RSC Advances

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/advances

Simultaneous spectrophotometric determination of ceftizidime and sulbactam using multivariate calibration methodes

Shiva Mahramyari[a], Eslam Pourbasheer[*a], Alireza Banaei[a], Mohammad Reza Ganjali[b] and Parviz Norouzi[b]

[a]*Department of Chemistry, Payame Noor University, Tehran, Iran*

[b]*Center of Excellence in Electrochemistry, Faculty of Chemistry, University of Tehran, P. O. Box 14155-6455, Tehran, Iran*

Abstract

The simultaneous spectrophotometric determination of ceftazidime (CTZ) and sulbactam (SBT) in the presence of the overlapping spectra were accomplished with the partial least squares (PLS) and genetic algorithm-partial least square (GA-PLS) approaches. In this study, the calibration set was based on the absorption spectra in the range of 230-350 nm for 25 different mixtures of CTZ and SBT. The calibration curve was linear over the concentration range of 2-30 and 4-46 μg mL^{-1} for CTZ and SBT, respectively. These two methods were tested by analyzing the synthetic mixtures of the CTZ and SBT and they were applied to the real samples, containing a commercial pharmaceutical preparation of the subjected drugs. Good results obtained by two methods. However, the accuracy of the GA-PLS method was better than that of PLS method.

Keywords: Ceftazidime; Sulbactam; Spectrophotometric; Partial least square; Genetic algorithm

1. Introduction

Ceftazidime, (CTZ), a beta-lactamase stable semi-synthetic third generation aminothiazolyl cephalosporin; is use in febrile episodes in patients with hematological malignancies [1-4]. CTZ is active against facultative or aerobic Gram-negative (Gram-negative bacterial infections are a major cause of morbidity and mortality in immune compromised patients) and also active against some Gram-positive pathogens[1].CTZ is identified by a widely antimicrobial spectrum. Several methods have been used for estimation of ceftazidime which includes spectrophotometry[5-7], high performance liquid chromatography (HPLC)[8, 9].

Sulbactam,(SBT), is a penicillanic sulfone[10] and a beta-lactamase inhibitor[11] with a poor absorption in the gastrointestinal tract[12]. Inducible beta-lactamases have been found in almost all Gram-negative bacteria, which are important causes of nosocomial infections [13]. SBT can be combined with one of many beta-lactam antibiotics. Sulbactam was analyzed successfully by spectrophotometry [14], capillary electrophoresis [15], HPLC[16] and gas chromatography-mass spectrometry (GC-MS) [16]. It should be mentioned that although above techniques provide accurate results, they may be unsuitable for the analysis of large sample sets, because the separation step is time-consuming.

Partial least squares (PLS) is a multivariate calibration model which for building regression models on the latent variable decomposition (analyzed) relating two sets, matrices X and Y[17, 18] which are very large data matrices. PLS involves a two-step procedure: (1) calibration, where the relation between spectra and reference component concentrations is established from a set of standard samples, and (2) prediction, in which the calibration results are employed to estimate the component concentrations in unknown samples[17, 19]. Various multi component appoint by applied PLS method to spectrophotometric data have been reported [20-22]. Derivative techniques have obtained to be very remedial in the resolution of binary and ternary mixtures, whereas multivariate calibration has been found to be the method of election for more complex mixtures [23, 24]. Until not so many years ago, partial least square (PLS) was discussed to be high insensitive to noise, and hence it was commonly stated that no trait selection at all was required[25, 26].

Genetic Algorithm (GA) is a useful method with variable selection problems[27]. This method of numerical optimization that simulate biological evolution based on the Darwin theory[28]. GA was the prediction ability of the model, specifically for PLS models[27]. This

method is very complex and the mathematical properties are unknown [29, 30]. The algorithm used in this article is an evolution of the algorithm described by Leardi and Gonzalez [31].

In the present study, the PLS and GA-PLS methods were used for simultaneous spectrophotometric determination of ceftazidime and sulbactam mixtures in different real samples.

