0.1111	0.1356	0.1908	0.1672	-	-
$F_{theoretical}$	0.1980		0.1980		0.1980		-	-
LOD	0.25	0.14	0.41	0.34	0.41	0.37	0.15	0.21
LOQ	0.82	0.46	1.38	1.12	1.38	1.24	0.50	0.69

Lable claim (mg): 5 mg CDP, 2.5 mg PZB per tablet; Results obtained are average of 6 replicate for each method; SE = Standard error; CL = Confidential limit; LOD = Limit of detection; LOQ = Limit of quantitation.

means of ANOVA test and F tests. The ANOVA test and F-test results were compared and shown in Table-6. The obtained F vales are not bigger than the critical value of F. This result indicates that there is not a statistically significant difference between the methods utilized in this study.

The ANOVA test was carried out on the PLS, CLS, first derivative and HPLC tablet results indicated no statistical difference among these procedures. Therefore, it was concluded that they were suitable for the quantification purposes. F-test was applied to the tablet analysis results. HPLC method was taken as a standard method and others compared with the HPLC results. It can be observed that the concentrations of the active compounds were predicted with highly acceptable errors and that all of the commercial preparations proved to comply with the manufacturers declared amounts of ingredients.

Conclusion

In conclusion, we have applied various methods such as PLS, CLS and first derivative to UV absorption spectra for the simultaneous evaluation of mixtures containing CDP and PZB. Comparison of the different procedures indicated that PLS provides the best results among the utilized methods. The obtained results were proved that these methods are not statistically different in their ability to evaluate the two analytes. However, from a practical point of view, spectrophotometric methods do not require data pretreatment. The selection of methods completely depends on ones mathematical background. If the results are required more accurate and needs less analysis time the method of selection would be PLS method, but it requires more sophisticated mathematical background to evaluate the results. Graphical

method provides acceptable results with less mathematical background and less time consumption. Although HPLC provides good results, but it is more expensive and requires usage of additional chemicals, time consuming, needs a lot of time for method development and data treatment and laborious and required a lot of experimental preparation steps. Finally, it is concluded that the short analysis time, the accuracy and the low cost are the main advantages of these four spectrophotometric methods for the simultaneous determination of CDP and PZB in quality control tests such as dissolution and assay.

REFERENCES

1. J. Gasparic and J. Zimak, *J. Pharm. Biomed. Anal.*, **13**, 259 (1983).
2. M.I. Toral, P. Richter, N. Lara, P. Jaque, C. Soto and M. Saavedra, *Int. J. Pharm.*, **189**, 67 (1999).
3. S.A. Ozkan, N. Erk and Z. Senturk, *Anal. Lett.*, **32**, 497 (1999).
4. M.I. Walash, M. Rizk and A. Elbrashy, *Talanta*, **35**, 895 (1988).
5. M. Bedair, M.A. Korany and M.E. Abdelhamid, *Analyst*, **109**, 1423 (1984).
6. A. Elbrashy, F.A. Aly and F. Belal, *Microchim. Acta*, **110**, 55 (1993).
7. B.A. Koechlin, M.A. Schwatrz, G. Krol and W. Oberhansli, *J. Pharmacol. Exp. Th.*, **148**, 399 (1965).
8. B.A. Koechlin and L. D'Arconte, *Anal. Biochem.*, **5**, 195 (1963).
9. E. Tanaka, M. Terada, S. Misawa and C. Wakasugi, *J. Chromatogr. B*, **682**, 173 (1996).
10. V. Soentjenswerts, J.G. Dubois, G. Atassi and M. Hanocq, *J. Chromatogr. A*, **662**, 255 (1994).
11. M.A. Abuirjeie and M.E. Abdelhamid, *Anal. Lett.*, **22**, 951 (1989).
12. I.M. Jalal, S.I. Sasa and A. Hussein, *Anal. Lett.*, **20**, 635 (1987).
13. S.E. Roberts and M.F. Delaney, *J. Chromatogr.*, **283**, 265 (1984).
14. T.B. Vree, A.M. Baars, Y.A. Hekster and E. Vanderkleijn, *J. Chromatogr.*, **224**, 519 (1981).
15. R. Saelzer, G. Godoy, M. Vega, M. De Diego, R. Godoy and G. Rios, *J. AOAC Int.*, **84**, 1287 (2001).
16. C. Sarbu and G. Cimpan, *J. Planar Chromatogr.-Modern TLC*, **9**, 126 (1996).
17. S.M. Yuen and G. Lehr, *J. AOAC Int.*, **74**, 461 (1991).
18. M. Divoll, D.J. Greenblatt and R.I. Shader, *Pharmacology*, **24**, 261 (1982).
19. E. Alex, M.D. Finkbeiner, K. Nabil, M.D. Bissada, T. Larry and P.H.D. Welch, *Urology*, **10**, 503 (1977).
20. K.C.H. Yiu, E.N.M. Ho and T.S.M. Wan, *Chromatographia*, **59**, 45 (2004).
21. F.P.W. Tang, G.N.W. Leung and T.S.M Wan, *Electrophoresis*, **22**, 2201 (2001).
22. G.L. Green and T.C. O'Haver, *Anal. Chem.*, **46**, 2191 (1974).
23. J.J. Berzas Nevado, C.G. Cabanillas and F. Salinas, *Talanta*, **39**, 547 (1992).

(*Received*: 7 January 2008; *Accepted*: 25 September 2008) AJC-6888