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Original Article

Application of Chemometrics in Simultaneous Spectrophotometric Quantification of Etophylline and Theophylline: The Drugs with Same Chromophore

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Abstract

Chemometric techniques in spectral analysis have gained importance in the quality control of the drugs mixtures and pharmaceutical formulations containing two or more drugs with overlapping spectra. Since theophylline and etophylline have common chromophore, they cannot be analyzed simultaneously using conventional UV methods. Simultaneous spectrophotometric determination of etophylline and theophylline was performed by partial least-squares (PLS) and principal component regression (PCR) methods. The absorbance values in the UV-Vis spectra were measured for the 71 wavelength points (from 230-300) in the spectral region 200-400 nm considering the intervals of 1 nm. The accuracy and the precision of the methods were determined and validated by analyzing synthetic mixtures of the drugs. The numerical calculations were performed with the 'Unscrambler 10.3 X software. The calibration ranges were found to be 6-30 µg/ml for etophylline and 2-10 µg/ml for theophylline. The chemometrics analysis methods were satisfactorily applied to the simultaneous determination of etophylline and theophylline in the tablet dosage form.

*Keywords: E*tophylline, Theophylline, Chemometrics, Spectrophotometry, partial least-squares, principal component Regression.

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1. Introduction

Etophylline is 7-(2-Hydroxyethyl)-1,3-dimethyl-3,7-dihydro-1H-purine-2,6-dione. It is non-selective phosphodiesterase inhibitor, antiasthmetic agent used in the treatment of acute attacks or status asthmatic in conjunction

with other drugs [1, 2]. Theophylline is respiratory smooth muscle relaxant, phosphodiesterase inhibitor, bronchodilator agent and vasodilator agent. Chemically it is 1, 3-dimethyl-3, 7-dihydro-1H- purine-2, 6-dione [3, 4]. Literature survey revealed that etophylline and theophylline in combination with other drugs were simultaneously estimated by HPLC [5-10]. Literature survey also revealed that HPLC [11-13] and HPTLC [14] methods have been reported for the simultaneous estimation of etophylline and theophylline in combined dosage forms.

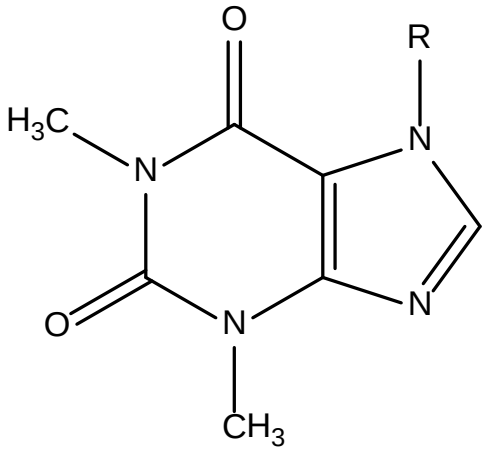

Figure 1. Structure of Theophylline (R = H) and Etophylline (R = CH_3CH_2OH).

No UV spectrophotometric method has been reported for simultaneous determination of etophylline and theophylline in combined dosage form. The present article discusses the attempts made to develop simple, sensitive, reproducible and economical chemometric methods for simultaneous determination of these drugs in combined dosage forms. Etophylline and theophylline both the drugs have same chromophore and complete overlapping spectra (Figure 1). Hence they cannot be quantified with any conventional UV method.

In recent years, multivariate calibrations, such as classical least-squares (CLS), inverse least-squares (ILS), partial least-squares (PLS) and principal component regression (PCR) have been started to apply to the analysis of the analytical data obtained in all the instrumentation. It is perhaps the area within chemometrics which has attracted the most interest so far [15-16]. The approach is useful in the simultaneous spectrophotometric determination of two or more components in a pharmaceutical formulation with overlapping spectra. These are extensively applied in quantitative spectral analysis to get selective information from unselective data [17].

The objective of work is to investigate the ability of PLS and PCR models to quantify the binary mixture of etophylline and theophylline with overlapped UV spectra and to apply the optimized models to pharmaceutical formulations. The proposed methods are simple and accurate, resulted in a significant reduction in analysis time and proved to be suitable for routine determination of the two components of the standard mixture.

