SIMULTANEOUS SPECTROPHOTOMETRIC DETERMINATION OF PARACETAMOL AND METHOCARBAMOL IN A PHARMACEUTICAL PREPARATION USING CHEMOMETRIC TECHNIQUES

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Abstract

Four chemometric methods, classical least-squares, inverse least-squares , principal component regression and partial least-squares, are described for the simultaneous spectrophotometric determination of paracetamol and methocarbamol in their combination. In the methods, the concentration data matrix were prepared by using the synthetic mixtures containing these drugs in methanol. The absorbance data matrix corresponding to the concentration data matrix was obtained by the measurements of absorbances wavelengths at 11 wavelengths in the range 240 – 290 nm as $\Delta\lambda$ = 5 in classical least-squares, principal component regression and, at 16 wavelengths in the range 250 – 280 nm as $\Delta\lambda$ = 2 nm in partial least-squares (PLS-1) technique and inverse least-squares technique in their zero-order spectra. Than, calibration was obtained by using the absorbance data matrix and concentration data matrix for the prediction of concentrations of paracetamol and methocarbamol in their binary mixture. The procedures do not require any separation step. Working range was found 1.6 – 13 μg /ml for paracetamol and 5 – 120 μg / ml in all the methods. The accuracy and the precision of the methods have been determined and they have been validated by analysing synthetic mixtures containing title drugs. These four methods were successfully applied to a pharmaceutical formulation, tablet, and the results were compared with each other.

Key Words: *Paracetamol, Methocarbamol, , Chemometric technique, Pharmaceutical preparation*

Bir Farmasötik Preparatta Parasetamol ve Metokarbamol'ün Kemometrik Teknikler Kullanılarak Aynı Anda Spektrofotometrik Miktar Tayinleri

Çalışmada, parasetamol - metokarbamol karışımında bu iki etken maddenin aynı anda dört kemometrik teknik (klasik en küçük kareler, ters en küçük kareler, temel bileşen regresyonu ve kısmi en küçük kareler) yardımıyla spektrofotometrik olarak miktar tayinleri gerçekleştirilmiştir. Yöntemlerde, bu etken maddelerin metanolde hazırlanmış sentetik karışımlarındaki konsantrasyonlara gore hazırlanmış veri matrisleri hazırlanmıştır. Absorbans veri matrisleri ise hazırlanan sentetik karışımların sıfırncı derece (orijinal) absorpsiyon spektrumlarında klasik en küçük kareler ve temel bileşen regresyonu teknikleri ile $\Delta\lambda$ = 5 nm olarak 240 –290 nm arasında 11 dalga boyunda, kısmi en küçük kareler (PLS-1) ve ters en küçük kareler tekniklerinde ise $\Delta\lambda$ = 2 nm olarak 250 – 280 nm arasında 16 dalga boyunda absorbanslar ölçülerek hazırlanmıştır. Daha sonra bu konsantrasyon ve absorbans veri matrisleri yardımıyla kalibrasyon hazırlanmış ve buna göre de ikili karışımlarını içeren numunelerdeki parasetamol ve metokarbamol'ün konsantrasyonları hesaplanmıştır. Yöntemlerin uygulanmasında önceden bir herhangi bir ayırma işlemine gerek yoktur. Çalışma aralığı uygulanan tüm yöntemlerde parasetamol için 1.6 – 13 μg/mL ve metobarbamol

için 5 – 120 μg/mL olarak bulunmuştur. Yöntemlerdeki doğruluk ve duyarlık tayin edilmiş ve bu maddeleri içeren sentetik karışımların analizleri yardımıyla validasyon gerçekleştirilmiştir. Bu dört yöntem başarı ile bir tablet formulasyonuna uygulanmış ve sonuçlar birbirleri ile karşılaştırılmışlardır.
Anahtar Kelimeler: *Parasetamol, Metokarbamol, , Kemometrik teknik, Farmasötik preparat*

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Introduction

Binary combinations of paracetamol (**PAR**) with methocarbamol (**MET**) are frequently prescribed in medicine as myorelaxant and placed in various dosage forms.

Simultaneous determination of paracetamol and methocarbamol in their binary mixture was realized by using spectrophotometry (1) derivative spectrophotometry (2), ratio spectra derivative spectrophotometry (3), gas chromatography (4) and HPLC (5,6). But we couldn't find any study for the analysis of this mixture applying chemometric techniques for any analytical method.

