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Chemometrics assisted quantitative estimation of synthetic and marketed formulations

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Abstract

Promising Four multivariate methods like CLS (Classical least square), ILS (Inverse least square), PCR (Principle component regression) and PLSR (Partial least square regression) were used for the determination of ternary mixture of Clidinium Bromide (CDB), Dicyclomine Hydrochloride (DICY) and Chlordiazepoxide (CDZ) in synthetic and market formulation. Overlapped data was quantitatively resolved by using chemometrics methods, *viz* CLS, ILS and PLSR methods. Calibrations sets were constructed by means of the absorption data matrix corresponding to the concentration data matrix. A prediction set design of the concentration data corresponding to the CDZ, DICY and CDB mixtures was prearranged statistically to maximize the information content from the spectra and to minimize the error of multivariate calibrations. By applying the respective algorithms for CLS, PLSR, PCR and ILS to the measured spectra of the calibration set, an appropriate model was obtained. This model was selected on the basis of % RSEP and % mean recovery values and the same was applied to the prediction set and tablet formulation. Mean recoveries of the marketed formulation set together with the figures of merit (calibration sensitivity, selectivity, limit of detection, limit of quantification and analytical sensitivity) were estimated. Validation of the proposed methods was successfully assessed for analysis of drugs in the various prepared synthetic mixtures and marketed formulation.

Keywords: Root mean squares error of cross-validation, figures of merit, classical least square, inverse least square, partial least square regression, multivariate.

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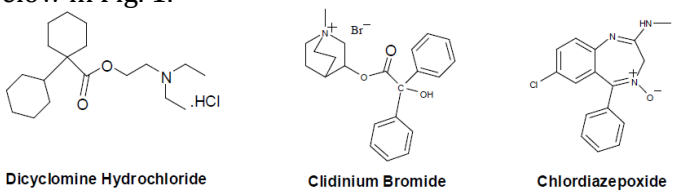
1. INTRODUCTION

The utmost difficulties with multi determination methods (HPLC and UV-Vis methods) come up when the analytes to be determined give partially or completely overlapped spectra. Multivariate calibration is a valuable tool in the analysis of multicomponent mixtures as it allows rapid and simultaneous determination of each and every component in the mixture with sensible accuracy and precision and devoid of the need of lengthy separation procedures. With the aid of modern instrumentation to acquire and digitize spectral information and dominant computers to process huge amounts of data, multivariate methods such as classical least squares (CLS), inverse least squares (ILS), partial least squares regression (PLSR) and principle component regression (PCR) are finding increasing use in quantitative analysis of complex mixtures, offering an interesting substitute to chromatographic techniques.

Dicyclomine hydrochloride (DICY) is an antispasmodic and anticholinergic agent. DICY is used in the cure of intestinal trouble called as irritable bowel syndrome [1] and also used for the antispasmodic action [2]. Clidinium bromide is anticholinergic and antisecretory agent which exerts its action by inhibiting the action of parasympathetic innervations hence reducing the secretions of abdomen acid and is also a mild antispasmodic. Chlordiazepoxide is a benzodiazepine. It also shows GABA facilitator action. It is used as anxiolytic [3], sedatives, hypnotics and skeletal muscle relaxants. Different marketed formulations are available for these ternary combinations like tablets of Normaxine, Curemaxine, Equital, Arvin fort etc. The combination of three drugs is extremely effective and used in the treatment of peptic ulcer, nervous dyspepsia, gastritis, irritable spastic colon, mucous colitis and acute enterocolitis.

Literature survey reveals that a few analytical methods have been used for the appraisal of Chlordiazepoxide, Dicyclomine hydrochloride and Clidinium bromide individually or combination [4] with other drugs. Chlordiazepoxide along with Clidinium Bromide assayed by means of non-aqueous method according to United States Pharmacopoeia (USP). Some few other methods for estimation for chlordiazepoxide and clidinium bromide in combined dosage form are derivative spectroscopy and spectrophotometry by means of multivariate calibration techniques and reverse phase high-performance liquid chromatography (RP-HPLC) techniques has been reported. Chlordiazepoxide also determined in single or along with its combination with other compound in pharmaceutical formulations using HPLC, UV/VIS spectrophotometry, derivative spectrophotometry [5], flow-injection potentiometry as well as voltammetry etc. Very few methods have been reported for estimation of Dicyclomine hydrochloride along with its combination with Clidinium bromide and Chlordiazepoxide [4].