2. Experimental

2.1. Reagents and instrumentation

Ceftazidime, sulbactam, potassium dihydrogen phosphate (KH_2PO_4), sodium hydroxide (NaOH) were supplied by Merck. Spectrophotometric measurements were performed on a di-beam Shimadzu UV-visible spectrophotometer with 1.0 cm quartz cells. The pH measurements were made with a Jenway pH meters (Germany). Ilettich EBA 20 centrifuges (Germany) selected for centrifuge of urine sample. Syringe filter with 0.20 μm pore size (made in USA) were used for filtering of real samples. The PLS and genetic algorithm were done under the Matlab R2009a software.

2.2. Preparation of real samples

2.2.1. Urine sample

The human urine samples are diluted with distilled water in the ratio of 1:3. Then, the cell debris and the particulate matter were removed from the urine using low-speed centrifugation (for 5 min at 1500 rpm). Certain amount of NaOH added to the final solution to be pH = 7. Moreover, appropriate amounts from the stock solutions of CTZ and SBT were added to 0.5 mL of the final prepared urine and completed to the final volume (10 mL volumetric flask) with buffer solution to get the desired concentration.

2.2.2. Water sample

To determine the actual examples, a few samples of water which included: river water, lake water and treated water is used to measure the drug was applied. River water collected from Ardebil city river. Lake water was sampled from Ardebil Shourabil lake. Tab water was obtained from the laboratory of Payam-e- noor university of Ardebil. River water, lake water and treated water filtered using a 0.2μm pore size syringe filter to remove suspended particulate matter. Appropriate amounts from the stock solutions of CTZ and SBT were

added to 0.5 mL of the final prepared water samples and completed to the final volume (10 mL volumetric flask) with buffer solution to get the desired concentration. These water samples can be stored 4°C in the dark at room temperature for one week in amber glass container was kept for analysis.

3. Results and discussion

3.1. Selection of the linear range

The absorption spectra of CTZ and SBT in buffer phosphate solution at pH=7 are shown in Fig. 1. As can be seen the spectra of these compounds are completely overlapped. The spectral overlapping of the drugs prevented the resolution of the mixtures by direct spectrophotometric measurements. Thus, the univariate analysis could not be applied to resolve their mixtures. The calibration curves were constructed with 14 points, with the absorbance versus the CTZ and SBT concentrations in the ranges of 2-30 and 4-46 μg mL^{-1}, respectively. As can be seen in Fig. 2, in these range the linear regression results showed high R^2 value of 0.995 and 0.996 for the CTZ and SBT respectively.

3.2. PLS and GA-PLS Methods

3.2.1. Calibration and prediction data set:

The first step for the simultaneous determination of CTZ and SBT by multivariate methods is the building of the calibration matrix. We prepared the calibration set with the absorption spectra. The PLS model calibration was optimized with the aid of the orthogonal array design (OAD) method[20]. A set of standard samples was equipped according to a five-level orthogonal array design which led to 25 samples, so that the concentration of each drug in the resulting solutions was in its own linear dynamic range. According to calibration set and OAD method, 25 experiments were carried out, which are shown in Table 1.

For prediction set, 7 mixtures were prepared randomly, which were not including in the calibration set, and were used as an independent test (Table. 2).

3.2.2. Optimal number of factors selection:

The optimum number of factors (latent variables), to be included in the calibration model was determined by computing the root mean squares error (RMSE) for cross-validated models. The cross-validation method was employed to eliminate only one samples at a time, and then the remaining standard spectra were calibrated by PLS[32]. Using this calibration sample concentration is predicted to left-out sample. This process was repeated until each standard had been left out once. A reasonable select for the optimum number of factors would be that number, which yielded the minimum RMSE. A solution to this problem has been suggested by Haaland et al.[25] Fig. 3 shows the plots of RMSE against the number of factors with the PLS method for CTZ and SBT. The optimum number of factors to collect whit PLS and GA-PLS models are selected 2 number of factors.

3.2.3. Statistical parameters

Some statistical parameters were selected to test the prediction ability of the PLS and GA-PLS methods for the simultaneous determination of the CTZ and SBT mixtures. This parameters are root mean squares error (RMSE) and relative error of prediction (REP%).

$$RMSE = \left[\frac{1}{n}\sum_{i=1}^{n}(x_i - \hat{x}_i)^2\right]^{0.5}$$

The RMSE values are an estimation of the absolute error of prediction for each component. Another profitable parameter was relative error of prediction (REP%), that shows the predictive ability of each component.

$$REP(\%) = \frac{100}{\bar{x}}\left[\frac{1}{n}\sum_{i=1}^{n}(x_i - \hat{x}_i)^2\right]^{0.5}$$

Where x_i is the true concentration of the analyte in the sample i, $\hat{x}_i$ represents the estimated analyte concentration in the sample i, $\bar{x}$ is the mean of the true concentration in the prediction set. n is the total number of samples used in the prediction set. The values of RMSE and REP(%) for the CTZ and SBT mixtures are collected in the Table 3.

