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Simultaneous Spectrophotometric Determination of Paracetamol and Dantrolene Sodium by Chemometric Methods

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Authors' contributions

This work was carried out in collaboration between all authors. Author IHH designed the study, wrote the protocol, the draft of the manuscript, managed the experimental process and the statistical analysis. Author RMT managed the literature searches and performed the spectroscopy analysis. Authors MSR, MS and DM managed the experimental process. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Simultaneous determination of Paracetamol (PAR) and Dantrolene Sodium (DAN) in pharmaceutical formulations was carried spectrophotometrically.
Study Design: Two different chemometric techniques namely Partial Least-Squares (PLS) and Classical Least Squares (CLS). In both techniques, a full factorial experimental design was done using three levels and both components in mixtures.

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Methodology: This design was used to prepare the concentration data matrix in solvent composed of 2.5 mM NaOH solution. The wavelength range of 220 – 500 nm was chosen with the intervals of Δλ= 1 nm, through which the corresponding absorbance data matrix was measured. Both of the absorbance and concentration data matrices were used to obtain regression. The regression was used for the prediction of the unknown concentrations of PAR and DAN in their mixture. Neither chemical separation steps nor prior graphical treatment of the overlapped spectra was required for this procedure.
Results: The linearity of calibration curve was fulfilled over concentrations of 1-20 and 1.5 – 15 µg/mL for PAR and DAN, respectively.
Conclusion: Validation was done for multivariate methods using both authentic mixtures and pharmaceutical preparations. Assessment of accuracy and precision was done for each method and results were compared.

Keywords: Paracetamol; dantrolene; spectrophotometric; chemometry.

ABBREVIATIONS

PAR : Paracetamol
DAN : Dantrolene Sodium
CLS : Classical Least Squares
PLS : Partial Least-Squares
HPLC : High Performance Liquid Chromatography
UV : Ultra Violet
UV-VIS : Ultra Violet – Visible
SVD : Singular Value Decomposition
PRESS : prediction error sum of squares
CI : Confidence Interval
RSD : Relative standard deviation
SEM : Standard Error of Mean

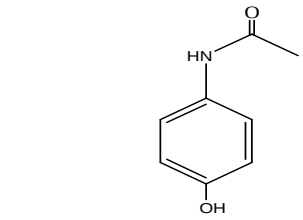
1. INTRODUCTION

Dantrolene sodium (DAN) is a skeletal muscle relaxant, that acts directly by preventing calcium from being released out of sarcoplasmic reticulum of skeletal muscle. Its chemical nomenclature is 1 (5- (p-nitrophenyl) furfurylmethylideneamino)imidazolidine-2,4-dione) sodium salt [1].

Paracetamol (PAR) is an anti-inflammatory drug that has analgesic and antipyritic activity. Its chemical nomenclature is N-(4-Hydroxy phenyl)-acetamide [1]; Fig. (1).

Several HPLC methods are used to determine DAN alone [2-7].

PAR is determined either alone or in combination with other drugs using different analytical methods including UV spectrophotometric [8-10], HPLC [11-13], electrochemical [14-15] and IR [16] methods. The two drugs in their mixture can be analytically determined by spectrophotometric simultaneous equations [17,18] and HPLC method [17,19] spectrophotometry based on zero order, first derivative and derivative ratio methods [20]. Our study aims to develop selective and sensitive methods for the determination of the two drugs by the use of Chemometric methods such as partial least squares PLS.



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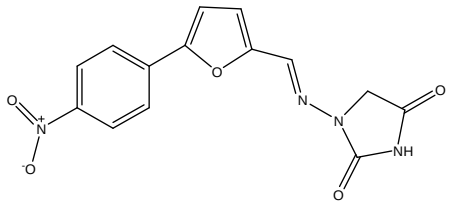

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Fig. 1. Chemical structures of PAR (a) and DAN (b)

2. MATERIALS AND METHODS

2.1 Apparatus

A JASCO V-530 double beam UV-VIS spectrophotometer with a fixed slit width (2 nm) and scan speed at 1200 nm/min connected to a computer loaded with Spectra Manager Program (JASCO) was used for spectral acquisition and elaboration of the data obtained. Quartz cuvettes, 1-cm path length, were used for measuring the light absorption in ultra violet and visible regions (200-500 nm).

2.2 Materials and Reagents

All the experiments were performed using pharmaceutical-grade authentic standards of PAR (Sigma Pharmaceuticals, Kowesnna, Egypt) and DAN (Adwia Pharmaceuticals, 10th Ramadan, Egypt), which were certified to contain 99.9 and 99.8% (w/w), respectively, on a dried basis.