Therefore our prime objective is to develop a selective, sensitive and accurate method for estimating these three components simultaneously by means of UV/Vis spectrophotometric combined with multivariate technique for simultaneous evaluation of CDB, CDZ and DICY containing bulk drugs and combined tablet [6] dosage forms in routine analysis and the developed method is validated according to the ICH guidelines to estimate. Structures of all three drugs were shown below in Fig. 1.



Dicyclomine Hydrochloride Clidinium Bromide Chlordiazepoxide

Figure 1: Structure of dicyclomine hydrochloride, clidinium bromide and chlordiazepoxide

1.1. Figures of Merit

For assessing analytical methods different parameters like, Sensitivity (SEN), selectivity (SEL), limit of detection (LOD) and limit of quantification (LOQ) are significant figures of merit. The potential of analytical method for quantifying analytes entirely depends upon these figures of merit and validation of analytical method based on the parameters established by the International Conference Harmonization (ICH) [7].

2. MATERIALS AND METHODS

In modern years, chemometrics calibration techniques, such as classical least squares (CLS), inverse least squares (ILS), principle component regression (PCR) and partial least square regression (PLSR) started to be applied to the analysis of analytical data obtained in all instrumentations. The major advantages of these techniques are higher speed of processing data regarding the values of concentrations and absorbance's of formulations with strongly overlapping spectra; errors of the calibration sets are decreased by measuring the absorbance values at many points in the wavelength range of zero-order spectra. In this work, lab prepared mixtures and formulations containing CDZ, DICY and CDB were investigated and resolved by four multivariate methods using zero-order spectra.

2.1. Classical Least Squares

This method assumes that Beer's law model with the absorbance at each frequency being comparative to the component concentrations [8]. Beer's law model for *m* calibration standards containing *l* chemical components with the spectra of *n* digitized absorbance's is given by:

$$A = C * K + E_1 \quad (1)$$

where *A* is the *m* × *n* matrix of calibration spectra, *C* is the *m* × *l* matrix of component concentration, *K* is the *l* × *n* matrix of absorptivity-path length products, and E_1 is the *m* × *n* matrix of spectral errors.

Analysis based on the spectrum of unknown components concentration (samples) is given by equation (2)

$$c_0 = (KK^T)^{-1}K * A \quad (2)$$

where c_0 is vector of predicted concentrations and K^T is transpose of the matrix *K*.

2.2. Inverse Least Squares:

This method treats these concentrations as a function of absorbance. The inverse of Beer's law model for *m* calibration standards with spectra of *n* digitized absorbance is given by:

$$c_0 = a^T * P \quad (3)$$

where c_0 and *a* represents concentration and spectrum of unknown analyte respectively. Since in ILS [8] the number of frequencies cannot exceed the total number of calibration mixtures used, stepwise multiple linear regressions have been used for the selection of frequencies.

2.3. Partial Least Square Regression:

PLSR is used to analyse strongly collinear and noisy data with numerous *X* variables (independent variables) and also simultaneously model the several response variables [9] i.e *Y* (dependent variables). MLR in which modelling of *Y* by means of *X* is done as long as when data is few and fairly uncorrelated [10]. However, in modern instrumentation only *X* variables are in larger numbers and also strongly correlated so that they are usually noisy and incomplete [11].

X scores estimate linear combination of variable x_k with coefficient of weight (*W**)

$$T = X.W^* \quad (4)$$

However, the weight *W* can be transformed to *W** which directly relate to *X*.

2.4. Principal Component Regression:

In the spectral work, the following steps can explain the elemental concept of Principal component regression: (*i*) the original data obtained in absorbances (*A*) and concentration (*C*) of the analyte is reprocessed by mean-centering as *A*0 and *C*0, respectively. (*ii*) The covariance dispersion matrix of the centered matrix *A*0 is computed. The normalized eigenvectors and eigenvalues are calculated starting from the square covariance matrix. The numeral optimal principal component (eigenvectors) is selected by considering only the highest values of the eigenvalues [8]. The other eigenvalues and their corresponding eigenvectors were eliminated from this study. Using the ordinary linear regression:

$$a = C_{mean} - A^T{}_{mean} * b \quad (5)$$

where C_{mean} is the mean concentration of the calibration set *and* A^T mean represents the transpose of the matrix having the mean absorbance values.