Table 3 also provides the figures of merit such as limits of detection (LOD) and limit of quantitation (LOQ). The details of LOD and LOQ are reported in our previous work [27].

3.2.4. Variable selection

The calibration set consisted of 121 variables. The genetic algorithm was run for these 121 variables (in the selected range of 230-350 nm) using a PLS regression method. The maximum number of factors allowed is the optimal number of components determined by cross-validation on the model containing all the variables, and the selected variables were used for the running of PLS. For obtaining the optimum set of wavelengths for determination of CTZ and SBT, the GA procedure was repeated. Finally a wavelength was selected if the percent of selection for that variable exceeded a critical value. The selected wavelengths are 231, 230, 232 and 235 nm for SBT and 232, 230, 231 and 238 nm for CTZ. By the selected wavelengths the new PLS methods created and the results are reported in Table 3.

3.2.5. Determination of ceftazidime and sulbactam in synthetic mixtures

The predictive ability of the method was determined using seven two-component of CTZ and SBT mixtures (their compositions are given in Table 2). The results obtained applying PLS and GA-PLS algorithm to seven synthetic samples of CTZ and SBT mixtures listed in Table 2. The results of the RMSE and REP% for the PLS and GA-PLS methods are summarized in Table 3. The comparison of GA-PLS results with PLS shows that the values of RMSE and REP (%) in the GA-PLS method with two components are less than PLS method. The plots of the RMSE versus the number of factors are shown in Figure 3. Also, the plots of the predicted concentration versus actual values are shown in Figure 4 (R^2 values are also shown). As can be seen, the present study shows that the GA can be a good method for feature selection in spectral data sets. The results obtained on a data set of SBT and CTZ mixture demonstrate that the predictive ability of the models obtained with the wavelengths selected by the genetic algorithm is very often much better.

3.2.6. Determinationof Ceftazidime and Sulbactam in real samples

In order to test the applicability and matrix interferences of the offered method to the analysis of real samples, the method was applied in human urine sample and water samples which to included river, lake and tab waters. The results are shown in Table 4. The good agreement between these results and known values indicates the successful applicability of the proposed procedure for simultaneous determination of CTZ and SBT in real samples. According to the

Table 4, it can be seen that recovery values for the GA-PLS is better than the following PLS alone.

Also, determination of CTZ and SBT was conducted in different mixtures of their pharmaceutical formulations (Ceftazidime and Ampisol vials) and the study was repeated four times. The results obtained were complying with the label claim. As can be seen in Table 5, the calculated values are in satisfactory agreement with the declared values.

4. Conclusions

According to the results obtained in this work, application of the UV spectrophotometric method is an effective and accurate way for the simultaneous determination of ceftazidime and sulbactam in binary mixtures by multivariate calibration of real samples. This mixture is a difficult complex system, because of the high spectral overlapping observed between the absorption spectra of their components. For overcoming the drawback of the spectral interferences, PLS and GA-PLS multivariate calibration using the absorption spectra were applied to the CTZ and SBT concurrent analysis in their synthetic mixtures. Analysis of the results for binary mixtures showed that the use of GA-PLS leads to more accurate results than the PLS method. In addition, the application of this method was tested in different real samples and good results have been obtained.

