Using Chemometrics Methods for Determination of Aripiprazole and Quetiapine as Antipsychotic Drugs in Pharmaceutical Mixture and Biological Fluid by Spectrophotometry Method Based on Continuous Wavelet Transform and Multivariate Calibration

Alibakhshi, Mojdeh; Sohrabi, Mahmoud Reza*[+]; Davallo, Mehran
Department of Chemistry, North Tehran Branch, Islamic Azad University, Tehran, I.R. IRAN

ABSTRACT: *In this study, two multivariate calibration methods, including partial least squares (PLS) and principal component regression (PCR), as well as continuous wavelet transform (CWT) along with spectrophotometry technique were developed for the simultaneous analysis of Aripiprazole (ARI) and Quetiapine (QTP) in the pharmaceutical formulation and biological fluid. The linear range of ARI and QTP was 1-3 and 2-10 μg/mL, respectively for the proposed methods. The root means square error (RMSE) of ARI and QTP related to the test set was obtained 0.014, 0.0758, and 0.194, 0.882 for PLS and PCR methods, respectively. Also, the mean recovery of ARI and QTP was 99.95, 100.04%, and 97.38, 98.83% for PLS and PCR models, respectively. Among various families of wavelets in CWT technique, the Coiflet (Coif3) and Symlet (Sym2) families were selected to determine the value of ARI and QTP, respectively. In this method, the Limit of Detection (LOD) and Limit of Quantification (LOQ) values was found 0.0033, 0.0200, 0.2764, 0.3486 μg/mL for ARI and QTP, respectively. The mean recovery values of ARI and QTP in synthetic mixtures for CWT approach were 96.98%, 98.94%, respectively. A one-way analysis of variance (ANOVA) test was applied to compare the results of both mentioned chemometrics models and High-Performance Liquid Chromatography (HPLC) as a reference method. No significant difference was observed between these methods.*

KEYWORDS: *Spectrophotometry; Multivariate calibration; Continuous wavelet transform; Aripiprazole, Quetiapine, Biological fluid.*

INTRODUCTION

Antipsychotic drugs are mostly prescribed for patients with bipolar disorder, schizophrenia, and other mental disorders. This group of drugs is also prescribed to a lesser the amount for autism, anxiety, depression, as well as nausea in early pregnancy [1]. In fact, they can be used for both short-term (manic, dementia, agitated states in delirium)

* *To whom correspondence should be addressed.*
+ E-mail: sohrabi.m46@yahoo.com
1021-9986/2022/6/1870-1882 13/$/6.03

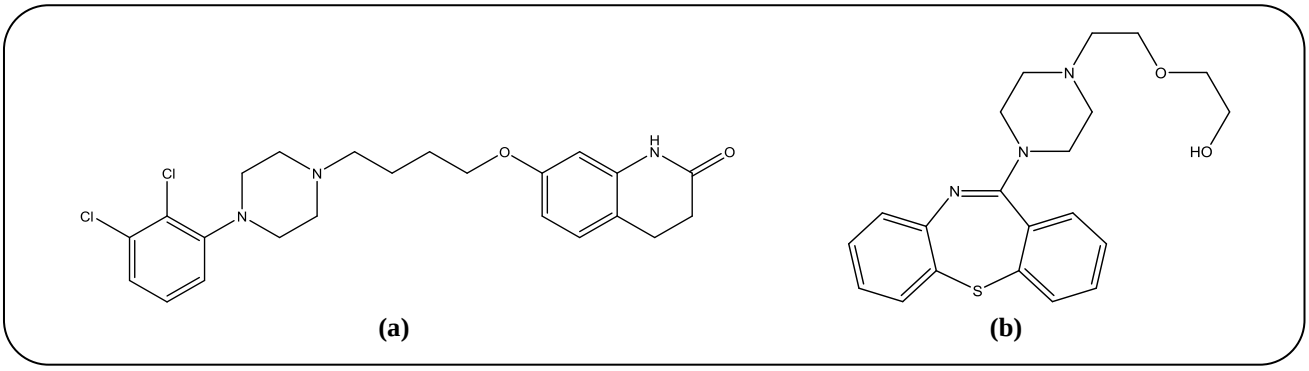

Fig. 1: Chemical structure of (a) ARI and (b) QTP.

and long-term treatments (schizophrenia, delusional disorders) [2]. schizophrenia usually occurs in the adult period, which is characterized by unusual thinking and a decrease in behavioral and social functions [3,4]. The First-Generation Antipsychotics (FGAs), including haloperidol, perphenazine, and chlorpromazine, as well as second-generation antipsychotics (SGAs) such as risperidone, quetiapine, aripiprazole, clozapine, and olanzapine, are prescribed for treatment of schizophrenia [5,6]. Bipolar disorder (BD) or bipolar depression, which is known as manic depression affects a person's mood in the sense that it can change from an intense state to another one. Antipsychotic drugs are used for BD treatment [7,8].

Central nervous system (CNS) disorders lead to autism spectrum disorder (ASD), which is a neurodevelopmental disorder. These patients have difficulty in social communication and interaction. Psychopharmacologic treatments, changes in diet, education, and antipsychotic drugs are various treatment methods for ASD. Food and Drug Administration (FDA) approved risperidone and aripiprazole for the treatment of pediatric patients with the autistic disorder [9,10].

Aripiprazole (ARI) with chemically named 7-[4-[4-(2, 3-dichlorophenyl) piperazine -1- yl] butoxy] - 3, 4-dihydro-1H-quinolin-2-one (Fig. 1a) is antipsychotic agent, which actives as a powerful partial agonist at dopamine D_2 and serotonin 5-HT_{1A} receptors. ARI is used to treat schizophrenia, acute mania, and bipolar disorder [11-13].

Quetiapine (QTP) chemically known as 2, (2-[2-(4-Dibenzo [b,f] [1,4]thiazepin-11-yl-1- piperazinyl) ethoxy] ethanol) fumarate (Fig. 1b) is dibenzothiazepine derivative. QTP is an atypical antipsychotic drug for treating schizophrenia, bipolar disorders, and major depression. Furthermore, cocaine use disorder can be treated with this drug [14,15]. Also, reports indicate that QTP has been used to treat children with autism [16]. Antagonizing of both 5-HT_2 and dopamine D_2 receptors leads to the antipsychotic activities of QTP [17].

