PARAFAC and PLS Applied to Spectrophotometric Determination of Tetracycline in Pharmaceutical Formulation and Biological Fluids

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A simple and rapid analytical procedure was proposed for determination of tetracycline in pharmaceutical formulation, urine and plasma based on chemometrics methods and spectrophotometric measurements. The calibration set was constructed with twenty solutions in concentration range 0.25—13.00 μg ml^{-1} for tetracycline. The procedure was repeated at nine different pH values. Partial least squares (PLS) models were built at each pH and used to determinate a set of synthetic tetracycline solutions. The best model was obtained at pH 8.00 (PLS-PH8). Parallel factor analysis (PARAFAC) model was applied to a three-way array constructed using all the pH data sets and enabled better results. The capabilities of the method for the analysis of real samples were evaluated by determination of tetracycline in pharmaceutical formulations and biological fluids with satisfactory results.

Key words tetracycline; partial least squares (PLS); parallel factor analysis (PARAFAC); determination; pharmaceutical formulation; biological fluid

Tetracycline (Fig. 1) is antibiotic of tetracycline group frequently given to animals destined for human consumption not only to prevent and treat certain diseases but also to fraudulently accelerate growth.[1)] Most procedures for the determination of tetracycline use high performance liquid chromatography (HPLC) with UV–Vis,[2)] fluorescence[3—5)] detectors. Also flow injection analysis with fluorescence detector,[6)] and fluorescence spectrophotometry,[7)] are employed. However, few methods have been developed so far for determination of tetracycline using ultraviolet–visible spectroscopy. Thus, with the increase of the production and consumption of drugs that employ tetracycline, it becomes interesting to develop new method for its determination. In the last decades, two- and three-way analysis was introduced in the field of analytical chemistry.[8,9)] Theory and application of PARAFAC[10,11)] and PLS[12)] in spectrophotometry has been discussed by several workers.[13—16)] In addition, several determinations based on the application of these methods to spectrophotometric data have been reported.[17—24)]

Experimental

Reagents and Apparatus All the chemicals were of analytical-reagent grade. Tetracycline, acetic acid, phosphoric acid, boric acid, hydrochloric acid, and sodium hydroxide were purchased from Fluka. Stock standard solution of tetracycline, 1000 μg ml^{-1} was prepared by dissolving the compound in water. This solution was stored in the dark and was found to be stable for at least three weeks changing in the its spectral profile. All the solutions were prepared in deionized water. Universal buffer solutions in pH range from 1.0—12.0 were prepared by ref. 25.

A Perkin Elmer (Lambda 25) spectrophotometer controlled by a computer and equipped with a 1-cm path length quartz cell was used for UV–vis spectra acquisition. Spectra were acquired between 220 and 450 nm (2 nm resolution). Figure 2 displays the UV–Vis absorption spectra for aqueous solutions of tetracycline at various pH values at 220—450 nm intervals. The most remarkable feature in this figure is spectra variations of tetracycline at different pH. A HORIBA M-12 pH-meter furnished with a combined glass-saturated calomel electrode was calibrated with at least two buffer solutions at pH 3.00 and 9.00. The data were treated in an AMD 2000 XP (256 Mb RAM) microcomputer using MATLAB software, version 6.5 (The MathWorks). The *N*-way toolbox for Matlab version 2.1, available at http://www.models.kvl.dk/source, was employed for PARAFAC calculations, while PLS calculus was carried out in the PLS-Toolbox, version 2.0 (Eigenvector Technologies).

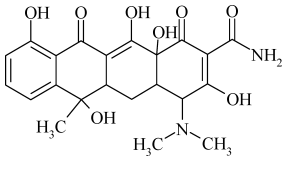

Fig. 1. Chemical Structure of Tetracycline

General Procedure Known amounts of standard solutions were placed in a 10-ml volumetric flask and completed to the final volume with deionized water and universal buffer in pH range from 1.0 to 12.0. The final concentration of these solutions varied between 0.25 to 13.00 μg ml^{-1} for tetracycline.

Sample (Pharmaceutical Formulations) Determination The pharmaceutical preparations assayed had the following composition per capsules: Razak (Iran), 250 mg of tetracycline. Five capsules of pharmaceutical formulation were weighed individually to an average weight. The capsules were finely powdered and mixed, and a mass corresponding to one capsules formulation was weighed and dissolved in 100 ml of methanol/water (10 : 90, v/v) in a volumetric flask. An aliquot of 100 μl of each sample was added into a cuvette containing 2.0 ml of the respective buffer with the specified pH. The spectra were obtained in the same conditions described previously. All these determinations were performed in triplicate.