Refrences

1. G. A. Albarellos, L. A. Ambros and M. F. Landoni, *The Veterinary Journal*, 2008, **178**, 238-243.
2. E. Dailly, A. Brun, M.-F. Kergueris, C. Victorri-Vignoli, N. Milpied and P. Jolliet, *International journal of Antimicrobial Agents*, 2006, **27**, 553-556.
3. M. R. Siddiqui, A. Tariq, M. Chaudhary, K. D. Reddy, P. S. Negi, J. Yadav, N. Srivastava, S. M. Shrivastava and R. Singh, *American Journal of Applied Sciences*, 2009, **6**, 1781-1787.
4. C. Rains, H. Bryson and D. Peters, *Drugs*, 1995, **49**, 577-617.
5. B. Hiremath and B. Mruthyunjayaswamy, *Acta Pharmaceutica*, 2008, **58**, 275-285.
6. A. d. H. Moreno and H. R. N. Salgado, *Analytical Letters*, 2008, **41**, 2143-2152.
7. V. Ródenas, M. S. García, C. Sánchez-Pedreño and M. I. Albero, *Journal of Pharmaceutical and Biomedical Analysis*, 1997, **15**, 1687-1693.
8. J. Ayrton, *Journal of Antimicrobial Chemotherapy*, 1981, **8**, 227-231.
9. J. Guitton, A. Laffont, J. Bruzeau, L. Rochet-Mingret, M. Bonnefoy and J. Bureau, *Journal of Chromatography B: Biomedical Sciences and Applications*, 1998, **719**, 151-157.
10. M.-N. Hung, P.-R. Hsueh, H.-T. Chang, W.-S. Lee, M.-Y. Chou, I.-S. Chen, J.-H. Wang, C.-F. Lin, J.-M. Shyr, W.-C. Ko, J.-J. Wu, Y.-C. Liu, W.-K. Huang, L.-J. Teng, C.-Y. Liu and K.-T. Luh, *International journal of Antimicrobial Agents*, 2007, **29**, 145-152.
11. Y. Zhou, J. Zhang, B. Guo, J. Yu, Y. Shi, M. Wang and Y. Zhang, *Journal of Chromatography B*, 2010, **878**, 3119-3124.
12. Q. Pei, G.-P. Yang, Z.-J. Li, X.-D. Peng, J.-H. Fan and Z.-Q. Liu, *Journal of Chromatography B*, 2011, **879**, 2000-2004.
13. F.-D. Wang, M.-L. Lin, W.-S. Lee and C.-Y. Liu, *International journal of Antimicrobial Agents*, 2004, **23**, 590-595.
14. J. Haginaka, J. Wakai, H. Yasuda, T. Uno and T. Nakagawa, *Analyst*, 1984, 109, 1057-1059.
15. G. Pajchel, K. Pawłowski and S. Tyski, *Journal of Pharmaceutical and Biomedical Analysis*, 2002, **29**, 75-81.
16. M. Siddiqui, A. Tariq, K. Reddy, P. Negi, J. Yadav, A. Bhatnagar, M. Chaudhary and R. Singh, *International Journal of Pharmacology*, 2010, **6**, 271-277.
17. J. A. Arancibia, G. M. Delfa, C. E. Boschetti, G. M. Escandar and A. C. Olivieri, *Analytica Chimica Acta*, 2005, **553**, 141-147.
18. H. Khajehsharifi, M. Sadeghi and E. Pourbasheer, *Monatshefte für Chemie-Chemical Monthly* 2009, ***140***, 685-691.
19. H. Martens, *Multivariate Calibration*, John Wiley & Sons, 1989.
20. R. G. Brereton, *Analyst*, 2000, **125**, 2125-2154.
21. V. Kaur, A. K. Malik and N. Verma, *Annali di Chimica*, 2007, **97**, 237-249.
22. S. Riahi, M. R. Ganjali, E. Pourbasheer, F. Divsar, P. Norouzi and M. Chaloosi, *Current Pharmaceutical Analysis,* 2008, ***4*****,** 231-237.
23. M. Blanco, J. Coello, H. Iturriaga, S. Maspoch and N. Villegas, *Analyst*, 1999, **124**, 911-915.
24. C. K. Markopoulou and J. E. Koundourelllis, *Journal of Pharmaceutical and Biomedical Analysis*, 2003, **33**, 1163-1173.
25. D. M. Haaland and E. V. Thomas, *Analytical Chemistry*, 1988, **60**, 1193-1202.
26. D. M. Haaland and E. V. Thomas, *Analytical Chemistry*, 1990, **62**, 1091-1099.