All chemicals were used as received without further modification or purification.

The studied pharmaceutical formulation, DantRelax compound capsules, manufactured by Chemipharm Co., Egypt, were purchased from a local drugstore. Each capsule was declared to contain 300 mg PAR and 25 mg DAN.

2.3 Standard Solutions and Calibration Curves

Stock solutions were prepared by dissolving 20 mg each PAR and DAN in 5 ml 0.05 M NaOH and then completed to 100 ml with distilled water to obtain finally a concentration of 0.2 mg/mL.

To a series of 10-ml volumetric flasks, aliquots of PAR and DAN solutions were added and then diluted to 10 ml with 2.5 mM NaOH. Two sets of standard solutions were prepared, one series of individual solutions and another series of 9 mixtures as summarized in Table 1. The first one of data sets will be used for calibration and another for prediction step. UV spectra of the solutions were then recorded in the range of 200-500 nm against a blank of 2.5 mM NaOH solution.

2.4 Pharmaceutical Sample Preparation

DantRelax compound capsule containing 300 mg of PAR and 25 mg of DAN (Chemipharm Co., Egypt, BN 141642A) was transferred to a 1000 ml volumetric flask and 50 ml of 0.05 M NaOH solution was added and the solution was shacked vigorously and diluted to the mark with distilled water. A volume, 0.6 ml, of the solution were transferred to 10-ml volumetric flasks and then diluted to the mark with 2.5 mM NaOH. UV spectra of the triplicate solutions were then recorded in the range of 200-500 nm against a blank of 2.5 mM NaOH solution. The reproducibility of the method was studied by performing the assay on the same day (intra-day precision) and three different days (inter day precision).

Table 1. Composition of PAR and DAN in mixtures and individual solutions

#	µg/ml			
	Mixture solutions		Individual solutions	
	PAR	DAN	PAR	DAN
1	2	1.5	2	1
2	9	1.5	4	3
3	18	1.5	6	5
4	2	8	8	7
5	9	8	10	9
6	18	8	12	11
7	2	15	18	13
8	9	15	20	15
9	18	15	-	-

3. RESULTS AND DISCUSSION

As shown in Fig. 2, the absorption spectra of PAR and DAN in aqueous 2.5 mM NaOH solutions were severely overlapped obstructing the resolution of the mixture containing the two drugs to direct absorbance measurements. Such a determination could theoretically be facilitated by the use of chemometric methods. The spectra of PAR and DAN scanned over the wavelength range 220–500 nm with 1 nm intervals were selected for chemometric analysis. Wavelengths below 220 nm were rejected due to the noise appeared on repeating the measurement on the sample. It is evident that using 2.5 mM sodium hydroxide solution as solvent for PAR and DAN experimentally gave reproducible and stable spectra compared to traditional solvent of methanol.

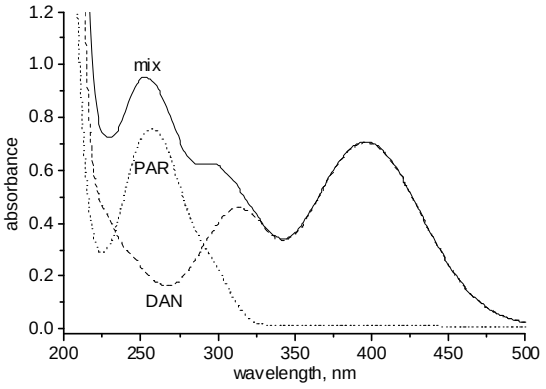

Fig. 2. Overlapped absorption spectra of PAR and DAN, 10 ug/ml each

Two different chemometric approaches namely classical least squares (CLS) and partial least squares (PLS) were evaluated to improve the recovery of these compounds.

A comparison of the different multivariate calibration methods for quantitative spectral analysis was done by Haaland and Thomas [21]. As the relative performance of different methods depends on the analyzed data set it was difficult to draw a general conclusion on superiority of one method over the other.

3.1 Classical Least Squares (CLS)

This method assumes Beer's law model with direct relation between absorbance at each wavelength and the component concentration.

$$A = KC + E$$

At 180 different wavelengths with dimension 2 components, the training set (calibration set) of absorptivity was used for constructing CLS model or (K) matrix. It's required that all the components in the calibration samples for the CLS method be known. The absorptivity matrix (K-matrix) was determined using the absorbance matrix A of the individual data set of mixtures (9x180) and their corresponding concentration matrix C (9x2) by the following equation:

$$K = (C^t . C)^{-1} . C^t . A$$

The prediction of unknown concentration C_u of the two components in both the mixture data set and pharmaceutical formulation samples was done by the obtained K-matrix as follows:

$$C_u = (K . K^t)^{-1} . K . A_u$$

Where A_u is spectra matrix of the unknown samples.

