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Research Article

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Spectrophotometric and Chemometric Methods for Simultaneous Determination of Alzheimer's drugs in Pharmaceutical Tablets

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ABSTRACT

In this study, precise, sensitive and accurate spectrophotometric-chemometric methods were developed for the Alzheimer's drugs in pharmaceutical tablets. The examined Alzheimer's drugs are donepezil and rivastigmine. The used chemometric methods are partial least squares regression (PLS) and principal component regression (PCR). PLS and PCR were successfully applied for chemometric analysis of donepezil and rivastigmine in synthetic mixtures and pharmaceutical tablets. A concentration set including binary mixtures of donepezil and rivastigmine formed to 10 different combinations were randomly prepared in 0.1 M HCl. The accuracy and precision of the developed method were validated by analyzing synthetic mixtures containing the examined drugs. As a result of the determination, high recoveries and low standard deviations were found. Absorbance and concentration values were used in Minitab and other chemometric programs to calculate estimated concentrations with PCR and PLS. The second step, in drug tablets (Exelon and Doenza), was calculated amounts for donepezil and rivastigmine.

Keywords: *Rivastigmine, Donepezil, PLS, PCR, Chemometry*

INTRODUCTION

Alzheimer's illness is the most common reason of dementia[1]. Alzheimer's illness is a neurodegenerative disorder characterized by progressive loss of memory followed by complete dementia[2]. In this study, donepezil and rivastigmine of Alzheimer's drugs were determined by spectrophotometric-chemometric methods. Donepezil is chemically2,3-dihydro-5,6-dimethoxy-2-[[1-(phenylmethyl)-4-piperidinyl]methly]-1H-inden-1-one hydrochloride[3]. It is an acetyl-cholinesterase inhibitor[4]. Donepezil is a piperidine-based reversible inhibitor, the predominant cholinesterase in the brain[5]. The most commonly used techniques for the determination of donepezil are LC[6], RP-HPLC[7], LC-MS/MS[8] and spectrophotometric [1,9] methods.

Rivastigmine is chemically (S)-3-[(1-dimethylamino)ethyl]-N-methylphenly-carbamate hydrogen tartrate [10]. It is a carbamate inhibitor of acetylcholinesterase used şn the treatment of Alzheimer's disease in adults[11]. It shows high brain region selectivity for the hippocampus and cortex[12]. HPLC[11], LC-ESI-MS/MS and LC-UV methods[10], spectrofluorimetric method[13], voltammetry[14], HPLC-MS and UPLC-MS/MS[15] methods are used techniques for the determination of rivastigmine.

Simultaneous quantitative analysis of pharmaceuticals is difficult to perform by the classical spectrophotometric method to overlapping spectra[16]. Because of this reason; in this study, multivariate calibration methods were applied as partial least squares regression (PLS) and principle component regression (PCR)[17,18].

Experimental

Reagents and chemicals

All materials used were analytical grade. Stock solutions of 25 mg/250 mL of donepezil (Sigma), rivastigmine (Sigma) were prepared with 0.1 M HCl. A training set and validation set containing the drugs in various proportions, ten synthetic mixtures synthetic mixtures (for validation) was made.

Low conductivity water (0.05 S/cm) was obtained using Millipore's Milli-Q Integral lab water purification system.

Apparatus

A Shimadzu UV-1700 PharmaSpec Spectrophotometer connected to an IBM PS with UV Probe Software was used for all measurements and data processing. A pair of 1.0 cm quartz cuvettes were used for absorbance measurements.

Procedure

Absorbance spectra, donepezil and rivastigmine both points and the corresponding spaces 200-400 nm were recorded. The calibration matrix and training and validation sets contain three component mixtures, at different rates and optimized, and the resulting spectra analysis and analysis of real samples to calculate concentrations have been calculated using PLS and PCR.

Samples of 7.0 and 45.00 (µg/ml) between drugs (alone or in combination) were placed in volumetric flasks, 25 mL and an aliquot containing 0.1 M HCl were added. The mixture was shaken for 20 minutes and filtered. Dry, tight standards were prepared in the same manner as described except those with the reagents and drug. The concentrations prepared from donepezil and rivastigmine for the PLS and PCR calibrations are listed in Table 1.