The method involves a calibration step in which the relation between spectra and component concentrations is estimated from a set of reference samples, and a prediction step in which the results of the calibration are used to

estimate the component concentrations in spectra of unknown sample.

2. Materials and Methods

2.1. Instruments and Software

Digitized UV/VIS absorbency spectra were collected using a UV-Visible spectrometer 1601 Techcomp with 1 cm quartz cells. The data acquisition was made with UV solitions software at a scan rate of 1000 nm min^{-1} and the slit width of 1 nm. The UV spectra of mixtures were recorded over the wavelength 230-300 nm with one data point per nm. All spectral measurements were performed using blank (distilled water) 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 10.3 X) was used.

2.2. Pharmaceutical Tablet formulations

A commercial pharmaceutical formulation (Deriphylline) tablet containing 77 mg of etophylline and 23 mg of theophylline was analyzed by the proposed chemometric methods.

2.3. Preparation of Stock Solutions

Stock solution were prepared by dissolving 100 mg of etophylline and 100 mg of theophylline in 100 ml volumetric flasks and the volume was made up with distilled water. The training set containing 2-10 µg/ml theophylline and 6-30 µg/ml etophylline working standard solution were prepared by diluting the stock solutions for each drug according to its linear calibration range. Two set 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 etophylline and theophylline solutions, containing appropriate amount of these drugs in the range of calibrations, were added and then then solutions were diluted to 10 ml with distilled water. UV spectra of the mixtures were recorded in the wavelength range 230-300 nm versus a solvent blank, and digitized absorbance was sampled at 1 nm intervals. All the solution was freshly prepared.

2.4. Sample Preparations

Twenty tablets were accurately weighed and powdered in mortar. The suitable amount of the powder equivalent to 77 mg and 23 mg of etophylline and theophylline were weighed and dissolved in distilled water in 100 ml calibrated flasks. 20 ml distil water was added and ultra sonicated for 10 minutes and the volume was made up to 100 ml with distilled water and shake well. Then, the solution was filtered through whatman® 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 distilled water. From the resulting solution take 10 ml in 100 ml volumetric flask and made up to 100 ml with distilled water .Then after take 3 ml in 10 ml volumetric flask and made up to 10 ml. Each sample solution was

prepared in triplicate and measured in random order.

The two drugs show an overlap in their absorption spectra.

3. Result and Discussion

The absorption spectra of etophylline and theophylline solution in distilled water recorded between 230 – 300 nm were shown in figure 2.

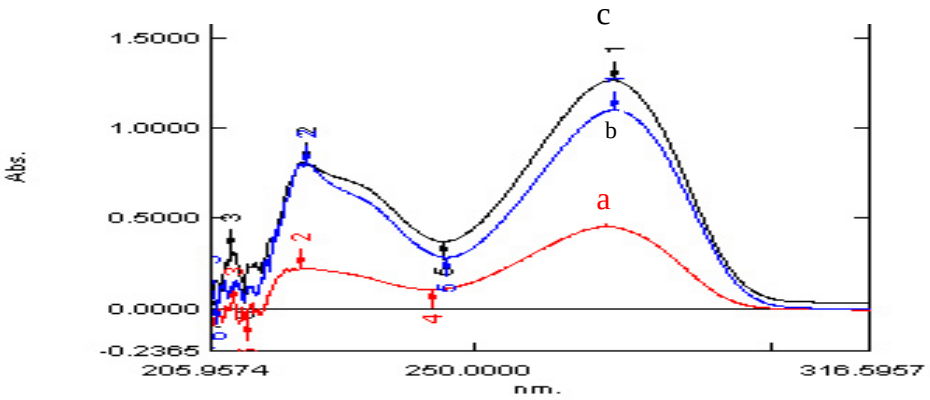

Figure 2. Overlaid Spectra of Etophylline and Theophylline (a) λ max: 273 nm for Theophylline, (b) λ max: 273 nm for Etophylline, (c) λ max: 273 nm for mixture.

