SIMULTANEOUS SPECTROPHOTOMETRIC DETERMINATION OF DIACEREIN AND CELECOXIB IN CAPSULES BY CHEMOMETRIC METHODS

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ABSTRACT

Simultaneous spectrophotometric determination of diacerein and celecoxib was performed by partial least-squares (PLS) and principal component regression (PCR) methods do not require any priori graphical treatment of the overlapping spectra of two drugs in the mixture. The absorbance values in the UV-Vis spectra were measured for the 64 wavelength points (from 225-288) in the spectral region 200–400 nm considering the intervals of 1 nm. The calibration range was found to be 2-10 µg/ml for diacerein, 4-20 µg/ml for celecoxib with a correlation coefficient of 0.9999(PLS), 0.9998(PCR) for diacerein and 0.9999(PLS), 0.9999(PCR) for celecoxib. The validation of the multivariate methods was realized by analyzing the synthetic mixtures of diacerein and celecoxib. The numerical calculations were performed with the 'Unscrambler 10.1 X' software. The chemometrics analysis methods were satisfactorily applied to the simultaneous determination of diacerein and celecoxib in the pharmaceutical formulation.

Key words: celecoxib, chemometrics, diacerein, spectrophotometry, partial least square, principal component regression.

INTRODUCTION

Diacerein (DIA) is chemically 1, 8-Diacetoxy-3-carboxyanthraquinone which is used as an analgesic, antipyretic and anti inflammatory Agent¹⁻². Celecoxib (CELE) chemically described as 4-[5-(4-methylphenyl)-3-(trifluoromethyl) pyrazol-1-yl]benzenesulfonamide is a selective cyclooxygenase-2 (COX-2) inhibitor approved for relief of the signs and symptoms of inflammation associated with rheumatoid arthritis and osteoarthritis³. From the literature survey it was found that HPLC and spectrophotometric methods are available for the determination of diacerein⁴⁻⁸ and celecoxib⁹⁻¹⁶ alone and combined dosage form with other drugs but there is no method available for the simultaneous estimation of diacerein and celecoxib in combined dosage form by chemometrics. So there is a need for a method capable of simultaneous estimation of diacerein and celecoxib in pharmaceuticals by chemometric methods.

The aim of this paper is to investigate the ability of PLS and PCR methods to quantify binary mixture of DIA and CELE with overlapping spectra and to apply the optimized models in pharmaceutical preparations. The proposed methods are simple, sensitive and reproducible method for the simultaneous estimation of diacerein and celecoxib from combined dosage form.

In recent years, multivariate calibrations, such as classical least-squares (CLS), inverse least-squares (ILS), principal component regression (PCR) and partial least-squares (PLS) are started to apply to the analysis of the analytical data obtained in all the instrumentations¹⁷⁻¹⁸. The same methods and their algorithms (NIPALS) have been applied to the simultaneous spectrophotometric determination of drugs in the pharmaceutical formulation containing two or more compounds with overlapping spectra. On the other hand the chemometric calibration methods as those enumerated above have been used extensively in quantitative spectral analysis to get selective information from unselective data. The main

advantages of these techniques are the following: a higher speed of processing data concerning the values of concentrations and absorbance of compounds with strongly overlapping spectra, the errors of calibration model are minimised by measuring the absorbance values at many points in the wavelength range of the zero-order and derivative spectra. Analytical methods using multivariate calibrations and their applications include the spectrophotometric, chromatographic and electrochemical for determinations of analytes in the mixtures.

MATERIALS & METHODS

Instruments and software

Digitized UV/VIS absorbency spectra were collected using a UV-visible spectrometer 2300 Techcomp with 1 cm quartz cells. The data acquisition was made with UV solutions software at a scan rate of 1000 nm min⁻¹ and the slit width of 2 nm. The UV spectra of mixtures were recorded over the wavelength 225–288 nm with one data point per nm. All spectral measurements were performed using blank solution as a reference. Partial least squares regression, and principal component regression were used for chemometric analysis of data. For all calculations Unscrambler for windows (Version 10.1 X) was used.

Pharmaceutical tablet formulations

A commercial pharmaceutical formulation (OSTIGARD - 100) capsule containing 50 mg of DIA and 100 mg of CELE was analysed by the proposed chemometric methods.

Standard solutions

Stock solutions of Diacerein and celecoxib of 10 mg were prepared in 100 ml volumetric flasks with methanol. The training set containing 2-10µg/ml diacerein and 4-20µg/ml celecoxib working standard solutions were prepared by diluting the stock solutions for each drug according to its linear calibration range. Two sets of standard solutions were

prepared, the calibration set contained 25 standard solutions and the prediction set contained 9 standard solutions. To a series of 10 ml volumetric flasks, aliquots of diacerein and celecoxib solutions, containing appropriate amount of these drugs in the range of calibrations, were added and then the solutions were diluted to 10 ml with methanol. UV spectra of the mixtures were recorded in the wavelength range 225–288 nm versus a solvent blank, and digitized absorbance was sampled at 1 nm intervals. All the solutions were prepared freshly and were protected from light.