3. EXPERIMENTAL

3.1. Apparatus, Hardware and Software:

The complete UV-Vis spectrophotometric measurements were made with a Shimadzu 1700 double beam UV-Visible spectrophotometer with a fix slit width of 1 nm coupled with a computer loaded with Shimadzu UV Probe software of version 2.31. All spectra were saved in CSV format and then data was statistically analysed by unscrambler® 10.2.

3.2. Solvent, Stock and Standard Solutions and Pharmaceutical Formulations:

Analytical standards of chlordiazepoxide (CDZ), clidinium bromide (CDB) and dicyclomine hydrochloride (DICY) were obtained from CONSERN Pharmaceutical Pvt. Ltd., India. All the stock solutions were prepared by dissolving all drugs in Analytical grade methanol at a concentration of 1 mg/ml. All the working solutions for analytical determination were prepared in Analytical grade methanol. Tablet formulation of CDZ, CDB and DICY in their combined tablet dosage forms were obtained from market (Tab. Normaxine®).

3.3. Data Matrix for Analytical Estimation:

D-optimal experiment design has been used for preparation of suitable prediction or calibration set [12] in which we employed 6 concentration levels (*l*=6) and a training set of 19 mixtures was obtained which was prepared in separate 10 ml volumetric flask by adding appropriate volume of solution of drugs Table 1. A validation set of 12 synthetic ternary mixtures was prepared and six replicated measurements in each time were carried out for evaluating inter and intra-day variations. The UV absorption spectra were recorded over the wavelength range of 210–380 nm. The data points of the spectra were collected at interval of 1 nm. PCR and PLS were applied for determination of ternary mixtures using two latent variables and the entire characteristic obtained from calibration set was given in (Table 3). Overlay spectra of these three drugs has been shown in Fig. 2

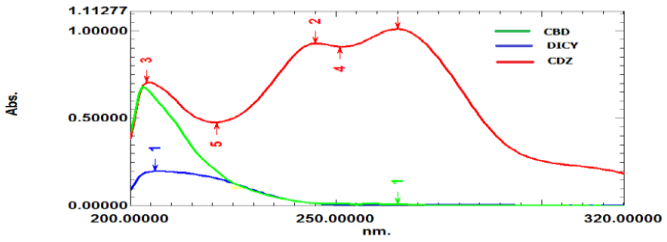


Figure 2: Overlay spectrum of CDZ (10 µg/mL), CDB (10 µg/mL) and DICY (10 µg/mL) in methanol.

So. No.	CDB	CDZ	DICY	So. No.	CDB	CDZ	DICY
1	5	6	20	**11**	2	3	5
2	3	4	10	**12**	12	13	55
3	20	21	95	**13**	16	17	75
4	15	16	70	**14**	14	15	65
5	18	19	85	**15**	9	10	40
6	4	5	15	**16**	7	8	30
7	19	20	90	**17**	11	12	50
8	17	18	80	**18**	8	9	35
9	6	7	25	**19**	10	11	45
10	13	14	60				

Table 1: Composition of the calibration (µg/mL)

The concentrations of these drugs were varied in the linearity range of every compound (4-16 µg/ml for CDB and CZD and 10-60 µg/ml for DICY). For the preparation of marketed sample twenty tablets were accurately weighed and crushed to a powder, a recognized quantity (equivalent to 2.5 mg CDB, 5 mg CZD and 10 mg DICY) was dissolved in methanol and followed by sonication for 8-10 minutes. Later than that the solution was filtered through a whatmann filter paper and transferred into 10 ml volumetric flasks.

4. RESULTS AND DISCUSSION

The spectra of the drugs in their ternary mixture displayed substantial overlapping as exposed in Fig 2. The other Conventional spectrophotometric methods failed to resolve them with acceptable accuracy. Therefore, there is a need for multivariate calibration methods for such kind of methodical estimations. This spectral overlapping was resolved successfully by Chemometrics methods.