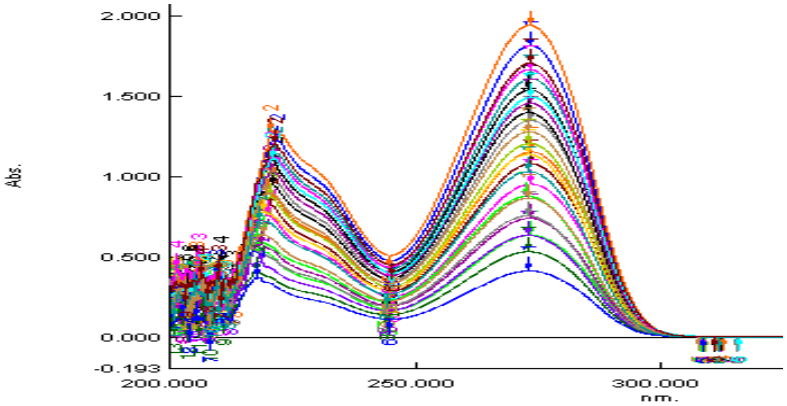

Figure 3. Calibration Spectra of Etophylline and Theophylline

3.1. Experimental Ddesign of Sample Sets

Calibration and test sets for component systems were designed according to factorialprinciple five-level factorial design was used to produce a calibration set (Training step) of 25 samples. Calibration spectra are shown in Figure 3.

A three-level set was derived to produce a prediction set (Validation step) of nine samples. Prediction spectra are shown in Figure 4. The compositions of the used calibration and validation sets are summarized in Tables 1 & 2 respectively.

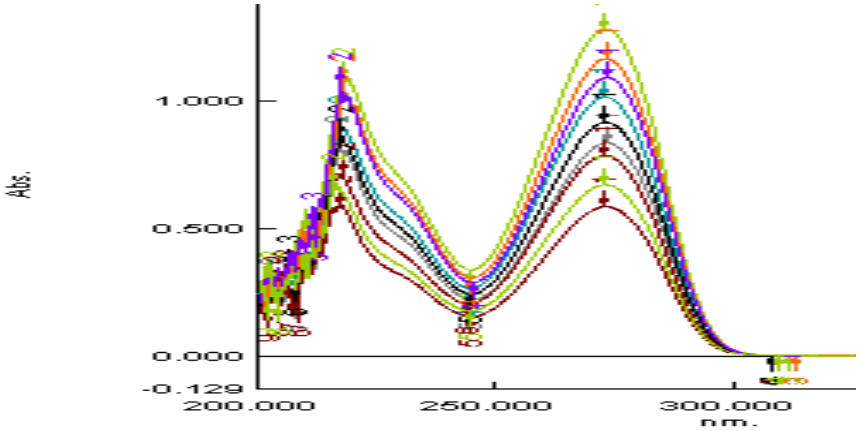

Figure 4. Prediction Spectra of Etophylline and Theophylline.

Table 1. Composition of calibration (training set) set for PLS and PCR method.

Sr. No.	Theophylline			Etophylline		
	Reference µg/ml	Predicted µg/ml		Reference µg/ml	Predicted µg/ml	
		PLS	PCR		PLS	PCR
1	2	2.09	1.96	6	6.05	6.22
2	2	1.95	1.88	12	11.83	11.91
3	2	2.05	2.10	18	17.99	17.95
4	2	1.91	2.11	24	24.02	23.72
5	2	2.02	1.97	30	30.00	30.12
6	4	4.03	3.99	6	6.16	6.12
7	4	4.01	4.02	12	12.09	12.05

8	4	4.02	4.09	18	17.88	17.78
9	4	4.03	4.21	24	24.01	23.75
10	4	3.88	3.71	30	30.07	30.30
11	6	6.02	5.80	6	5.85	6.16
12	6	6.01	6.08	12	11.90	11.84
13	6	5.98	6.18	18	18.03	17.74
14	6	5.99	5.98	24	23.88	23.92
15	6	5.86	5.90	30	30.07	30.05
16	8	8.10	8.02	6	6.00	6.10
17	8	7.85	7.78	12	12.16	12.23
18	8	7.88	8.13	18	17.96	17.58
19	8	8.21	8.29	24	24.03	23.88
20	8	8.12	7.85	30	29.94	30.20
21	10	9.96	9.96	6	5.92	5.97
22	10	9.87	9.96	12	12.00	11.86
23	10	9.86	9.78	18	18.13	18.28
24	10	10.16	10.40	24	23.95	23.68
25	10	10.02	9.73	30	29.95	30.36

Table 2. Composition of validation (prediction set) for PLS and PCR methods.