Chemometric quantitative calibration techniques in spectral analysis is gaining importance in the quality control of drugs in mixtures and pharmaceutical formulations containing two or more drugs with overlapping spectra due to not need any separation procedure in the drug determinations. In addition, these techniques can be successfully applied to all the analysis methods. We used these techniques for the simultaneous analysis of a binary and a ternary mixtures (7-12). Also these methods were used for the simultaneous analysis of multicomponent pharmaceutical preparations containing paracetamol (13-25).

In this study; four chemometric methods are proposed for the simultaneous spectrophotometric determination of paracetamol and methocarbamol in a pharmaceutical preparation, tablet.

Experimental

Apparatus

Shımadzu 1601 PC double beam spectrophotometer with a fixed slit width (2 nm) connected to a computer loaded with Shımadzu UVPC was used for all the spectrophotometric measurements.

In chemometric methods, original spectra of the solution of **PAR** and **MET** in methanol in 225 – 300 nm range were used.

Computer software and hardware

In chemometric procedures *Matlab 6.1* and *NCSS97* softwares were used and run on PC Pentium III , 128 MB RAM, 1500 MHz computer.

Materials

Paracetamol, and methocarbamol were kindly donated by Sanofi Doğu Pharm.Ind., Turkey and used without further purification.

All the materials used in spectrophotometric analysis were of analytical reagent grade.

Standard solutions

Solutions of 1 mg/mL of paracetamol, 1 mg/ml methocarbamol was prepared in methanol.

Sample preparation

20 tablet formulations selected were accurately weighed and powdered in a mortar for the commercial preparation. The amount of the tablets mass equivalent to one tablet contents of each were dissolved in 60 mL of solvent proposed , methanol. After 30 min. of mechanically shaking the solutions were filtered in a 100 mL volumetric flask. The residue was washed three times with 10 mL of solvent then the volume was completed to 100 ml with the same solvent (I). ***I*** was diluted 1/250 with methanol for the analysis of tablet selected.

Commercial pharmaceutical preparations

MİYOREL® (375 mg methocarbamol and 300 mg paracetamol / tablet) Sanofi-Doğu Pharm.Ind., Turkey (batch no: 0205018) was assayed.

Results and Discussion

Procedure

Fig.1 show the zero-order absorption spectra for **PAR** and **MET,** and their binary mixture in methanol. The spectra of both components were overlapped in 240-300 nm range. In the chemometric techniques for the determination of these drugs in their binary mixture optimum conditions were investigated and absorbance data matrix were obtained by the measurements of absorbances between 240.0 – 290.0 nm in the intervals with $\Delta\lambda = 5$ nm at 11 wavelengths in classical least-squares (CLS) (n=number of mixed standard=10) , principal component regression (PCR) (n=10) and partial least-squares (PLS-1) (n=10) and between 250.0 – 280.0 nm with the $\Delta\lambda = 2$ nm of intervals at 16 wavelengths in inverse least-squares (ILS) (n=16) method in the zero-order absorption spectra of **PAR** + **MET** mixture in methanol. In the techniques, calibration was obtained by using the absorbance data matrix mentioned above and the concentration data matrix prepared as the concentrations in the mixtures for prediction of the unknown concentrations of **PAR** and **MET** in their binary mixtures. We observed that good results were obtained by using standardized data in calculation procedures. All the data were examined for homoscedasticity before applying all the regression analysis.

To select the number of factors, in order to model the system without overfitting the concentration data in the PLS–1 and PCR algorithms, a cross-validation method, leaving out one sample at a time was employed using training sets. In PLS-1 technique; three factors for both **PAR** and **MET**, and in PCR technique; two factors for **PAR** and three factors for **MET** in **PAR** + **MET** mixture were found optimum for the determinations . We obtained the prediction error sum of squares (PRESS) minimum with these factors. Regression coefficients in PCR and PLS-1 methods were illustrated in Table 1.