3.2 Partial Least Squares (PLS)

The components under investigation could be determined using PLS method even in the presence of unknown components such as interfering substance giving these two methods an advantage over CLS. However the PLS method consists of two steps compared to CLS method based on direct regression of the concentrations onto the spectroscopic responses.

The first step constitutes simultaneous decomposition of the spectroscopic data matrix A and the chemical data matrix C using either principal component analysis PCA or singular value decomposition SVD methods into a minimum number of linear products of two smaller matrices which are independent. This step is named principal component scores (eigenvalues) and loadings (eigenvectors). This is followed by calibration step which includes regression of the obtained scores against the concentrations as a [22].

The fact that each eigenvalue represents the relative importance of the associated eigenvector reveals the advantage arise from this decomposition.

A major factor indicated by large eigenvalue represents meaningful information. On the contrary a very small eigenvalue is considered as an unimportant factor representing mainly noise and can be neglected. This minimum number of eigenvectors/eigenvalues is collectively known as factor number. The experimental error is due to the remaining eigenvectors. under fitting or over fitting concentration data is risked if the data is either deficiently or excessively introduced.

To overcome under/over fitting the concentration data while evaluating the factor number, a pre-processing step is done by mean centring the raw data of the calibration samples [23] where the centring makes the following computations numerically well conditioning, without co-linearity, with minimum noise and no constant background. Calculation of the prediction error sum of squares (PRESS) of concentrations and leaving out one sample at a time is done using the cross validation method [20]. This calculation is used to measure the efficiency for a calibration fit model. The optimum number of factors could be the number that yielded the minimum PRESS.

$$\mathbf{PRESS} = \sum_{i=1}^{m} \left(\hat{c}_i - c_i\right)^2$$

Where ĉi is the calculated concentration and ci is the actual concentration for the ith sample left out of the calibration during cross validation.

The spectral data and their corresponding concentration data of 8 individual samples obtained 3 factors as optima values for determining PAR and DAN components as shown in Fig. 3. A minimum value for each component was obtained by prediction error sum of squares PRESS.

3.3 Validation of Training Set

The calibration and determination of the different concentrations of mixtures containing the two components can be predicted by the individual data set. As shown in Table 2, using PLS method, PAR and DAN which gave results with higher accuracy and precision than using CLS.

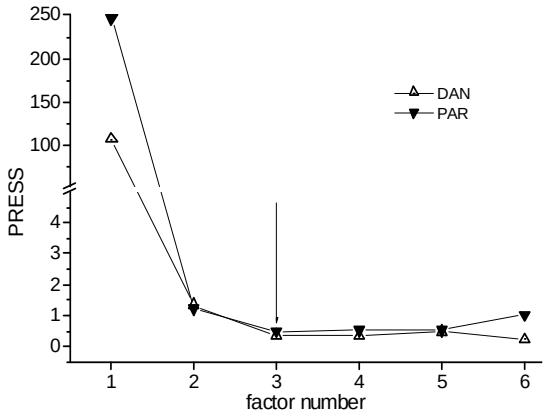

Fig. 3. Variation of PRESS with factor number per component

Confidence Interval CI is used to detect whether the source of error is due to systematic error (due to chemical interference or faulty calibration and expressed as accuracy, bias) or random error (express as RSD). The CI for the mean $\overline{x}$ is the interval (above and below) around true value x_t, with a given degree of certainly (or confidence level) [24]:

$$\overline{x} - x_t = \pm \frac{ts}{\sqrt{n}}$$

Where t is critical t-test value at confidence level and degrees of freedom (n-1) and s is the standard deviation If the left side is greater than the right the error is systematic while if it is reverse the error is random. The confidence interval CI revealed that at 95% confidence, the source of error is likely random on determining PAR and DAN by PLS as depicted in Table 2. Comparing between two unpaired groups (independent) assuming that variance of two populations (s_1^2 and s_2^2) are unequal, t-test is found similar accuracy by CLS or PLS while F-test gave precision by PLS better than CLS.

The proposed method PLS was then applied for the simultaneous determination of the two components in formulation samples as given in Table 3.