Sr. No.	**Theophylline**			**Etophylline**		
	Reference µg/ml	Predicted µg/ml		Reference µg/ml	Predicted µg/ml	
		PLS	PCR		PLS	PCR
1	3	3.46	3.46	9	9.01	9.01
2	3	3.44	3.44	15	15.02	15.02
3	3	3.37	3.37	21	21.29	21.29
4	5	4.96	4.96	9	9.07	9.07
5	5	5.18	5.18	15	14.69	14.69
6	5	5.33	5.33	21	20.44	20.44
7	7	6.92	6.92	9	9.19	9.19
8	7	7.02	7.02	15	14.77	14.77
9	7	6.98	6.98	21	21.02	21.02

3.2. Selection of Optimum Number of Factors and the Spectral Region.

The most frequently employed validation criterion is to divide the dataset into two subsets, a calibration set and validation set. 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 accuracy of the prediction equations was given by squared coefficient of determination in prediction (r^2) and Root Mean Square Error of Prediction (RMSEP). The analysis of results (Table 3) shows that both PLS and PCR models have good accuracy. However PLS model is more accurate and stable because it has least RMSEC and RMSEP values, correlation factor (r^2) has closest approach to unity.

Table 3. Summary of statistics in PLS and PCR methods.

	RMSEP		**RMSEC**		**r^2**		**Intercept**		**Slope**	
	PLS	PCR	PLS	PCR	PLS	PCR	PLS	PCR	PLS	PCR
Etophylline	0.2555	0.3369	0.0887	0.2150	0.999	0.999	0.001	0.011	0.999	0.999
Theophylline	0.2294	0.2439	0.0957	0.1699	0.998	0.996	0.006	0.021	0.998	0.996

3.3. Market Sample Analysis (Assay)

The proposed PLS and PCR methods were applied to the simultaneous determination of etophylline and theophylline in commercial tablets. Determination of six replicates was made. Satisfactory results were obtained for each drug in good agreement with the label claims. Assay spectra are shown in Figure 5. The results are presented in Table 4.

3.4. Precision

The method was found to be precise with six sample preparations for the quantification of etophylline and theophylline. The precision and intermediate precision variations were calculated in terms of relative standard deviation. The low values of % RSD indicate that method is having high repeatability (Table 5).

Table 4. Analysis of tablet formulation (Assay) (n=6).

Formulation	Label claim	PLS mg/tab Found	% Purity	PCR mg/tab Found	% Purity
Deriphylline	ETO 77 mg	78.2 mg	101.55%	78.2 mg	101.55%
	Theo 23 mg	22.6 mg	98.26%	22.6 mg	98.26%

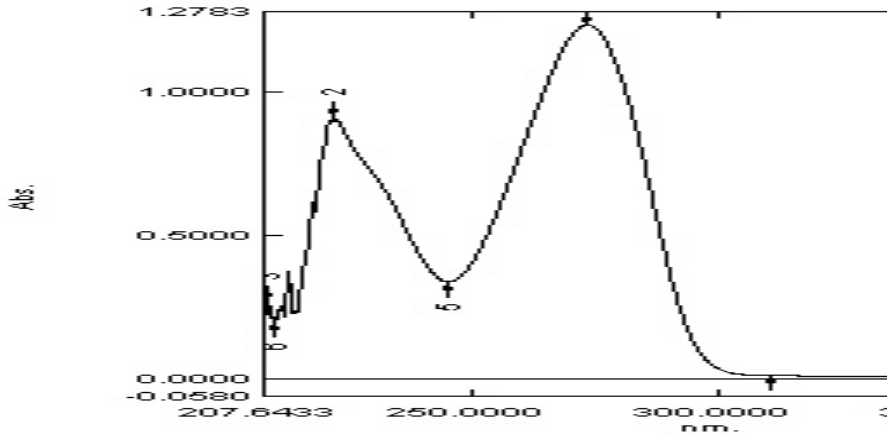

Figure 5. Assay Spectra of Etophylline and Theophylline.

Table 5. Results of system precision and method precision.