Sample preparations

Twenty capsules were accurately weighed and powdered in a mortar. An amount of the powder equivalent was weighed and dissolved in methanol in 100 ml calibrated flasks. 20ml of methanol was added and ultra sonicated for 25 minutes and the volume was made up to 100 ml with methanol and shake well. Then, the solution was filtered through what man filter paper No. 41 and the residue was washed three times with 10 ml of solvent, and then the volume was completed to 100 ml with methanol. The resulting solution was diluted to 1:2 in a 100 ml calibrated flasks. Both techniques were applied to the prepared sample solutions.

TABLE 1 COMPOSITION OF CALIBRATION SET (TRAINING SET) FOR PLS AND PCR METHODS

S.NO	Diacerein			Celecoxib		
	Reference $\mu\text{g/ml}$	Predicted $\mu\text{g/ml}$		Reference $\mu\text{g/ml}$	Predicted $\mu\text{g/ml}$	
		PLS	PCR		PLS	PCR
1	2	2.03	2.03	4.00	4.08	4.08
2	2	2.02	2.02	8.00	7.97	7.97
3	2	2.01	2.01	12.00	11.96	11.96
4	2	2.00	2.00	16.00	16.15	16.15
5	2	1.99	1.99	20.00	19.94	19.94
6	4	4.08	4.08	4.00	4.07	4.07
7	4	4.06	4.06	8.00	7.96	7.96
8	4	4.05	4.05	12.00	11.95	11.95
9	4	4.04	4.04	16.00	16.14	16.14
10	4	4.03	4.03	20.00	19.94	19.94
11	6	5.97	5.97	4.00	4.06	4.06
12	6	5.96	5.96	8.00	7.96	7.96
13	6	5.95	5.95	12.00	11.95	11.95
14	6	5.94	5.94	16.00	16.14	16.14
15	6	5.93	5.93	20.00	19.93	19.93
16	8	8.11	8.11	4.00	4.06	4.06
17	8	8.10	8.10	8.00	7.95	7.95
18	8	8.09	8.09	12.00	11.94	11.94
19	8	8.08	8.08	16.00	16.13	16.13
20	8	8.07	8.07	20.00	19.92	19.92
21	10	9.96	9.96	4.00	4.05	4.05
22	10	9.94	9.94	8.00	7.94	7.94
23	10	9.93	9.93	12.00	11.93	11.93
24	10	9.92	9.92	16.00	16.12	16.12
25	10	9.91	9.91	20.00	19.91	19.91

TABLE 2 COMPOSITION OF VALIDATION (PREDICTION SET) FOR PLS AND PCR METHODS

S.NO	Diacerein			Celecoxib		
	Reference $\mu\text{g/ml}$	Predicted $\mu\text{g/ml}$		Reference $\mu\text{g/ml}$	Predicted $\mu\text{g/ml}$	
		PLS	PCR		PLS	PCR
1	3	3.09	3.09	6.00	6.07	6.07
2	3	3.08	3.08	10.00	9.96	9.96
3	3	3.07	3.07	14.00	14.25	14.25
4	5	4.97	4.97	6.00	6.06	6.06
5	5	4.96	4.96	10.00	9.96	9.96
6	5	4.94	4.94	14.00	14.25	14.25
7	7	7.01	7.01	6.00	6.06	6.06
8	7	7.00	7.00	10.00	9.95	9.95
9	7	6.99	6.99	14.00	14.24	14.24

TABLE 3 SUMMARY OF STATISTICS IN PLS AND PCR METHODS

Drug	RMSEP		RMSEC		r^2		Intercept		Slope	
	PLS	PCR	PLS	PCR	PLS	PCR	PLS	PCR	PLS	PCR
DIA	0.0546	0.0546	0.0623	0.0623	0.9999	0.9998	0.0422	0.0422	0.9941	0.9941
CELE	0.0148	0.0148	0.082	0.082	0.9999	0.9999	0.0377	0.0377	0.9976	0.9976

Dia-diacerein, Cele- celecoxib, RMSEP-Root mean square error of prediction, RMSEC-Root mean square error of calibration and r^2 - Correlation coefficient.

TABLE 4 ANALYSIS OF TABLET FORMULATION

Formulation	Label claim	PLS mg/cap found*	PCR mg/cap found*
OSTIGARD 100	Dia 50mg	49.66	49.67
	Cele 100 mg	99.74	99.75

*Each value is a mean of six readings

TABLE 5 PRECISION DATA

S.NO	System precision				Method precision			
	Diacerein		Celecoxib		Diacerein		Celecoxib	
	PLS	PCR	PLS	PCR	PLS % purity	PCR % purity	PLS % purity	PCR% purity
1.	5.95	5.95	11.95	11.95	99.48	99.48	99.91	99.91
2.	5.94	5.94	11.92	11.92	99.05	99.05	99.47	99.47
3.	5.95	5.95	11.95	11.95	99.59	99.59	100.02	100.02
4.	5.96	5.96	11.97	11.97	99.65	99.65	100.08	100.08
5.	5.95	5.95	11.95	11.95	99.13	99.13	99.55	99.55
6.	5.96	5.96	11.97	11.97	99.39	99.39	99.81	99.81
Mean	5.95	5.95	11.98	11.98	99.38	99.38	99.81	99.81
S.D	0.0087	0.0087	0.02	0.02	0.2460	0.2460	0.2471	0.2471
%RSD	0.1462	0.1462	0.15	0.15	0.2475	0.2475	0.2475	0.2475