There are many techniques in the literature for the simultaneous determination of antipsychotic drugs in pharmaceutical formulation and human plasma. For example, High-Performance Liquid Chromatography (HPLC) [18,19], ultra-high performance liquid chromatography [20], and liquid chromatography-tandem mass spectrometry (LC–MS/MS) [21-23]. Complicated instruments, environmental pollutant solvents, high cost, and time-consuming are some disadvantages of chromatography methods [24]. In contrast, the spectrophotometric procedure is simple and inexpensive. However, the spectral overlap of mixtures is known as a drawback associated with this method [25]. In order to solve the problem of spectral overlap related to the components, the chemometrics method along with the spectrophotometry technique can be used without complicated preparation steps [26].

In this study, for the first time, Partial Least Squares Regression (PLSR) and Principal Component Regression (PCR) as multivariate calibration models, as well as Continuous Wavelet Transform (CWT) combined with spectrophotometry method as simple, low-cost, and An accurate procedure was proposed to determine ARI and QTP in synthetic mixtures and biological fluids simultaneously. Finally, the results of the proposed methods on the commercial tablet were compared with the HPLC as a reference method using a one-way analysis of variance (ANOVA).

THEORETICAL BACKGROUND

Partial least squares

PLS is a multivariate calibration method extensively used in the chemometrics community [27]. The calculation of the relationship between two matrices, including the concentration matrix (Y) and the absorbance matrix (X) are performed using PLS. The basis of this method is the design of the principal multivariate on smaller matrices (T,U) with orthogonal columns. The correlation between the response matrix (Y) information to the principled variance in the descriptor matrix (X) is described by the PLS method, as shown in the equations below.

$$X = \bar{X} + TP' + E \quad (1)$$

$$Y = \bar{Y} + UC' + F \quad (2)$$

$$U = T + H\,(\text{the inner relation}) \quad (3)$$

Herein $\bar{X}$ and $\bar{Y}$ denote the mean value matrices. The matrices of scores are shown by T and U. P and C are the loadings and weights matrix, respectively. E, F, and H represent the residual matrices [28,29]. The fast estimation of mixture components without the need for prior separation or sample pre-treatment can be performed [30].

Principal component regression

PCR possesses two steps: 1) a principal component analysis (PCA); 2) Multiple Linear Regression (MLR) between the concentration matrix (Y) and the PCA related to the absorbance matrix (X). According to Eq (4), PCA decreases the dimension of matrix X into the smaller matrices (T and P').

$$X = \bar{X} + TP' + E \quad (4)$$

Where T is the score value of the calculated PCA. P' and E indicates the descriptor loadings and matrix of residuals, respectively [29].

Continuous wavelet transform

In order to analyze the non-fixed time series signals in the time-frequency amplitude, CWT can be used as a powerful tool, which varies from the Short-Time Fourier Transform (STFT) method. In wavelet analysis, long-time and short-time windows are used for accurate information with low frequency and high frequency, respectively. Unlike the STFT, the wavelet analysis includes the area of the time scale. Also, the CWT is not restricted to sinusoidal analyzing function. The relationship between the analyzed continuous-time signal x(t) and the wavelet function is investigated by a CWT, which was introduced by Eq (5).

$$Cw\,(a,b) = \int_{-\infty}^{+\infty} x(t)\psi_{a,b}{}^{*}\,dt = \frac{1}{\sqrt{a}}\int_{-\infty}^{+\infty} x(t)\psi^{*}\,\left(\frac{t-b}{a}\right)dt) \quad (5)$$

Herein *Cw(a,b)* denotes the function of the *a* and *b* variables. *a* and *b* represent the dilation of wavelet (scale) and wavelet translation, respectively. The complex conjugate related to the analyzing mother wavelet ψ (t) and energy normalized factor are shown by ψ^{*}(t) and the coefficient of $\frac{1}{\sqrt{a}}$, respectively [31].

EXPERIMENTAL SECTION

Instrument and software

A double-beam spectrophotometer (OPTIZEN 3220 U) was used to measure the absorption of samples. Separation of compounds was performed on Varian 9001 HPLC with C18 (250×4.6 mm, 5 µm) column. MATLAB 2015a (version 8.6) was applied to write PLS and PCR programs. Microsoft Office Excel 2013 was used to plot some diagrams and calculate related to the proposed methods.

Materials

Both pure ARI and QTP drugs were kindly provided by Shimi Darou Co, (Tehran, Iran). ARI (5 mg) and QTP (200 mg) tablets were purchased from Sobhan Darou Co (Tehran, Iran). Ethanol with 96% purity was prepared from Merck.

Preparation of standard solutions

Stock solutions of ARI and QTP were individually prepared by dissolving 5 mg of each component in ethanol and then adjusted to the mark in a 50 mL volumetric flask. Afterward, standard solutions in the concentration range of 1-3 and 2-10 µg/mL were made from stock solutions for ARI and QTP, respectively. Finally, the absorption spectra of the samples were recorded in the wavelength range of 200 to 500 nm.

Preparation of synthetic mixtures

According to the ranges related to the standard solutions, 16 mixtures with different concentrations

of ARI and QTP were randomly prepared. Then, the absorption of all mixed solutions was recorded.

Real sample preparation

First, the urine was filtered and centrifuged. It was then adjusted to the mark. Stock solutions of 10 and 25 µg/mL of ARI and QTP were prepared. Next, mixtures of 2.5 and 2 µg/mL of ARI and QTP were prepared and 0.5 mL of urine was added to the solution, then adjusted to the mark with ethanol. Eventually, their absorption spectra were recorded in the range of 200-500 nm.

Chromatographic condition

The separation of ARI and QTP was carried out on C18 (250×4.6 mm, 5 µm) column. Acetate buffers (pH= 5.5) and acetonitrile (55:45, v:v) were selected as the mobile phase with a flow rate of 1 mL/min. The detector adjustment was performed at a wavelength of 254 nm [32,33].

RESULTS AND DISCUSSION

Spectral characteristics

The UV absorption spectra of ARI and QTP have demonstrated in Fig. 2. The maximum wavelength was 216 and 213 nm for ARI and QTP. There is a strong overlap between the two substances and separation is necessary to determine these components. In this study, chemometrics approaches, including PLS, PCR, and CWT along with the spectrophotometry method were used to solve this problem without the separation step.