TABLE 1. Regression coefficients in PCR and PLS techniques

PCR				**PLS**			
PAR		**MET**		**PAR**		**MET**	
Regression coefficients	Standard error	Regression coefficients	Standard error	Regression coefficients	Standard error	Regression coefficients	Standard error
5.6753	0.0090	-12.8209	0.0012	8.4700	0.0010	-6.5900	0.0013
9.2659	0.0111	-32.6546	0.0014	8.8700	0.0011	-4.6800	0.0017
11.1342	0.0125	-41.3834	0.0016	9.5100	0.0018	-4.9400	0.0009
9.7479	0.0117	-33.8803	0.0015	8.9000	0.0009	-6.0200	0.0003
6.0238	0.0090	-15.3511	0.0012	8.8500	0.0014	-7.3300	0.0009
0.4816	0.0051	12.5700	0.0006	7.3400	0.0013	8.4300	0.0008
-5.6092	0.0012	44.0484	0.0001	-6.6600	0.0009	8.0700	0.0003
-9.7284	0.0015	65.6743	0.0002	-5.7300	0.0012	8.7200	0.0018
-9.4825	0.0019	62.6663	0.0002	-4.8000	0.0013	5.2200	0.0013
-5.4572	0.0007	37.6333	0.0008	-2.3200	0.0006	3.7500	0.0008
0.4364	0.0015	1.0318	0.0002	0.9900	0.0010	0.4600	0.0003

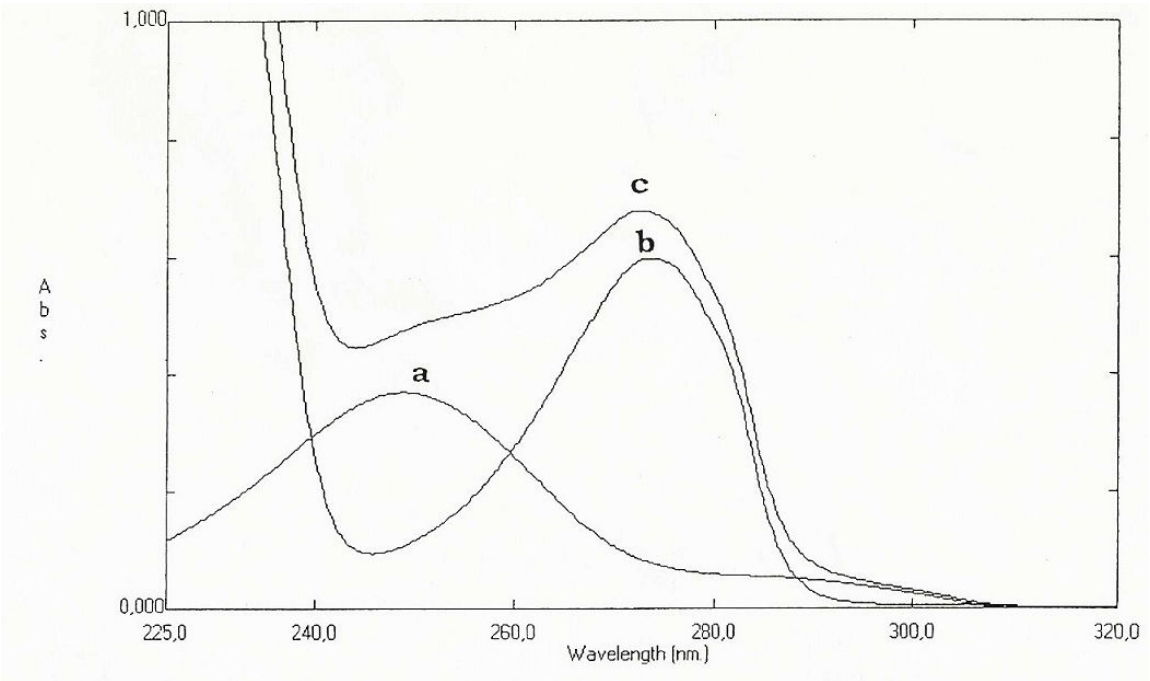

Figure 1. Zero-order absorption spectra of a) 3 μg/mL solution of paracetamol, b) 20 μg/mL solution of methocarbamol in methanol c) solution of 3 μg/mL paracetamol + 20 μg/mL methocarbamol mixture in methanol

The numerical values were calculated by using softwares mentioned in materials section.

Mean recoveries and relative standard deviations for the CLS, PCR, ILS and PLS techniques for **PAR + MET** mixture were found as 98.8 % and 2.99 %, % 100.5 and % 2.33 , 99.9 % and 1.07 , % 100.5 and % 2.33, for **PAR** and 100.6 % and 2.20, ,% 99.7 and % 1.71, % 99.9 and % 0.71 , % 99.7 and % 1.71 for **MET** respectively in the synthetic mixtures of both drugs (Table 2).