27. A. Beheshti, S. Riahi, E. Pourbasheer, M. R. Ganjali and P. Norouzi, *Journal of Food Science*, 2010, **75**, C135-C139.
28. H. Khajehsharifi and E. Pourbasheer, *Journal of the Chinese Chemical Society*, 2008, **55**, 163-170.
29. S. Riahi, M. R. Ganjali, E. Pourbasheer and P. Norouzi, *Current Pharmaceutical Analysis,* 2007, ***3***, 268-272.
30. A. G. Gonzalez, M. A. Herrador and A. G. Asuero, *Talanta*, 1999, **48**, 729.
31. R. Leardi and A. Lupiáñez González, *Chemometrics and Intelligent Laboratory Systems*, 1998, **41**, 195-207.
32. M. Stone, *Journals of the Royal Statistical Society B*, 1974, **36**, 111.

Table 1. Concentration data of the different mixtures used in the calibration set for the determination of Ceftazidime and Sulbactam

Mixture	Ceftazidime (μg ml^{-1})	Sulbactam (μg ml^{-1})
M1	2	4
M2	2	14.5
M3	2	25
M4	2	35.5
M5	2	46
M6	9	4
M7	9	14.5
M8	9	25
M9	9	35.5
M10	9	46
M11	16	4
M12	16	14.5
M13	16	25
M14	16	35.5
M15	16	46
M16	23	4
M17	23	14.5
M18	23	25
M19	23	35.5
M20	23	46
M21	30	4
M22	30	14.5
M23	30	25
M24	30	35.5
M25	30	46

Table 2. Added and found (μg ml^{-1}) results of the seven synthesis mixtures of ceftazidime and sulbactam by PLS and GA-PLS methods

Added		Found by PLS		Recovery (%)		Found by GA-PLS		Recovery (%)	
CTZ	SBT	CTZ	SBT	CTZ	SBT	CTZ	SBT	CTZ	SBT
3	5	2.944	5.264	98.136	105.284	2.962	5.326	98.727	106.511
4	42	4.322	42.064	108.057	100.152	4.416	41.616	110.406	99.086
27	6	27.122	6.478	100.451	107.969	27.127	6.316	100.464	105.269
10	15	9.855	14.852	98.546	99.016	9.931	14.497	99.309	96.646
15	20	14.934	20.182	99.558	100.909	14.984	19.806	99.891	99.032
20	30	19.693	30.275	98.465	100.915	19.720	30.202	98.599	100.673
29	45	28.616	45.803	98.676	101.783	28.696	45.361	98.950	100.803
LOD		0.064	0.089			0.046	0.072		
LOQ		0.192	0.267			0.138	0.216		

Table 3. Statistical parameters of the obtained models using the PLS and GA-PLS

Component	NPC[a]	RMSE[b]	REP(%)[c]
Ceftazidime[d]	2	0.236	1.528
Sulbactam[d]	2	0.392	1.685
Ceftazidime[e]	2	0.229	1.484
Sulbactam[e]	2	0.341	1.465

[a] Number of principal components
[b] Root mean squares error
[c] Relative error of prediction
[d] Using PLS
[e] Using GA-PLS

Table 4. Recovery study of simultaneous determination of CTZ, and SBT in real samples by PLS and GA-PLS methods

Real samples	Added ($\mu g\ ml^{-1}$)		Found by PLS ($\mu g\ ml^{-1}$)		Recovery (%)		Found by GA-PLS ($\mu g\ ml^{-1}$)		Recovery (%)	
	CTZ	SBT	CTZ	SBT	CTZ	SBT	CTZ	SBT	CTZ	SBT
Urine										
(I)	10	20	9.817	19.021	98.166	95.105	9.734	19.908	97.345	99.538
(II)	20	10	20.170	13.193	100.850	131.933	20.487	11.080	102.434	110.800
(III)	15	15	15.089	17.896	100.596	119.31	15.384	15.660	102.577	104.397
Lab water										
(I)	10	20	9.948	20.147	99.481	100.737	9.971	20.459	99.714	102.297
(II)	20	10	19.760	10.656	98.799	106.561	19.729	10.733	98.644	107.334
(III)	15	15	14.809	15.702	98.735	104.681	14.736	16.653	98.238	111.020
River water										
(I)	10	20	10.065	20.460	100.649	102.299	9.990	20.966	99.899	104.830
(II)	20	10	19.685	10.528	98.427	105.282	19.765	10.426	98.826	104.257
(III)	15	15	14.803	15.624	98.688	104.161	14.790	15.804	98.602	105.364
Lake water										
(I)	10	20	9.747	21.349	98.467	106.743	9.738	21.724	97.376	108.620
(II)	20	10	19.943	10.228	99.717	102.283	20.061	9.514	100.307	95.140
(III)	15	15	14.789	15.178	98.595	101.186	14.914	14.247	99.429	94.981