Analysis of Biological Fluids (Urine and Plasma) Urine spiked with tetracycline was obtained by following procedure; an aliquot of pure tetracycline was added into 10 ml urine sample. A 1 ml of the resulting urine solution was mixed with 5 ml (0.2 M) sodium carbonate buffer and 10 ml butyl chloride. The mixture was rotated for 20 min and centrifuged at 2000 rpm for 10 min. The butyl chloride layer was separated and then evaporated till

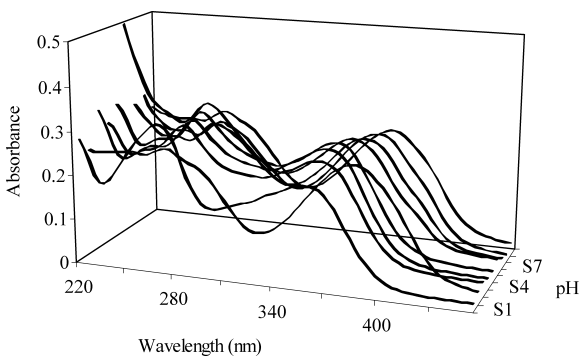

Fig. 2. Absorption Spectra of Tetracycline at Different pH Values: (1) 2.10, (2) 3.00, (3) 4.00, (4) 5.10, (5) 6.00, (6) 7.10, (7) 8.00, (8) 9.10 and (9) 10.00

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dryness.[26] Resultant residue was dissolved in universal buffer (at different pH) into a 10 ml volumetric flask, diluted to the mark.

Plasma spiked with tetracycline was obtained by diluting aliquots of the stock standard tetracycline solution with the human plasma. A 1 ml aliquot of this spiked solution was diluted to 5 ml with ethanol in 10 ml centrifuge tube. The precipitated protein was separated by centrifugation for 15 min at 2000 rpm. The clear supernatant layer was filtrated by Whatman filter to procedure protein free-spiked human plasma,[27] and then it was added into 10 ml volumetric flask and diluted to mark by universal buffer (at different pH).

Chemometrics Procedures The data (Fig. 2) has been arranged in a three-way array 30×115×9, composed of 30 solutions, with different tetracycline concentrations (Table 1), in the rows, 115 wavelengths in the columns and 9 pH values in the slices. No preprocessing (centering or auto scaling) was applied to the data. Unconstrained PARAFAC models of the tetracycline data at different pH were developed using one to nine components and the percentage of fit was used as the initial approach to select the number of factors. Five factors for unconstrained PARAFAC model furnished the best model for the deconvolution of the data. The decomposition of the three-way data by PARAFAC gives rise to three loading matrices, one of which, **C**, corresponds to the sample mode. The **C**-loading are the relative concentrations of the tetracycline in the solutions. In the calibration step, these loadings are regressed against the real concentrations of tetracycline to get a linear calibration. Linear regression results and standard deviation of results, line equations and correlation coefficient are summarized in Table 2. In the prediction step, this regression line can then be used to predict the concentration of tetracycline in future test samples (Table 2). In PLS method, according to an experimental design (Table 1), twenty solutions were used to construct the models (calibration set) and another ten solutions to validate them (validation set). The models were validated using cross-validation. The results obtained by applying PARAFAC and PLS to ten synthetic samples are listed in Tables 2 and 3. Tables 2 and 3 also shows the root mean square error of prediction (RMSEP) and relative standard error of prediction (RSEP). The prediction results for tetracycline are very good.

Determination of Tetracycline in Pharmaceutical Formulations and Biological Fluids In order to show the analytical applicability of the present methods, first calibration curve obtained from PARAFAC and PLS model at PH8 were applied to determination of tetracycline in real samples (pharmaceutical formulations) and complex matrices, *i.e.* urine and human plasma. The results showed that satisfactory recovery for tetracycline could be obtained (Tables 4, 5) using the recommended procedures. Results of the determination are summarized in Tables 4 and 5. The data obtained by these methods reveal the capability of the methods for determination of tetracycline in real samples such as pharmaceutical formulations and complex matrices such as urine and plasma without considerable error. The average recoveries in pharmaceutical formulations (Razak capsule) and complex matrices (urine and human plasma) are summarized in Tables 4 and 5, respectively.