The predictive ability of a model can be defined in various ways. The most general expression is the standard error of prediction (SEP). In order to test the proposed techniques, the sets of synthetic mixtures containing the binary mixtures of drugs in variable composition were prepared. The results obtained in the application of CLS, PCR, ILS and PLS-1 methods to the same binary mixture are indicated in Table 3. The standard errors of prediction were found acceptable (Table 3).

Another statistical value is the SEC (standard error of calibration). SEC were also found acceptable in CLS, PCR, ILS and PLS-1 methods in the synthetic mixtures containing these drugs in variable compositions prepared were illustrated in Table 3. The standard error of calibration were found completely acceptable (Table 3).

In Table 3, r is defined as the correlation between constituent concentrations and shows the absorbance effects relating to the constituent of interest. r values obtained in the methods close to 1 mean no interference was coming from the other constituents in this set of synthetic mixtures.

The numerical values were calculated by using softwares mentioned in 2.2 of experimental section.

Working range

Working range was 1.6 – 15.0 μg/ml for **PAR** and 5.0 – 120.0 μg/ml for **MET** in **PAR** + **MET** mixture in the methods.

Precision

The precision was determined by means of a one-way ANOVA including 10 replicates carried out on three successive days using four chemometric methods (CLS, PCR, ILS and

TABLE 2. Recovery results for **PAR** and **MET** in synthetic mixtures by chemometric techniques

	CLS (*n= 10)		**ILS** (n= 16)		**PCR** (n= 10)		**PLS–1** (n= 10)	
	PAR	**MET**	**PAR**	**MET**	**PAR**	**MET**	**PAR**	**MET**
Mean recovery % (±CI* for P=0.05)	98.8 (±1.59)	100.6 (±1.17)	99.9 (±0.57)	99.9 (±0.38)	100.5 (±1.24)	99.1 (±0.91)	100.5 (±1.24)	99.7 (±0.91)
RSD %	2.99	2.20	1.07	0.71	2.33	1.71	2.33	1.71

*CI=confidence interval

**n= number of mixed standard samples

PLS-1) for synthetic mixtures. Snedecor F values below the tabulated levels were obtained in all cases ($F = 4.21$, $n_1 = 2$, $n_2 = 27$; Table 4), so there were no significant differences between the result obtained in the determination of each drug in the presence of other on different days. The highest % RSD values were obtain for CLS method for the between days and within days results for both **PAR** and **MET** .

Robustness

The robustness of a method is its ability to remain unaffected by small change in parameters. Water content of methanol up to ± % 2 did not have a significant effect on the methods. Also changing scanning speed as 0.1 – 0.5 nm/s did not effect the results.

Applications

Comparison of the spectra of **PAR** and **MET** in standard and drug formulation solutions showed that the wavelength of maximum absorbances in the zero-order spectra did not change and also after addition of known amount of these active ingredients to the

TABLE 3. Summary of statistics in CLS, PCR , ILS and PLS methods for **PAR+MET** in the mixture

	SEP			
mixture	**CLS**	**ILS**	**PCR**	**PLS**
PAR	0.74	0.07	0.12	0.12
MET	0.13	0.15	0.50	0.50
	SEC			
PAR	0.79	0.08	0.13	0.13
MET	0.14	0.16	0.54	0.54
	r			
PAR	0.9998	0.9998	0.9995	0.9995
MET	0.9979	0.9999	0.9999	0.9999
	Intercept			
PAR	0.0094	0.0158	0.0048	0.0048
MET	0.4201	0.0335	0.0432	0.0432
	Slope			
PAR	0.982	0.995	0.999	0.999
MET	0.992	0.998	0.998	0.998

TABLE 4. Analysis of variance (ANOVA) for the proposed methods

parameters	Classical least squares		Inverse least squares		Principle component regression		Partial least squares	
	PAR	**MET**	**PAR**	**MET**	**PAR**	**MET**	**PAR**	**MET**
Between-days variance	0.79	0.86	1.00	1.00	1.09	0.45	0.78	0.08
Within-days variance	1.97	2.01	1.00	1.00	1.20	1.56	1.11	0.28
F ratio	0.40	0.43	1.00	1.00	0.91	0.29	0.70	0.29
Mean value	10.1	40.1	10.0	40.0	10.2	40.3	10.1	40.3
Between-days RSD (%)	0.35	0.26	0.11	0.10	0.10	0.22	1.11	0.20
Within-days RSD (%)	0.30	0.46	0.11	0.10	0.19	0.28	0.28	0.21

Between-day and within-day degrees of freedom 2 and 27 respectively. The critical *F* ratio value for 2 and 27 degrees of freedom and a confidence level of 95 % is 4.21 .