PLS and PCR analysis

In order to perform the PLS and PCR methods, the absorption related to the synthetic samples was considered as the input matrix (X). Also, the actual concentrations of each component were selected separately as the target (y) matrices. The input and target matrix for synthetic mixtures including ARI and QTP were 301×16 and 16×1, respectively. The determination of the optimal number of components corresponding to each component was examined by the cross-validation (k-fold cross-validation) method. The results of the Mean Squares Error Prediction (MSEP) versus the number of components for ARI and QTP are shown in Fig. 3. Components with the least MSEP were selected as the optimum components for the mentioned drugs. As can be seen, the number of optimal

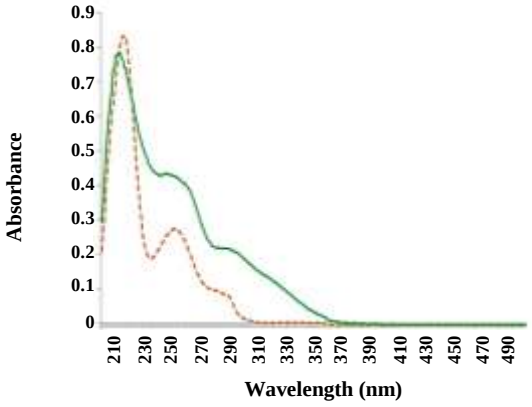

Fig. 2: The absorption spectra of ARI and QTP.

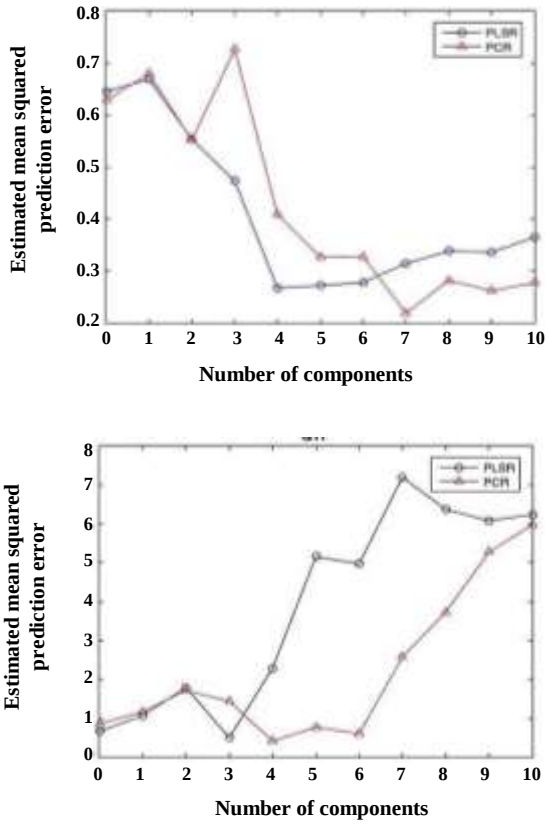

Fig. 3: MSEP versus several components for ARI and QTP in PLS and PCR methods.

components for ARI and QTP related to the PLS method were 4 (MSEP=0.2672) and 3 (MSEP=0.9939), respectively. In the PCR method, the number of components 7 and 4 with MSEP of 0.2188 and 0.8111 were chosen for ARI and QTP, respectively.

Of the 16 mixtures prepared, 10 and 6 mixtures were considered as training and test sets, respectively. Recovery

Table 1: Recovery and RMSE results of the 6 synthetic mixtures for PLS and PCR methods.

Mixtures	Add ($\mu g/ml$)		Found ($\mu g/ml$)		Recovery (%)	
	ARI	QTP	ARI	QTP	ARI	QTP
PLS						
1	1	10	1.00464	9.94374	100.46	99.43
2	3	10	3.00485	10.13026	100.16	101.30
3	2	2.5	1.96792	2.53197	98.39	101.27
4	2.5	6.25	2.49999	6.23552	99.99	99.76
5	3	6.25	3.00366	6.29002	100.12	100.64
6	1.5	5	1.50927	4.89258	100.61	97.85
Mean Recovery					99.95	100.04
RMSE					0.014	0.0758
PCR						
1	1	10	1.01226	8.77818	101.22	87.78
2	3	10	2.78444	8.31208	92.81	83.12
3	2	2.5	2.08467	2.63720	104.23	105.48
4	2.5	6.25	2.32051	6.53332	92.82	104.53
5	3	6.25	2.63467	6.59705	87.82	105.55
6	1.5	5	1.58086	5.32799	105.39	106.55
Mean Recovery					97.38	98.83
RMSE					0.194	0.882

percentage, mean recovery percentage, and root mean square error related to these 6 mixtures are given in Table 1. The results show that the error of the components in the PLS method (ARI=0.014, QTP=0.0758) was less than the error in the PCR method (ARI=0.194, QTP=0.882). Therefore, PLS had a better prediction than PCR.

The RMSE is introduced as follows:

$$RMSE = \left[\sum_{i=1}^{n} \frac{\left(y_{pred} - y_{obs} \right)^2}{n} \right]^{1/2} \quad (6)$$

Herein y_{pred} and y_{obs} are estimated values and the actual values of the samples, respectively. Also, n represents the number of samples [34].

The predicted values against actual values related to the ARI and QTP for PLS and PCR methods are exhibited in Fig. 4 (a) and (b). The coefficient of determination (R^2) close to 1 in the PLS method indicated the close correlation between actual and predicted values.

The residual values for these models are illustrated in Fig. 5. As can be seen, the residual values for the PLS method are scattered around the zero point, which indicated that PLS was more acceptable than PCR.