Table 1. Concentration Data of the Calibration and Prediction Set of Tetracycline for PARAFAC and PLS Models ($\mu g\,ml^{-1}$)

Calibration	Concentration	Calibration	Concentration	Prediction	Concentration
C1	0.25	C11	7.10	P1	0.45
C2	0.50	C12	7.80	P2	0.75
C3	0.90	C13	8.30	P3	1.00
C4	1.60	C14	8.90	P4	2.10
C5	2.50	C15	9.90	P5	4.20
C6	3.10	C16	10.30	P6	6.10
C7	4.60	C17	11.10	P7	7.50
C8	5.10	C18	12.20	P8	8.30
C9	5.70	C19	12.80	P9	10.00
C10	6.40	C20	13.00	P10	12.50

Table 2. Statistical Parameters of the Linear Relationship between the Proportion Loadings Calculated by PARAFAC and the True Concentration of Tetracycline and Added and Found Results of the Prediction Set of Tetracycline Using PARAFAC Method

	First loading of C-loading (1st calibration)	Second loading of C-loading (2nd calibration)	Third loading of C-loading (3rd calibration)	Fourth loading of C-loading (4th calibration)	Fifth loading of C-loading (5th calibration)
Number of data points	20	20	20	20	20
Intercept	−0.3062	−0.5144	−0.4125	−0.0776	−0.1853
Standard deviation of intercept	0.0231	0.0322	0.0423	0.0154	0.1008
Slope	2.5424	1.8517	−1.6147	1.0978	1.0529
Standard deviation of slope	0.0074	0.0045	0.0039	0.0043	0.0038
Correlation coefficient	0.9988	0.9985	0.9954	0.997	0.9979
Standard deviation of regression	0.0456	0.0503	0.0344	0.0423	0.0309
Added Tetracycline			Found ($\mu g\,ml^{-1}$)		
0.45	0.45	0.48	0.51	0.40	0.39
0.75	0.76	0.71	0.70	0.79	0.79
1.00	1.02	0.93	1.11	1.11	1.70
2.10	2.11	2.15	2.22	2.03	2.23
4.20	4.20	4.26	4.30	4.88	4.03
6.10	6.09	6.03	5.97	5.87	5.74
7.50	7.57	7.65	7.68	7.05	7.71
8.30	8.28	8.22	8.92	8.91	8.66
10.00	10.02	10.11	10.15	10.38	10.72
12.50	12.44	12.34	13.31	12.76	13.05
RMSEP	0.0316	0.0922	0.3398	0.3637	0.4081
RSEP (%)	0.4754	1.3861	5.1093	5.4685	6.1351

Table 3. Added and Found Results of the Prediction Set of Tetracycline Using PLS Method at Different pH

Added	Found ($\mu g\,ml^{-1}$)								
	PLS-PH2 (pH=2.10)	PLS-PH3 (pH=3.00)	PLS-PH4 (pH=4.00)	PLS-PH5 (pH=5.10)	PLS-PH6 (pH=6.00)	PLS-PH7 (pH=7.10)	PLS-PH8 (pH=8.00)	PLS-PH9 (pH=9.10)	PLS-PH10 (pH=10.00)
0.45	0.37	0.38	0.39	0.40	0.38	0.41	0.42	0.41	0.52
0.75	0.82	0.83	0.81	0.86	0.79	0.79	0.79	0.8	0.81
1.00	1.09	1.08	1.16	1.15	1.08	1.05	1.01	1.08	1.11
2.10	2.29	2.33	2.21	2.32	2.19	2.15	2.07	2.18	1.89
4.20	3.86	3.92	4.00	3.88	3.93	4.10	4.11	4.33	4.05
6.10	5.81	5.83	5.85	5.93	6.01	6.00	6.08	5.95	6.19
7.50	7.89	7.12	7.10	7.23	7.19	7.34	7.32	7.13	7.22
8.30	8.61	8.66	8.92	8.84	8.45	8.46	8.43	8.12	8.12
10.00	10.98	10.29	10.41	10.37	10.31	10.29	10.21	10.33	9.42
12.50	13.29	12.86	13.22	12.74	12.69	12.64	12.28	12.88	12.17
No. of Factor	4	3	3	4	3	2	2	3	3
RMSEP	0.6603	0.2244	0.4359	0.2453	0.1116	0.0579	0.0486	0.1514	0.2060
RSEP (%)	9.9265	3.3736	6.5528	3.6884	1.6773	0.8705	0.7312	2.2768	3.0969

Table 4. Determination of Tetracycline in Pharmaceutical Preparations Using the PARAFAC and PLS-PH8 Models

Pharmaceutical preparations	Label claim (mg)	Amount found (PARAFAC)	Recovery (%)	Amount found (PLS-PH8)	Recovery (%)
Razak (sample 1)[a)]	250	246.5 (±4.05)[b)]	98.6	215.3 (±5.56)[b)]	86.1
Razak (sample 2)[c)]	250	245.9 (±3.89)	98.4	207.9 (±5.03)	83.2

a) Capsule (from Razak, Ltd., Iran). *b*) Value in parentheses are relative S.D. for $n=3$. *c*) Different sample.