ICH (International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use) states that method precision may be considered at three levels: Repeatability, intermediate precision and reproducibility [25]. Repeatability is defined as the precision under the same operating conditions over a short time interval (intra-day precision). The intermediate precision and reproducibility are calculated the same way but under different conditions of different days, operators or equipments [26]. The former is performed by the same operator in different

Table 2. Results obtained by CLS for determining PAR and DAN using either individual or mixture data sets as training set

Taken, ug/ml		Found CLS, %		Found PLS, %	
PAR	**DAN**	**PAR**	**DAN**	**PAR**	**DAN**
2	1.5	109.19	109.84	94.52	105.96
9	1.5	105.55	97.35	100.63	96.59
18	1.5	108.05	97.87	104.00	97.56
2	8.0	98.28	109.11	97.70	107.01
9	8.0	97.05	95.60	99.92	97.94
18	8.0	96.23	97.80	99.51	99.14
2	15.0	96.22	99.53	97.43	108.01
9	15.0	97.33	99.15	99.03	101.77
18	15.0	95.68	97.09	97.52	98.50
Accuracy (bias), RE %		0.40	0.37	-0.53	0.81
Precision RSD, %		5.53	5.29	2.63	4.47
Confidence interval CI at *P=.05* and n=9		3.61	3.46	1.72	2.92
t-test = 2.20 at *P=.05* and df=11		0.455	0.193		
F-test =3.44 at *P=.05* and df=8		4.436	1.403		

Table 3. Recoveries of PAR and DAN in capsules obtained by PLS1 on the same day and on the three successive days

Taken, µg/ml 18 PAR+1.5 DAN	Found, % using PLS1 with auto scaling 3 factors							
	Intra-days[1]						Inter-days[2]	
	DAN			PAR			DAN	PAR
Capsule samples	1	2	3	1	2	3	Three days	
C1	101.01	106.40	102.29	95.51	96.30	94.30	103.23	95.37
C2	95.28	94.98	94.98	90.56	89.85	89.85	95.08	90.09
C3	101.01	100.54	101.53	95.51	95.26	94.54	101.03	95.11
C4	103.82	103.44	102.93	96.24	94.66	94.70	103.40	95.20
C5	103.47	105.14	106.40	94.47	94.98	96.30	105.01	95.25
Accuracy, bias,%	-0.92	-2.10	-1.63	5.54	5.79	6.06	-1.55	5.80
Precision, RSD	3.39	4.45	4.09	2.40	2.67	2.57	3.83	2.44
CI at *P=.05*	4.21	5.53	5.08	2.98	3.31	3.19	4.75	3.03

Table 4. Comparison between recoveries of PAR and DAN in capsules obtained by chemometric method and reported method

Items	Paracetamol		Dantrolene sodium	
	Proposed method	Reported method [b]	Proposed method	Reported method [c]
Mean [a]	99.752	99.884	99.852	100.16
SD	1.28529	1.261935	1.242143	0.953022
RSD%	1.288485	1.263401	1.243984	0.951499
SEM	0.5748	0.5644	0.5555	0.4262
Variance	1.65197	1.59248	1.54292	0.90825
n	5	5	5	5
Student´s t-test [d]	0.1639		0.4399	
F value [e]	1.037		1.699	

[a]*Average of three experiments*
[b]*Reported method for PAR*
[c]*Reported method for DAN*
[d]*The corresponding tabulated value of t equals to 2.306 at P=.05*
[e]*The corresponding tabulated value of F equals to 6.39 at P=.05*

days, while the latter is performed by different operators in different days. Only repeatability (intra-day precision) and intermediate precision (inter-day precision) were evaluated in this validation. It is expressed as percent relative standard deviation (RSD). The assay of 3 replicates of specific concentrations was performed to determine the repeatability of PLS method on the same day (intra-day precision). Also the same method was used to determine the intermediate precision over three different days (inter day precision). The RSD for all analytes was between 2.40-4.45% for intra- and inter-day results, which confirm good precision (Table 3). Comparison between recoveries of PAR and DAN in capsules obtained by chemometric method and reported method [20] (Table 4).

4. CONCLUSION

The proposed multivariate calibration method PLS was found to be simple, rapid, sensitive and give results with high precision and accuracy. This method proved that it could be successfully applied for the determination of PAR in the presence of DAN in dosage form without the need of any preliminary separation step. This method fulfils the linearity over concentrations of 1-20 and 1.5 – 15 µg/mL for PAR and DAN, respectively. Validation of the multivariate methods was done by using both authentic mixtures and pharmaceutical dosage forms.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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