CWT analysis

At first, the data corresponding with standard solutions of ARI and QTP were individually transferred from excel into the wavelet toolbox. Afterward, optimum signal processing and suitable calibration plots were found by assessing different wavelet families. Then, plotting $C_{a,b}$ coefficients against wavelength in the range of 200 to 400 nm was performed to find the spectrum of CWT assigned to each component. Finally, the zero-cross point method was applied for CWT spectra of two components (ARI and QTP), which are located in the presence of each other. Eventually, the Coiflet wavelet family of three order

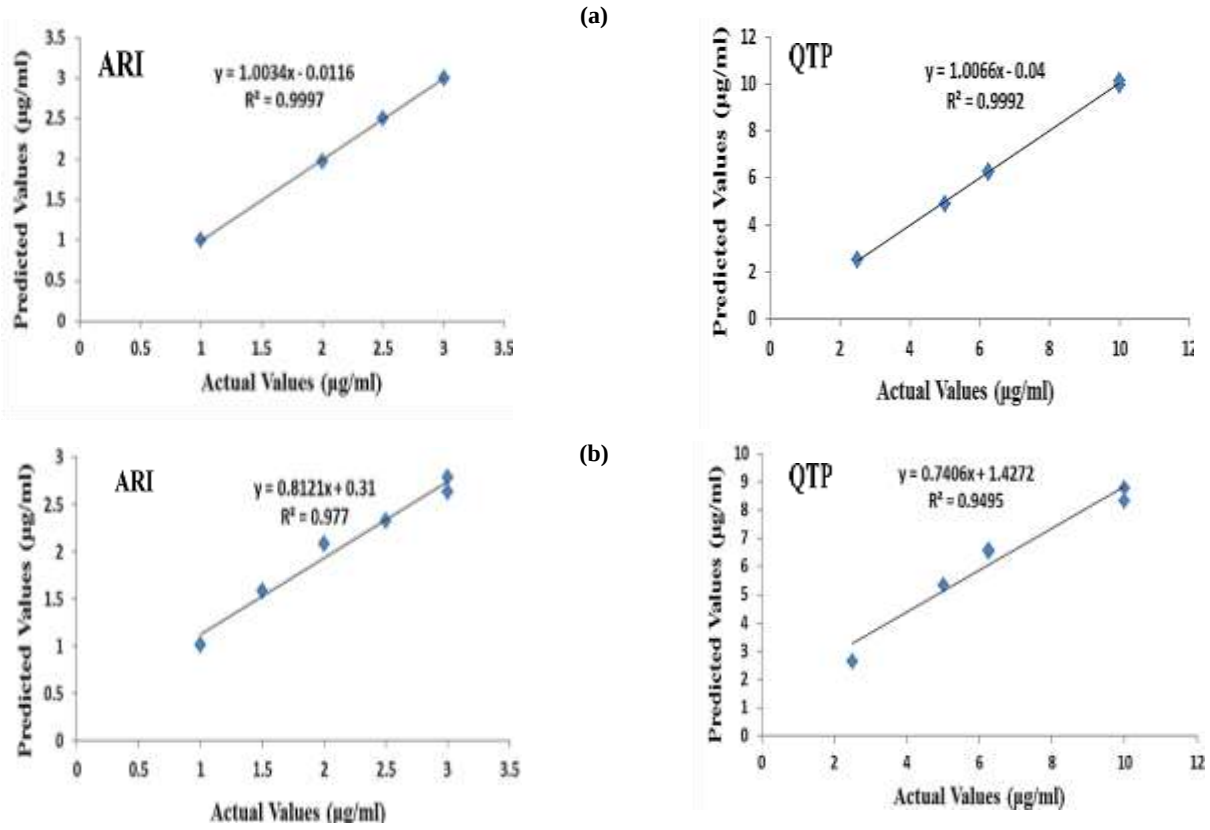

Fig. 4: Actual values versus predicted values for ARI and QTP in (a) PLS and (b) PCR methods.

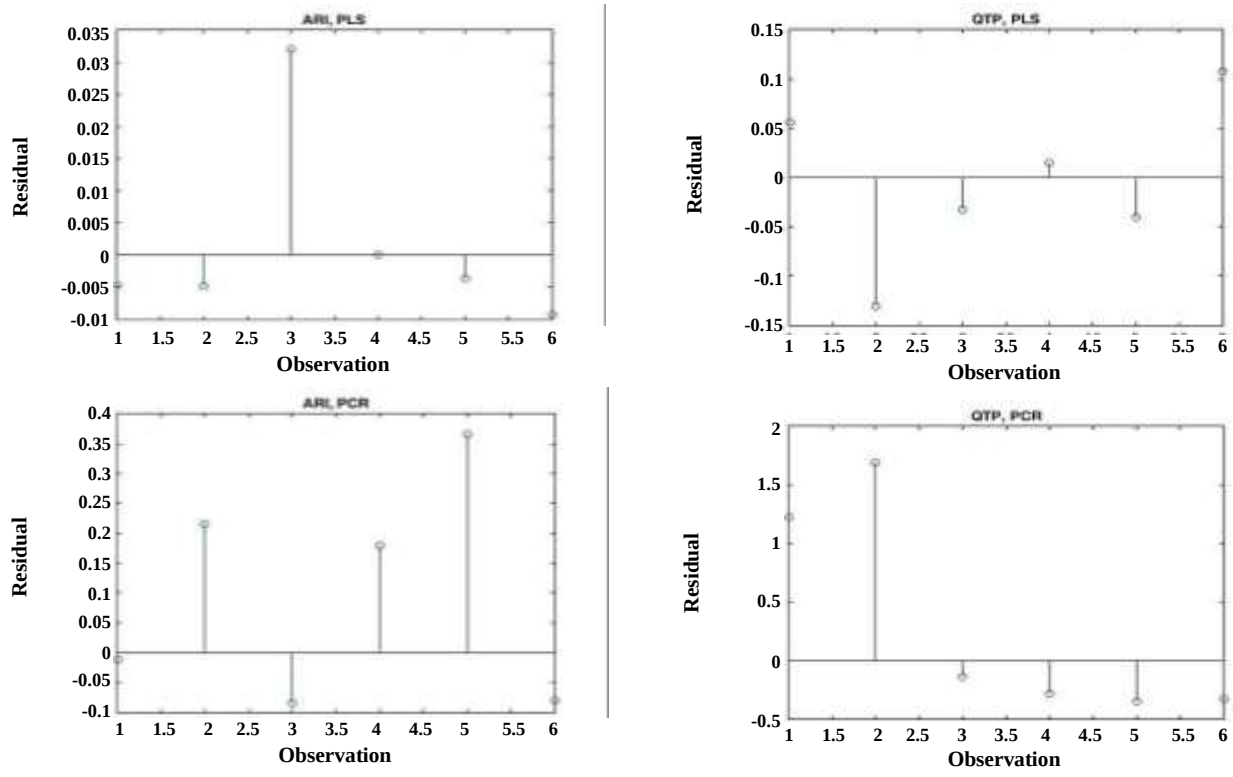

Fig. 5: Residual of prediction by PLS and PCR method for ARI and QTP.