TABLE 5. Assay results of commercial preparation (MİYOREL ® TABLET) (mg)

	PAR (Label claim= 300 mg/capsule)		**MET** (Label claim= 375 mg/capsule)	
	mean ± SD**	*t* values	mean ± SD	*t* values
Classical least squares (CLS)	298.2 ± 6.12	CLS – ILS = 0.56 CLS – PCR = 1.49 CLS – PLS = 1.49 ILS – PCR = 0.76 ILS – PLS = 0.76 PCR – PLS = 0.10 HPLC- CLS = 0.83 HPLC-ILS = 0.67 HPLC-PCR = 0.23 HPLC-PLS = 0.24	370.3 ± 6.80	CLS – ILS =1.61 CLS – PCR = 1.16 CLS – PLS = 1.16 ILS – PCR = 1.44 ILS – PLS = 1.44 PCR – PLS = 0.15 HPLC- CLS = 0.65 HPLC-ILS = 0.61 HPLC-PCR = 0.33 HPLC-PLS = 0.41
Inverse least squares (ILS)	297.8 ± 8.23		370.3 ± 3.77	
Principal component regression (PCR)	295.5 ± 1.16		368.3 ± 0.46	
Partial least Squares (PLS-1)	295.5 ± 1.16		368.3 ± 0.46	
****HPLC	296.6 ± 0.59		369.0 ± 1.75	

*Obtained results are average of ten tablets for five techniques;
**SD=standard deviation,
***Theoretical value for *t* at P : 0.05 level = 2.26
****Literature method (2)

commercial formulations powder were found the amount of these drugs did not change. It has been decided that excipients placed in the commercial preparations selected (lactose, starch, avicel, povidon, sodium dodecylsulfate, aerosil and magnesium stearate) did not interfere the quantitation of **PAR** and **MET** in the methods. All the results obtained by using the methods described above were compared with each other and no significant difference was observed between the amount of drugs found as theoretical values for *t* at P = 0.05 level for commercial formulation (Table 5). Also, the results obtained using chemometric methods proposed for the determination of **PAR** and **MET** in the tablet preparation selected was also compared with those obtained by the HPLC method (3) and no significant difference was observed. Amounts in the assay using chemometric techniques were found in coincidence with the HPLC methods used as reference for **PAR** and **MET.**

Conclusion

The proposed methods, four chemometric techniques used in spectrofotometric analysis, could be applied with great success for the simultaneous determination of **PAR** and **MET** and in their binary mixtures and in the pharmaceutical formulations selected containing these mixture without interference of each other. Satisfactory results were obtained by these methods but, they need softwares for the mathematical calculations. Using only zero-order spectra in the procedures and not need any other graphical mode, such as derivative and ratio mode in the instruments are the advantages for the chemometric methods when compared with the derivative and ratio spectra derivative spectrophotometric methods proposed previously for this mixture (1,2). By not needing any time consuming sample preparation procedures and using only ordinary methanol as solvent , our methods are easier and cheaper when compared with the HPLC methods. These ranges were given in the literature (2) as 2 – 30 μg/ml for both drugs in ratio spectra derivative spectrophotometric method, but these are impossible. Because, we observed that the spectrum for **MET** illustrated in this article (2) was incorrect and spectrum of **PAR** appeared in the same figure was also incorrect. All the methods proposed in this article

were compared with each other and with an HPLC method (literature method (2)). These four new methods were found suitable for simple and precise routine analysis of the pharmaceutical preparation selected. Good agreement was seen in the assay results of pharmaceutical preparation, tablet, for all the methods proposed in the text . By the fact that results obtained were similar for CLS, ILS, PCR and PLS-1 methods, we conluded that one of them could be used for the spectrophotometric analysis of these mixtures although the CLS and ILS methods are simpler than PCR and PLS-1 techniques in practise.

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