TABLE 6 RECOVERY STUDIES

% of Target	Diacerein					Celecoxib				
	PLS			PCR		PLS			PCR	
	Added mg	Found mg	% Recovery	Found mg	% Recovery	Added mg	Found mg	% Recovery	Found mg	% Recovery
80	4.8	4.75	99.03	4.75	99.03	10.00	9.99	99.9	9.99	99.9
	4.8	4.76	99.14	4.76	99.14	10.00	9.96	99.6	9.96	99.6
	4.8	4.73	98.53	4.73	98.53	10.00	10.01	100.1	10.01	100.1
	Mean	4.75	98.90	4.75	98.90	Mean	9.98	99.8	9.986	99.86
	SD	0.0152	0.32	0.0152	0.32	SD	0.025	0.32	0.025	0.32
	%RSD	0.3218	0.33	0.3218	0.33	%RSD	0.252	0.33	0.252	0.33
100	6	5.93	98.76	5.93	98.76	12.00	11.90	99.18	11.90	99.18
	6	5.94	99.02	5.94	99.02	12.00	11.93	99.44	11.93	99.44
	6	5.91	98.51	5.91	98.51	12.00	11.87	98.93	11.87	98.93
	Mean	5.926	98.76	5.926	98.76	Mean	11.9	99.19	11.9	99.19
	SD	0.0152	0.25	0.0152	0.25	SD	0.03	0.25	0.03	0.25
	%RSD	0.2577	0.26	0.2577	0.26	%RSD	0.252	0.26	0.252	0.26
120	7.2	7.15	99.30	7.15	99.30	14.00	14.36	102.57	14.36	102.57
	7.2	7.13	98.98	7.13	98.98	14.00	14.31	102.24	14.31	102.24
	7.2	7.11	98.81	7.11	98.81	14.00	14.29	102.07	14.29	102.07
	Mean	7.13	99.03	7.13	99.03	Mean	14.32	102.30	14.32	102.30
	SD	0.02	0.25	0.02	0.25	SD	0.036	0.26	0.036	0.26
	%RSD	0.2805	0.25	0.2805	0.25	%RSD	0.251	0.25	0.251	0.248

Figure 1 Overlaid UV spectra of Diacerein, Celecoxib and Mixture

Figure 2 Calibration spectra of Diacerein and Celecoxib

Figure 3 Prediction spectra of Diacerein and Celecoxib

Figure 4 Recovery spectra of Diacerein and Celecoxib

RESULTS & DISCUSSIONS

The absorption spectra of DIA and CELE solutions in methanol recorded between 225 - 288 nm were shown in Figure 1. The two drugs show an overlap in their absorption.

Experimental design of sample sets

Calibration and test sets for two component systems were designed according to factorial principle five-level factorial design was used to produce a calibration set (Training step) of 25 samples. Calibration spectra are shown in Figure 2. A three-level set was derived to produce a prediction set (Validation step) of nine samples. Prediction spectra are shown in Figure 3. The compositions of the used calibration and Validation sets are summarized in Tables 1 & 2 respectively.

Selection of optimum number of factors and the spectral region

The most commonly employed validation criterion is to divide the dataset into two subsets, a calibration set and a validation set. The calibration model is calculated using the

calibration set. Then, the root mean square errors of calibration and validation, RMSEC – root mean square error of calibration and RMSEP – root mean square error of prediction, are calculated using the calibration model under investigation to predict the samples in the calibration set and validation set, respectively. The results are presented in Table 3.

Market Sample Analysis (Assay)

The proposed PLS and PCR methods were applied to the simultaneous determination of DIA and CELE in commercial capsules. Determination of six replicates was made. Satisfactory results were obtained for each drug in good agreement with the label claims. The results are presented in Table 4.

Precision

The method was found to be precise with six sample preparations for the quantification of DIA and CELE. The precision and intermediate precision variations were calculated in terms of relative standard deviation and the

results were found to be less than 2.0% and the results are presented in Table 5.

Recovery Studies

To check the validity of the proposed methods, recovery studies were carried out by addition of the standard to the pre-analysed formulation. (Standard addition technique) Recovery spectra are shown in Figure 4 and the results are presented in Table. 6.

CONCLUSIONS

The most striking features of spectrophotometric method are its simplicity and rapidity without requiring time-consuming sample preparation. Chemometric calibration techniques in spectral analysis are widely used in quality control of drugs in mixtures and multi- component pharmaceutical formulations with overlapping spectra, as separation procedures in the drug determinations are not required. A comparative study of the use of PLS and PCR for the simultaneous spectrophotometric determination of diacerein and celecoxib has been accomplished.

High percentage of recovery shows that the methods are free from interference of the excipients used in the commercial formulation. Results also showed that the developed methods can be applied to a routine analysis, quality control of mixtures and commercial preparations containing these drugs.

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