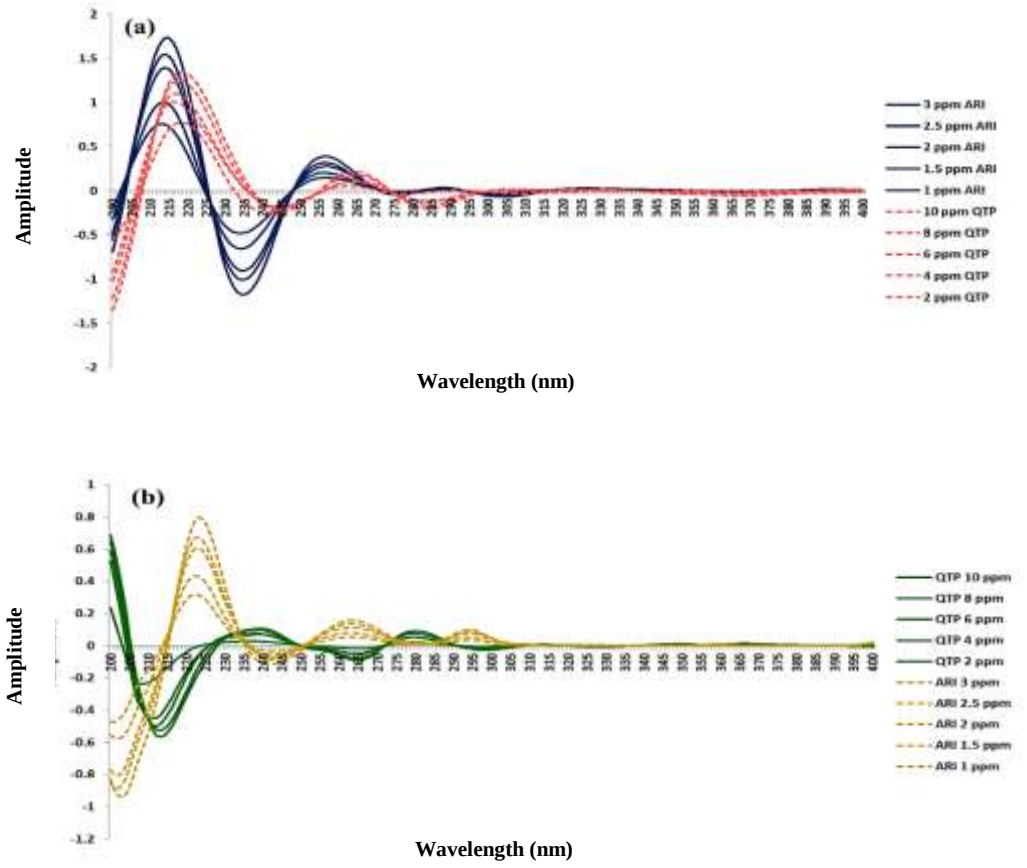

Fig. 6: Spectra of (a) CWT-Coif3 and (b) CWT- Sym2 related to the different concentrations of ARI and QTP.

(coif3) with scaling factor (a)=30 in the wavelength of 237 nm (related to the zero cross-point of QTP), Symlet wavelet family of two order (Sym2) via a=15 in the wavelength of 215 nm (related to the zero cross-point of ARI) were selected as the best families for the simultaneous estimation of ARI and QTP, respectively. The amplitude versus wavelength of the selected families related to the mentioned components was plotted (Fig. 6 a and b).

Also, amplitude versus concentration was plotted for obtaining the calibration graphs of ARI and QTP (Fig. 7). The highest R^2 equal to 0.9845 and 0.9242 was achieved for ARI and QTP, respectively.

The statistical parameters of CWT method were calculated using calibration curves and given in Table 2. Calculation of Limits of detection (LOD) and limit of quantification (LOQ) was done by Eqs (7) and (8).

$$LOD = y_B + 3s_B \quad (7)$$

$$LOQ = y_B + 10s_B \quad (8)$$

Where y_B and s_B represent the blank signal and standard deviations of the blank signal, respectively [35]. As shown in Table 2, LOD and LOQ values are admissible ranges.

In addition, the synthetic mixtures containing ARI and QTP were evaluated to validate this method. The obtained results include recovery (%), the mean recovery (%), and RMSE, which are presented in Table 3.

The predicted concentrations versus actual concentrations of the ARI and QTP in 16 synthetic mixtures are separately exhibited in Fig. 8. The high values of R^2 indicated good linearity. According to the high R^2, acceptable recoveries, and minimum RMSE, this method can be suggested for the simultaneous determination of ARI and QTP.

HPLC analysis

The chromatogram of ARI and QTP is shown in Fig. 9. The retention times were found 8.43 and 3.71 min for ARI and QTP, respectively.

Table 2: The obtained statistical results of calibration standard solutions graphs by the CWT method.

Parameter	CWT	
	ARI	QTP
Wavelength (nm)	237	215
Linear range (μg mL^{-1})	1-3	2-10
Slope (a)	0.0283	0.0016
Intercept (b)	0.0013	0.1308
Regression coefficient (R^2)	0.9874	0.9837
Correlation coefficient (r)	0.9936	0.9918
LOD (µg/mL)	0.1573	0.1608
LOQ (µg/mL)	0.5213	0.2308

Table 3: Recovery and RMSE obtained by the CWT method in 16 synthetic mixtures.