Table 1. Concentration Set for Donepezil and Rivastigmine

No	Donepezil ppm	Rivastigmine ppm
1	7	9
2	7	18
3	7	36
4	14	9
5	14	18
6	14	36
7	27	9
8	27	18
9	27	27
10	28	9

CHEMOMETRIC METHODS

In this study; partial least squares (PLS) and principal component regression (PCR) were used as chemometric methods. PLS[19,20] and PCR[21,22] are multivariate calibration methods which have many of the full spectra avantages and have been successfully applied to the spectrophotometric analysis of multicomponent mixtures. PLS method is based on spectral variations without regard for the component concentrations. In PLS method, the spectral decomposition is weighted to the concentration. The significant difference in predictive abilities of these two approaches in that PLS seems to predict better than PCR methods[23].

The Minitab 17 program (İnova, Ankara, Turkey) was used for the analysis of all the concentration and absorbance data and to do the statistical calculations. Minitab is a statistical analysis software. In addition to statistical research, statistics can be used to learn[24].

Pharmaceutical Preparations

Commercial drug preparations; Exelon and Doenza per tablet were analyzed by principal component regression (PCR) and partial least squares regression (PLS) chemometric methods.

For this purpose, 1-gram sample of drug was mechanically mixed into 0.1 M HCl and transferred into 25mL bottles. All the techniques were applied to the final solution.

RESULTS AND DISCUSSION

Donepezil and rivastigmine are both in the visible region of high absorbent substances. Figure 1. shows the absorbance-wavelength (nm) curves. The spectrum of donepezil and rivastigmine are in the range of 200-400 nm.

Figure 1. shows absorption spectra for Donepezil and rivastigmine hydrochloride mixtures in 0.1 M HCl

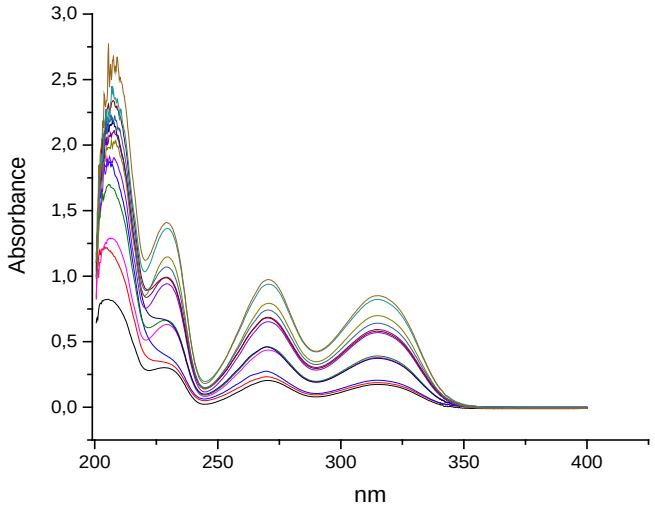

Fig 1. The spectrum of donepezil and rivastigmine mixtures

Our objective of this study is to develop a lower-cost but more quick and reliable analytical method using chemometry. With this approach, active ingredients can be analyzed without pre-separation, and loss of time and work due to the trial and error method will be prevented.

The PLS method and absorption spectra can be used individually or be overlapping for multiple simultaneous detections of very linear components. Some statistical parameters were given for validation of calibrations for synthetic mixtures of drugs.

Table 2. Composition of prediction set and recovery results obtained in synthetic mixtures for PLS method

	Donepezil			**Rivastigmine**		
Mixture No	**Actual (ppm)**	**Predicted (ppm)**	**% Recovery**	**Actual (ppm)**	**Predicted (ppm)**	**% Recovery**
1	7	6.99	99.85	9	9.01	100.11
2	7	6.87	98.14	18	17.97	99.83
3	7	6.92	98.85	36	35.56	98.77
4	14	13.96	99.71	9	8.98	99.77
5	14	13.84	98.85	18	17.97	99.83
6	14	13.92	99.42	36	35.86	99.61
7	27	26.95	99.81	9	8.96	99.55
8	27	25.89	95.88	18	17.94	99.66
9	27	26.45	97.96	27	26.87	99.51
10	28	27.94	99.78	9	8.95	99.44
			Mean=99.04 SS=1.15			Mean=99.43 SS=0.68

Table 3. Composition of prediction set and recovery results obtained in synthetic mixtures for PCR method