RESULT FOUND											
(CLS)			(ILS)			(PCR)			(PLSR)		
CDB	**CDZ**	**DICY**	**CDB**	**CDZ**	**DICY**	**CDB**	**CDZ**	**DICY**	**CDB**	**CDZ**	**DICY**
101.7	102.16	100.9	99.6	99.1	98.4	100	101.5	100	100	101.6	100.4
102.59	100.03	97.76	97.8	99.75	98.5	101.75	99.7	100.3	100.3	101.75	100.25
99.65	100.05	102.26	98.1	98.1	99.8	99	101.8	100.6	101.5	99.5	99.9
101.33	100.12	100.3	98.8	97.7	97.9	102	102	100.3	101.1	100.5	101.1
96.26	99.13	108.59	98.1	98.3	97.6	100	100	99.9	100.7	100	100
98.49	100.14	99.82	97.7	98.7	98.7	103	101.33	100.7	99.6	99.3	99.9
98.16	100.1	99.81	98.14	98.1	99.4	100	100	100	100	100	100
98.41	99.48	99.54	97.5	99.5	98.1	101.3	99.9	99	101.8	100.6	101.2
102.69	99.98	98.11	97.8	97.8	99.5	101.33	100.5	102	102	100.3	100.66
93.65	98.42	100.54	98.9	97.2	99.1	100	99.6	100	101.3	99.9	100.7
99.83	99.94	102.83	98.1	93.1	97.9	99.8	99.7	101.3	101.3	100.7	99.6
100.43	100.16	100.53	98.2	99.2	98.9	101.3	99.9	99.7	101.1	100.6	101
% Mean recovery											
99.43	99.98	100.9	98.23	98.05	98.65	100.79	100.49	100.32	100.89	100.4	100.39
% RSEP											
0.509	0.872	1.007	0.501	0.883	1.027	0.431	0.584	0.761	0.422	0.572	0.74

Table 2: Mean % (Recovery and RSEP) of data of lab prepared mixtures by CLS, ILS, PCR and PLSR

Methods	CDB			CDZ			DICY		
	Range (nm)	r^2	n^a	Range (nm)	r^2	n^a	Range (nm)	r^2	n^a
CLS	210-349	0.9966	-	210-349	0.9951	-	210-350	0.9932	-
ILS	210-330	0.9954	-	210-330	0.9974	-	210-330	0.9914	-
PCR	203-350	0.9988	2	203-350	0.9999	2	203-350	0.9971	2
PLS	203-303	0.9975	2	203-303	0.9990	2	203-303	0.9976	2

Table 3: Characteristics of the calibration model (n^a is the number of latent variables employed)

4.1. Multivariate Calibration Methods

The superiority of chemometrics was dependent on the selection of optimum wavelength ranges. In order to select each analyte most appropriate spectral working region, a number of factors to be used in the PCR and PLS methods and minimum prediction residual error sum of squares (PRESS) search guided by moving a window of variable sizes were employed. For each wavelength interval, model numbers of factors were constructed and leave-one-out cross-validation strategy was employed.

$$PRESS = \sum(Y_{pred} - Y_{true})^2 \quad (6)$$

where Y_{pred} and Y_{true} are predicted and true concentrations in µg ml^{-1} respectively.

The root mean squares error of cross-validation (RMSECV) was designed for each method as given below:

$$RMSECV = \sqrt{\frac{PRESS}{n}} \quad (7)$$

where n is equal to total no of samples in calibration set. The RMSECV was used for probing the errors in the predicted concentrations. It indicates both accuracy and precision of predictions. It was recalculated upon varying the number of latent variables in PCR and PLS. Two factors were found to be optimum for each component by the PLS and PCR methods. Calibration information related to various multivariate methods was given in Table 3, which summarized the data of the optimum spectral regions, the number of factors required for the different methods and analytes and their associated statistical parameter r^2 which was found greater than 0.999 in most of cases and indicating excellent linear relationships between predicted and actual concentration values over the ranges of interest for most of the procedures and analytes.