No.	Actual (μg mL^{-1})		Found (μg mL^{-1})		Recovery (%)	
	ARI	QTP	ARI	QTP	ARI	QTP
1	1	10	1.02	9.42	102.94	94.26
2	3	10	2.86	9.84	95.37	98.45
3	2	10	1.89	9.85	94.56	98.52
4	3	2.5	2.88	2.50	96.11	100.16
5	2	2.5	1.99	2.58	99.86	103.31
6	3	7.5	2.81	7.62	93.98	101.62
7	1	7.5	0.94	7.17	94.97	95.62
8	2	7.5	1.88	7.75	94.10	103.34
9	2.5	7.5	2.36	7.55	94.48	100.67
10	1.5	7.5	1.51	7.20	101.25	96.05
11	3	5	2.83	5.18	94.43	103.75
12	2.5	6.25	2.54	6.36	101.86	101.80
13	3	6.25	2.83	5.90	94.40	94.54
14	1	6.25	0.97	6.32	97.79	101.22
15	1.5	5	1.51	4.78	101.12	95.67
16	2	5	1.89	4.71	94.57	94.20
Mean recovery (%)					96.98	98.94
RMSE					0.1075	0.2447

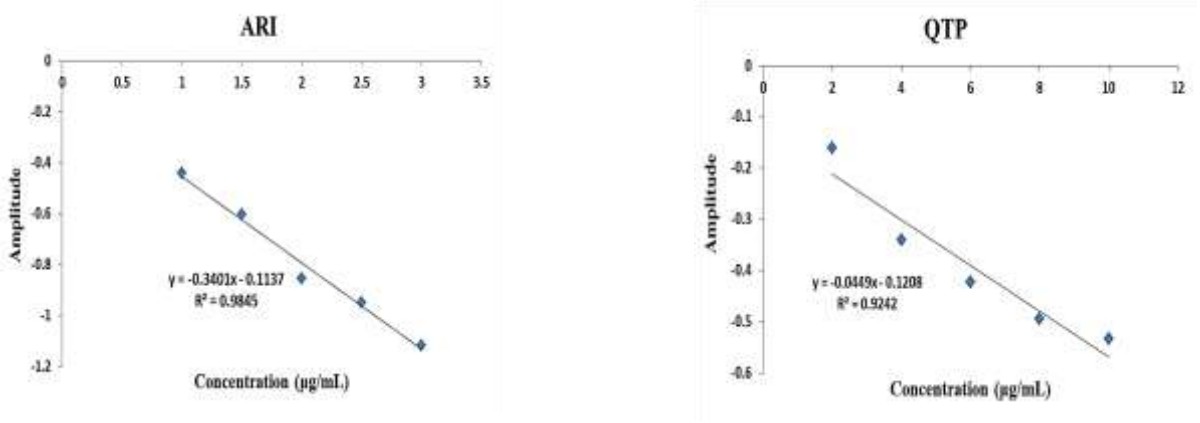

Fig. 7: Coif3 and Sym2 linear calibration graphs for determination of ARI and QTP.

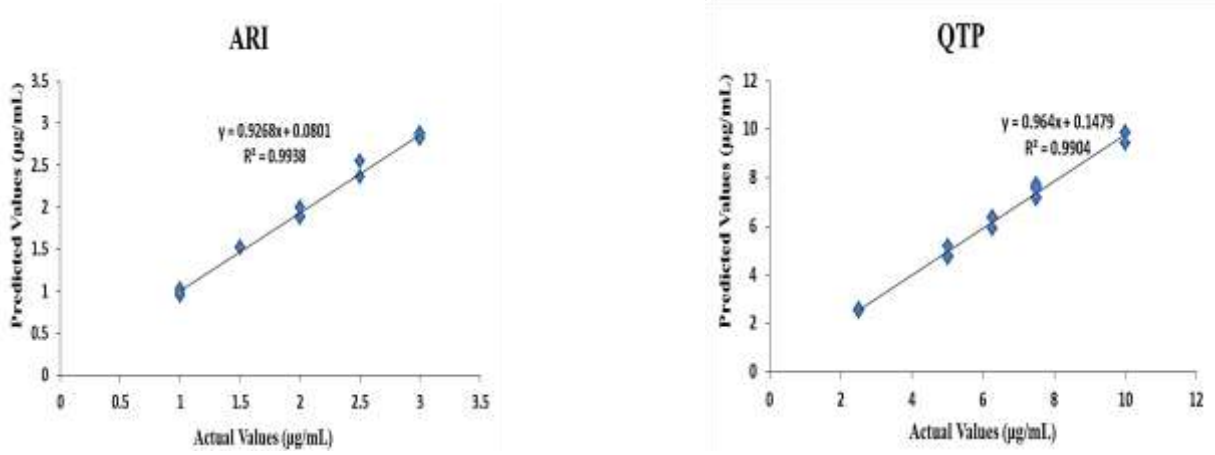

Fig. 8: Predicted values versus actual values for ARI and QTP in CWT method.

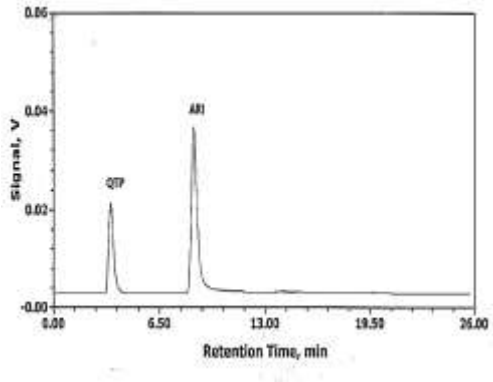

Fig. 9: Obtained chromatogram from the commercial tablet containing: 5 mg of ARI and 200 mg of QTP.

Real samples analysis

In order to evaluate the proposed methods, the analysis of the real samples (tablet and urine), including ARI and QTP, was evaluated by the desired chemometrics methods. Also, to validate the methods, these drugs were analyzed by HPLC as a reference method. The results of the analysis with three replications, including the average recovery percentage and Relative Standard Deviation (RSD) for various methods are presented in Tables 4 and 5. No significant errors were observed in both the tablet or urine samples. The recovery value of PLS model was better than the PCR and CWT methods.

In order to investigate the significant differences between the proposed methods and the reference method in tablet sample analysis, the ANOVA test was used. The obtained results, including the Sum of Square (SS), Mean Square (MS), degrees of freedom (df), F calculated, and F critical are summarized in Table 6 with three repetitions. According to the lower value of F-calculated than F-critical, there is no significant difference between these methods at the 95% confidence level. Therefore, it can be inferred that the precision of the suggested methods is almost equal to the

Table 4: Results of analyzing tablet sample by proposed methods 5 mg ARI and 200 mg QTP in each tablet.

	ARI		QTP	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
PLS	98.08±0.100	2.04	99.12±0.273	0.116
PCR	93.54±0.107	2.32	98.67±0.457	0.228
CWT	92.71±0.047	1.02	98.39±0.247	0.125
HPLC	96.73±0.121	2.50	98.91±0.763	0.384

Table 5: Results of analyzing the urine sample by proposed methods.