Sr. No.	System precision				Method precision			
	Etophylline		Theophylline		Etophylline		Theophylline	
	PLS	PCR	PLS	PCR	PLS	PCR	PLS	PCR
1	23.48	23.48	6.78	6.78	101.64	101.64	98.26	98.26
2	23.47	23.47	6.77	6.77	101.60	101.60	98.11	98.11
3	23.45	23.45	6.78	6.78	101.51	101.51	98.26	98.26
4	23.49	23.49	6.78	6.78	101.68	101.68	98.26	98.26
5	23.40	23.40	6.77	6.77	101.29	101.29	98.11	98.11
6	23.48	23.48	6.78	6.78	101.64	101.64	98.26	98.26
Mean	23.46	23.46	6.77	6.77	101.56	101.56	98.22	98.21
SD	0.0331	0.0331	0.00516	0.00516	0.1443	0.1443	0.0774	0.0774
%RSD	0.141	0.141	0.0762	0.0762	0.1421	0.1421	0.07887	0.07887

3.5. Recovery Studies

To check the validity of the proposed methods, recovery studies were carried out by addition of the standard to the pre-analyzed formulation (Standard addition technique). Recovery spectra are shown in Figure 6 and results are presented in Table 6. Low values of % RSD indicate that methods are having good recovery at all three levels.

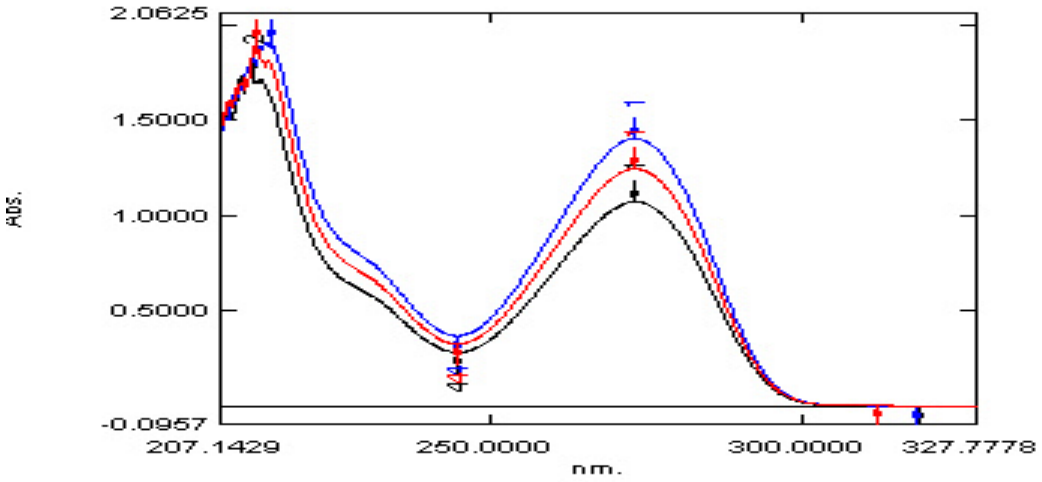

Figure 6. Recovery spectra of Etophylline and Theophylline.

Table 6. Results of recovery studies (n=3).

Drug	Level	Amount of Sample (mg)	Amount of Std added (mg)	Method	Average of Amount Detected (mg)	% Recovery ± SD	% RSD
	80%	3.45	2.76	PLS	6.18	99.51±0.0057	0.0058
				PCR	6.17	99.35±0.0057	0.0058
	100%	3.45	3.45	PLS	6.80	98.55±0.01	0.0101
THEO				PCR	6.80	98.55±0.01	0.0101
	120%	3.45	4.14	PLS	7.55	99.47±0.0057	0.0058
				PCR	7.54	99.34±0.0057	0.0058
	80%	11.55	9.24	PLS	20.65	99.32±0.01	0.0101
				PCR	20.64	99.27±0.0115	0.0116
ETO	100%	11.55	11.55	PLS	23.27	100.73±0.0057	0.0057
				PCR	23.28	100.77±0.0057	0.0057

120%	11.55	13.86	PLS	25.27	99.44±0.0056	0.0057
			PCR	25.27	99.44±0.0056	0.0057

4. Conclusion

The most striking features of chemometric methods are their 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 multicomponent pharmaceutical formulations with overlapping spectra, as separation procedures in the drug determinations are not required. 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 two drugs.

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