Parameters	CDB				CDZ				DICY			
	CLS	ILS	PCR	PLS	CLS	ILS	PCR	PLS	CLS	ILS	PCR	PLS
PRESS	0.079	0.027	0.341	0.151	0.028	0.018	0.03	0.025	0.041	0.030	0.019	0.027
RMSECV	0.078	0.048	0.17	0.113	0.049	0.034	0.051	0.047	0.06	0.05	0.039	0.047
SEL	0.39	0.44	0.61	0.71	0.57	0.52	0.72	0.73	0.52	0.49	0.73	0.79
SEN(ml µg^{-1})	2.31	2.42	2.96	2.97	2.16	2.35	2.91	2.95	2.45	2.31	2.91	2.89
LOD(µg ml^{-1})	0.612	0.693	0.601	0.605	0.212	0.233	0.242	0.287	1.503	1.814	0.991	0.963
LOQ(µg ml^{-1})	1.897	2.140	1.863	1.875	0.658	0.722	0.751	0.861	4.510	5.623	3.071	2.889

Table 4: Statistical parameters obtained for CBZ, CDB and DICY through CLS, ILS, PCR and PLS

Percentage relative error of prediction (REP), signifying the quality of fit of all the validation data, can be calculated by using following equation:

$$\%\ REP = RMSECV * \frac{100}{\bar{c}} \quad (8)$$

where $\bar{c}$ is average concentration in validation set.

The validation of calibration model was performed on a validation set including 12 different mixtures of three analytes. Results obtained were collected in terms of over- all mean of the % recovery values and % RSEP and depicted in Table 2. The prediction error of a single component in the mixtures was calculated as the RSEP shown in Eq. (9).

$$RSEP(\%) = \frac{\sum_{j=1}^{N}(C_p - C_j)^2}{\sum C_j^2} * 100 \quad (9)$$

where, ^{N} is the number of samples, C_j and C_p is the concentration of the component in the $_{jth}$ mixture and its approximate concentration respectively. These values be acceptable and within confines as per ICH guidelines. A choice of analytical figures of merits and r^2 values are given in Table 3. Within utmost cases r^2 values are found to be more than 0.9977 which showed a high-quality linear relationship between absorbance and concentration.

Pharmaceutical product	Mean recovery ± S.D			
	CLS	ILS	PCR	PLS
CDZ	99.23 ± 0.73	99.35 ± 0.71	100.20 ± 0.76	100.27 ± 0.64
CDB	99.32 ± 0.69	99.26 ± 0.62	100.43 ± 0.65	100.32 ± 0.41
DICY	98.33 ± 0.74	99.13 ± 0.39	100.27 ± 0.71	100.12 ± 0.68
% Recovery[a]				
CDZ	99.84 ± 0.56	100.11 ± 0.35	100.12 ± 0.34	100.13 ± 0.45
CBD	100.03 ± 0.87	100.00 ± 0.25	100.03 ± 0.57	100.04 ± 0.61
DICY	99 .31± 0.54	100.01 ± 0.23	100 .01± 0.44	100.09 ± 0.57

Table 5: Determination of CDZ, CDB and DICY in pharmaceutical product using the proposed methods

5. APPLICATION IN PHARMACEUTICAL SAMPLES

The proposed method was evaluated in the assay commercial tablets (Normaxine) and the results obtained by the application of the different chemometrics methods on the pharmaceutical formulation were shown in table 5. Six replicate determinations were carried out on each experiment. These results confirm acceptable to the label claim and signify the high accuracy and precision of the proposed method when applied to tablets.

6. CONCLUSION

In our routine analysis, the major interest of analyst is to establish methods capable of analyzing a large number of samples in a short time period with due accuracy and precision. Large amount of data can be generated by spectrophotometric techniques within a short period of analysis; however, when coupled with chemometrics tools, the quality of the spectral information can be noticeably increased, converting this hyphenated technique into a powerful and highly convenient analytical tool.

In this paper, a comparative swot up the four more or less equivalent multivariate calibration methods used for the resolution of CDZ, CBD and DICY in their multicomponent mixtures provided a clear picture of high resolving power and low cost of this technique.

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