	ARI				QTP			
	Added	Found[a]	Recovery (%)	RSD (%)	Added	Found[a]	Recovery (%)	RSD (%)
PLS	2.5	2.39	95.65±0.006	0.260	2	1.89	94.93±0.005	0.296
PCR	2.5	2.34	93.85±0.010	0.452	2	1.82	91.31±0.006	0.330
CWT	2.5	2.35	94.29±0.006	0.280	2	1.83	91.63±0.006	0.355

[a] *Mean value of the three measurements*

Table 6: One-way ANOVA results by applying the HPLC and proposed methods to the real sample.

Source of variation	SS	df *	MS	F Calculated	F Critical
Between groups					
ARI	0.01	2	0.008	0.35	4.25
QTP	0.45	2	0.22	0.41	4.25
Within groups					
ARI	0.22	9	0.02		
QTP	4.94	9	0.54		
Total					
ARI	0.24	11			
QTP	5.39	11			

SS, the Sum of Squares; df, the degree of freedom; MS, mean squares.

[*] *Degree of freedom for between groups: h-1; Within Groups: h (n-1); Total: hn-1; h, number of methods; n, number of samples of each method.*

to the precision of the HPLC technique and can be a suitable alternative to the reference method.

CONCLUSIONS

In this work, the spectrophotometric technique based on chemometrics methods provides the simultaneous determination of ARI and QTP in pharmaceutical formulations and biological fluids. The proposed methods were used without chemical separations, which proves the high potential of the PLS, PCR, and CWT methods for the simultaneous estimation of the mentioned drugs with remarkable spectral overlap. According to the results, it can be said that the PLS model has performed better than the other two proposed methods. Both of these drugs are effective as described, and the urine matrix is a complex matrix due to its content, but urine analysis did not interfere with the analysis of these two drugs, and the two components were easily measurable. The developed methods are rapid, simple, cost-effective, and precise, which can be a useful substitute for routine analysis.

Received : Jan. 2, 2021 ; Accepted : May 18, 2021

REFERENCES

[1] Reutfors J., Cesta C.E., Cohen J.M., Bateman T., Brauer R., Einarsdóttir K., Engeland A., Furu K., Gissler M., Havard A., Hernandez-Diaz S., Huybrechts K.F., Karlstad Q., Leinonen M.K., Li J., Man K., Pazzagli L., Schaffer A., Schink T., Wang Z., Yu Y., Zoega H., Bröms G, Antipsychotic Drug Use in Pregnancy: A Multinational Study from Ten Countries, *Schizophrenia Research*, **220**: 106-115 (2020).

[2] Gardner D.M., Baldessarini R.J., Waraich P., Modern Antipsychotic Drugs: A Critical Overview, *Canadian Medical Association Journal*, **172**: 1703-1711 (2005).

[3] Coelho da Silva D.G., Sales Kanazawa L.K., Dalla Vecchia D., Schizophrenia: Effects of Aripiprazole in Metabolic Syndrome, *Brazilian Journal of Pharmaceutical Sciences*, **55**: e17840 (2019).

[4] Hana X., Shan X., Du Y., Pang S., Hu L., Development and Evaluation of Novel Innovative Multi-Channel Aripiprazole Orally Disintegrating Tablets, *Journal of Drug Delivery Science and Technology*, **55**: 101446 (2020).

[5] Liang F., Terry A.V., Bartlett M.G., Determination of Aripiprazole in Rat Plasma and Brain Using Ultra-Performance Liquid Chromatography/Electrospray Ionization Tandem Mass Spectrometry, *Biomedical Chromatography*, **26**: 1325-1332 (2012).

[6] Bruijnzeel D., Suryadevara U., Tandon R., Antipsychotic Treatment of Schizophrenia: An Update, *Asian Journal of Psychiatry*, **11**: 3-7 (2014).

[7] Abdelwahab N.S., Ahmed A.B., Omar M.A., Derayea S.M., Abdelrahman M.M., Green Chromatographic Methods for Simultaneous Determination of Quetiapine and the Co-Administrated Paroxetine in Rat Plasma with Application to Pharmacokinetic Study, *Microchemical Journal*, **152**: 104317 (2020).

[8] Soeiro‐de‐Souza M.G., Videira Dias V., Missio G., Balanza‐Martinez V.., Valiengo L, Carvalho A.F., Alberto Moreno R., Role of Quetiapine Beyond its Clinical Efficacy in Bipolar Disorder: from Neuroprotection to the Treatment of Psychiatric Disorders (Review), *Experimental and Therapeutic Medicine*, **9**: 643-652 (2015).

[9] Wu Z.Y., Huang S., Zou J., Wang Q., Naveed M., Bao H., Wang W., Fukunaga K ., Han F, Autism Spectrum Disorder (ASD): Disturbance of the Melatonin System and its Implications, *Biomedicine & Pharmacotherapy*, **130**: 110496 (2020).

[10] Tural Hesapcioglu S., Ceylan M.F., Kasak M., Pınar Sen C., Olanzapine, Risperidone, and Aripiprazole Use in Children and Adolescents with Autism Spectrum Disorders, *Research in Autism Spectrum Disorders*, **72**: 101520 (2020).

[11] Eldidamony A.M., Hafeez S.M., Abdel Hafez M.M., Spectrophotometric Determination of Aripiprazole, Clozapine and Sulpiride by Ion- Pair Extractionin Precision of the HPLC in Tablets and Biological Fluids, *International Journal of Pharmacy and Pharmaceutical Sciences*, **7**: 178-184 (2015).

[12] Song M, Xu X, Hang T, Wen A, Yang L, Development of an LC–MS/MS Method for the Simultaneous Quantification of Aripiprazole and Dehydroaripiprazole in Human Plasma, *Analytical Biochemistry*, **385**: 270-277 (2009).

[13] Hwang P.L., Wei S.Y., Yeh H.H., Ko J.Y., Chang C.C., Chen S.H., Simultaneous Determination of Aripiprazole and its Active Metabolite, Dehydroaripiprazole, in Plasma by Capillary Electrophoresis Combining On-Column Fieldamplified Sample Injection and Application in Schizophrenia, *Electrophoresis*, **31**: 2778-2786 (2010).

[14] Derayea S.M., Ahmed A.B., Abdelwahab N.S., Abdelrahman M.M., Omar M.A., Innovative Spectrofluorometric Protocol Based on Micro-Environment Improvement for Determination of Quetiapine in Dosage Forms and Rat Plasma, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, **233**: 118196 (2020).