Table 5. Determination of Tetracycline in Urine and Human Plasma Using PARAFAC and PLS-PH8 Models ($\mu g\,ml^{-1}$)

Type of samples	Added (ppm)	Amount found (PARAFAC)	Recovery (%)	Amount found (PLS-PH8)	Recovery (%)
Plasma sample 1	5.0	4.73 (±2.46)[a)]	94.6	4.12 (±4.06)	82.4
Plasma sample 2	10.0	9.24 (±2.88)	92.6	8.16 (±4.56)	81.6
Urine sample	10.0	9.36 (±3.06)	93.6	8.03 (±4.44)	80.3

a) Value in parentheses are relative S.D. for $n=3$.

References

1) Ghosh J., "A Textbook of Pharmaceutical Chemistry," S. Chang and Company Ltd., New Delhi, 2003.
2) Cinquina A. L., Longo F., Anastasi G., Giannetti L., Cozzani R., *J. Chromatogr. A*, **987**, 227—233 (2003).
3) Pena A. L. S., Lino C. M., Silverira I. N., *J. AOAC Int.*, **86**, 925—929 (2003).
4) McCracken R. J., Blanchflower W. J., Haggan S. A., Kennedy D. G., *Analyst*, **120**, 1763—1766 (1995).
5) Croubels S., Peteghem C., Baeyens W., *Analyst*, **119**, 2173—2178 (1994).
6) Sultan S. M., Suliman F. O., Duffuaa S. O., Abu-Abdoum I. I., *Analyst*, **117**, 1179—1183 (1992).
7) Garcia I., Sarabia L. A., Ortiz M. C., *Anal. Chim. Acta*, **501**, 193—203 (2004).
8) Martens H., Naes T., "Multivariate Calibration," John Wiley, Chichester, 1989.
9) Smilde A. K., *Chemom. Intell. Lab. Syst.*, **15**, 143—157 (1992).
10) Bro R., "Multi-Way Analysis in the Food Industry," Ph.D. Thesis, Amsterdam, 1998.
11) Bro R., *Chemom. Intell. Lab. Syst.*, **38**, 149—171 (1997).
12) Brereton R. G., *Analyst*, **125**, 2125—2154 (2000).
13) Sena M. M., Poppi R. J., *J. Pharm. Biomed. Anal.*, **34**, 27—34 (2004).
14) Sena M. M., Fernandes J. C. B., Rover L., Jr., Poppi R. J., Kubota L. T., *Anal. Chim. Acta*, **409**, 159—170 (2000).
15) Kalivas J. H., *J. Chemometr.*, **13**, 111—132 (1999).
16) Kalivas J. H., *Anal. Chim. Acta*, **428**, 31—40 (2001).
17) Ghasemi J., Niazi A., *Microchem. J.*, **68**, 1—17 (2001).
18) Ghasemi J., Niazi A., Safavi A., *Anal. Lett.*, **34**, 1389—1399 (2001).
19) Ghasemi J., Amini R., Niazi A., *Anal. Lett.*, **35**, 533—544 (2002).
20) Ghasemi J., Niazi A., Leardi R., *Talanta*, **59**, 311—317 (2003).
21) Ghasemi J., Shahabadi N., Seraji H. R., *Anal. Chim. Acta*, **510**, 121—126 (2004).
22) Ghasemi J., Niazi A., *Talanta*, **65**, 1168—1173 (2005).
23) Niazi A., Ghasemi J., Yazdanipour A., *Anal. Lett.*, **38**, 2377—2392 (2005).
24) Ghasemi J., Niazi A., *Anal. Chim. Acta*, **533**, 169—177 (2005).
26) Lurie J. J., "Handbook of Analytical Chemistry," Mir Publishers, Moscow, 1978.
27) Salem A. A., Barsoum B. N., Izake E. L., *Spectrochim. Acta Part A*, **60**, 771—780 (2004).
28) Ghoneim M. M., Mabrouk M. M., Hassanien A. M., Tawfik A., *J. Pharm. Biomed. Anal.*, **25**, 933—939 (2001).