	Donepezil			Rivastigmine		
Mixture No	**Actual (ppm)**	**Predicted (ppm)**	**% Recovery**	**Actual (ppm)**	**Predicted (ppm)**	**% Recovery**
1	7	6.97	99.57143	9	8.89	98.77778
2	7	6.32	90.28571	18	17.85	99.16667
3	7	6.85	97.85714	36	36.01	100.0278
4	14	13.89	99.21429	9	9	100
5	14	13.94	99.57143	18	17.89	99.38889
6	14	13.97	99.78571	36	35.87	99.63889
7	27	26.96	99.85185	9	8.84	98.22222
8	27	26.87	99.51852	18	17.78	98.77778
9	27	26.23	97.14815	27	26.79	99.22222
10	28	27.94	99.78571	9	8.94	99.33333
			Mean =98.64 SS=2.65			Mean =99.12 SS=0.67

In this study, the statistical parameters were found to produce a satisfactory validity for the PLS and PCR methods. The PLS and PCR methods have reliable accuracy, and higher precision for calibration, the residual prediction error sum-of-squares (PRESS) was calculated as:

$$PRESS = \sum_{i=1}^{n} (C_i^{added} - C_i^{found})^2 \quad (1)$$

C_i^{added} : Actual Concentration, the added concentration of drug

C_i^{added} : Predicted Concentration, the calculated concentration of drug

The RMSEC can provide a good measure of how well the calibration model performs.

According to the actual and predicted concentrations of the samples, PRESS values of metronidazole and metoclopramide were informed and listed in Table 4.

Some statistical parameters determined the effectiveness of the calibration. The standard error of prediction (SEP) was calculated using the following expression:

$$SEP = \sqrt{\frac{\sum_{i=1}^{n} (C_i^{added} - C_i^{found})^2}{n-1}} \quad (2)$$

C_i^{added} : Actual Concentration, the added concentration of drug

C_i^{found} : Predicted Concentration, the calculated concentration of drug

n: the total number of synthetic mixtures

Table 4. Statistical parameter values for calibration step- simultaneous determination of Donepezil and Rivastigmine using partial least square and principal component regression methods

Parameters	**Methods**	**Donepezil**	**Rivastigmine**
SEP	PLS	0.076	0.032
	PCR	0.069	0.039
PRESS	PLS	0.16	0.024
	PCR	0.112	0.019

Analysis of pharmaceutical formulation (mg/tablet)

Table 5. lists the experimental results of the two numerical methods for pharmaceutical formulation and as you can see, the obtained results are very close to each other.

Table 5. Determination of Donepezil and Rivastigmine in pharmaceutical formulation using PLS and PCR methods

	Donepezil (gram)		**Rivastigmine (gram)**	
NO	PLS	PCR	PLS	PCR
1	4.89	4.9	4.48	4.45
2	4.87	4.95	4.45	4.46
3	4.95	4.96	4.47	4.42
4	4.96	4.97	4.46	4.4
5	4.9	4.98	4.43	4.45
Mean	4.914	4.952	4.458	4.436
Standard Deviation	0.039	0.031	0.019	0.025

To compare the performances of the investigated chemometric techniques according to UV spectrophotometric method for real samples, we applied Snedecor's F-test.

The method used to compare the differences between the one-way ANOVA test was applied to the actual samples for each food drug. In this study, Snedecor's F-values were calculated and compared with the F value. The same computation process was repeated for each drug. Table 6 shows ANOVA results. The experimental (calculated) F-values did not exceed the F-value in the variance analysis. Among all these methods, it was concluded that there was a meaningful difference. All the statistical parameters and numeric values are suitable for simultaneous identification in the actual samples.

Table 6. The Results of the one-way ANOVA test (PLS and PCR)

		$F_{calculated}$- PLS		**$F_{critical}$-PLS**	
		Donepezil	**Rivastigmine**	**Donepezil**	**Rivastigmine**
Between groups		0.00316	0.000376	4.11	
Within groups	46				
Total	47				
		$F_{calculated}$- PCR		$F_{critical}$-PCR	
Between groups		0.00255	0.00056	4.11	
Within groups	46				
Total	47				

CONCLUSIONS

The partial least squares method and principle component regression all successfully applied at the same time were able to identify drugs in synthetic solutions and pharmaceutical formulation. For all the values, low prediction errors and high correlation coefficients emphasize the high linear relationship between the predicted and actual concentrations. The results obtained with this binary mixture and some ratios of component concentrations show excellent predictive ability with these methods.

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