[15] Vignali C., Freni F., Magnani C., Moretti M., Siodambro C., Groppi A., Maria Osculati A.M., Morini L., Distribution of Quetiapine and Metabolites in Biological Fluids and Tissues, *Forensic Science International*, **307**: 110108 (2020).

[16] Posey D.J., Stigler K.A., Erickson C.A., McDougle C.J., Antipsychotics in the Treatment of Autism, *The Journal of Clinical Investigation*, **118**: 6-14 (2008).

[17] Li M., Zhang S., Shi A., Qi W., Liu Y., Determination of Quetiapine in Human Plasma by LC–MS/MS and its Application in a Bioequivalence Study, *Journal of Chromatography B*, **1060**: 10–14 (2017).

[18] Atilakaraca S., Yeniceli Ugur D., Development of a Validated HPLC Method for Simultaneous Determination of Olanzapine and Aripiprazole in Human Plasma, *Marmara Pharmaceutical Journal*, **22**: 493-501 (2018).

[19] Silva Gracia M., Koppl A., Unholzer S., Haen E., Development and Validation of a HPLC-UV Method for the Simultaneous Determination of the Antipsychotics Clozapine, Olanzapine and Quetiapine, Several Beta-Blockers and Their Metabolites, *Biomedical Chromatography*, **31**: e3968 (2017).

[20] Dziurkowsk E., Wesolowski M., Simultaneous Quantification of Antipsychotic and Antiepileptic Drugs and their Metabolites in Human Saliva Using UHPLC-DAD, *Molecules*, **24**: 2953 (2019).

[21] Tonooka K., Yoshida L., Tomobe K., Kunisue Y., Terada M., Shinozuka T., Sensitive Liquid Chromatography/Tandem Mass Spectrometry Method for the Simultaneous Determination of Risperidone, Olanzapine, Quetiapine, Clozapine, Ziprasidone, Perospirone, Aripiprazole and Blonanserin in Human Serum, *American Journal of Analytical Chemistry*, **9**: 88-97 (2018).

[22] Koller D., Zubiaur P., Saiz-Rodríguez M., Abad-Santos F., Wojnicz A., Simultaneous Determination of Six Antipsychotics, Two of their Metabolites and Caffeine In Human Plasma By LC-MS/MS Using a Phospholipid-Removal Microelution-Solid Phase Extraction Method for Sample Preparation, *Talanta*, **198**: 159–168 (2019).

[23] Chen X., Liang C., Cui L., Le J., Qian Z., Zhang R., Hong Z., Chai Y., A Rapid LC-MS/MS Method for Simultaneous Determination of Quetiapine and Duloxetine in Rat Plasma and its Application to Pharmacokinetic Interaction Study, *Journal of Food and Drug Analysis*, **27**: 323-331 (2019).

[24] Mofavvaz S., Sohrabi M. R., Heydari A., Application of UV/vis Spectrophotometry Based on Using Least Squares Support Vector Machine and Continuous Wavelet Transform Methods for the Simultaneous Analysis of Antibiotics Drugs in Tablet Formulation: Comparison with HPLC Method, *Optik-International Journal for Light and Electron Optics*, **220**: 165246 (2020).

[25] Mofavvaz S., Sohrabi M.R., Nezamzadeh-Ejhieh A., New Model for Prediction Binary Mixture of Antihistamine Decongestant Using Artificial Neural Networks and Least Squares Support Vector Machine by Spectrophotometry Method, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, **182**: 105–115 (2017).

[26] Arabzadeh V., Sohrabi M.R., Goudarzi N., Davallo M., Using Artificial Neural Network and Multivariate Calibration Methods for Simultaneous Spectrophotometric Analysis of Emtricitabine and Tenofovir Alafenamide Fumarate in Pharmaceutical Formulation of HIV Drug, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, **215**: 266–275 (2019).

[27] Li C.Q., Fang Z., Xu Q.S., A Partition-Based Variable Selection in Partial Least Squares Regression. *Chemometrics and Intelligent Laboratory Systems*, **198**: 103935 (2020).

[28] Naguib I.A., Abdelaleem E.A., Hassan E.S., Ali N.W., Gamal M., Partial Least Squares and Linear Support Vector Regression Chemometric Models for Analysis of Norfloxacin and Tinidazole with Tinidazole Impurity, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, **239**: 118513 (2020).

[29] Boeris M.S., Luco J.M., Olsina R.A., Simultaneous Spectrophotometric Determination of Phenobarbital, Phenytoin and Methylphenobarbital in Pharmaceutical Preparations by Using Partial Least-Squares and Principal Component Regression Multivariate Calibration, *Journal of Pharmaceutical and Biomedical Analysis*, **24**: 259–271 (2000).

[30] Hassaninejad-Darzi S.K., Es'haghi, Zarin S.M., Torkamanzadeh M., Rapid and Simultaneous Determination of Montelukast, Fexofenadine and Cetirizine Using Partial Least Squares and Artificial Neural Networks Modeling, *Iran. J. Chem. Chem. Eng. (IJCCE)*, **36 (3)**: 81-96 (2017).

[31] Komorowski D., Pietraszek S., The Use of Continuous Wavelet Transform Based on the Fast Fourier Transform in the Analysis of Multi-channel Electrogastrography Recordings, *Journal of Medical Systems*, **40**: 10 (2016).

[32] Sastry B.S., Gananadhamu S., Devalarao G., RP-HPLC Determination of Aripiprazole in Pharmaceutical Formulations. *Asian Journal of Chemistry*. **21**: 6643-6646 (2009).

[33] Belal F., Elbrashy A., Eid M., Jeehan Nasr J., Stability-Indicating HPLC Method for the Determination of Quetiapine: Application to Tablets and Human Plasma, *Journal of Liquid Chromatography & Related Technologies*, **31**: 1283–1298 (2008).

34] Zhao Z., Wang J., Sun B., Arowo M., Shao L., Mass Transfer Study of Water Deoxygenation in a Rotor-Stator Reactor Based on Principal Component Regression Method, *Chemical Engineering Research and Design*, **132**: 677-685 (2018).

[35] Miller J.N., Miller J.C., "Statistics and Chemometrics for Analytical Chemistry", Sixth ed., ISBN- 978-0-273-73042-2. (2010)