Table 5. Simultaneous determination of STZ, and SBT in two pharmaceutical formulations using PLS and GA-PLS models

Pharmaceutical formulation	Label claim (g/Vial)		Predicted by PLS [a]		Predicted by GA-PLS	
	STZ	SBT	STZ	SBT	STZ	SBT
Ceftizidime	2	-	1.98±0.01	-	1.93±0.01	-
Ampisol	-	1		0.99±0.01	-	0.99±0.02

[a] Mean values and relative standard deviation of four determinations.

Figure Captions

Fig. 1. Absorption spectra of CTZ (3µg ml^{-1}) and SBT(3µg ml^{-1}).

Fig.2. Analytical curve for the univariate determination of CTZ and SBT.

Fig.3. Plots of RMSE versus number of factors for CTZ and SBT.

Fig.4. Plots of predicted concentration versus actual concentration for CTZ and SBT by PLS and GA-PLS methods.

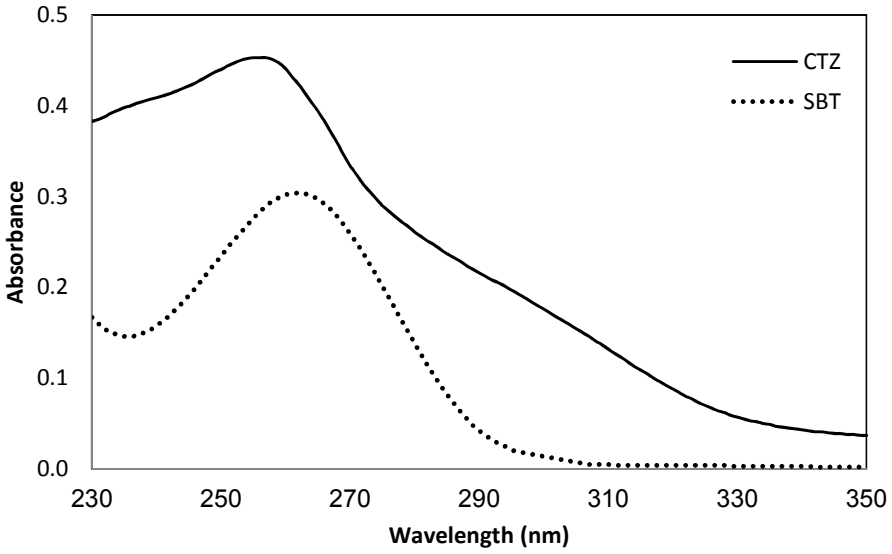
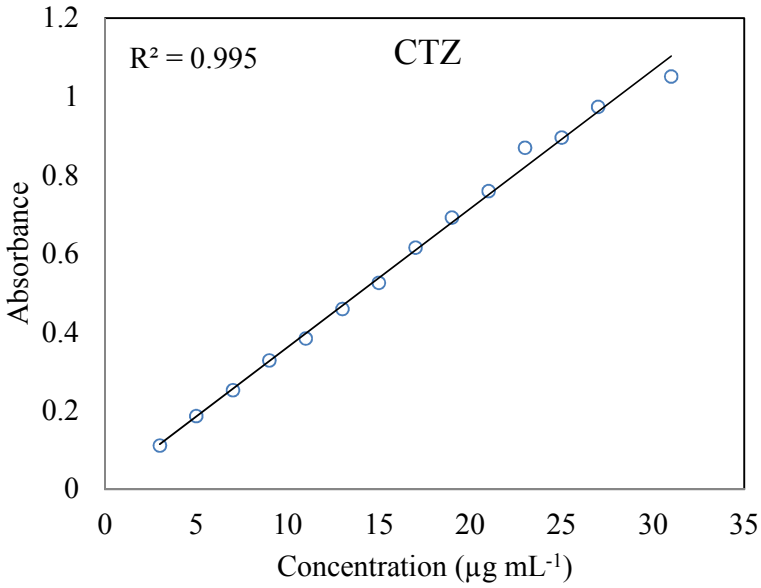
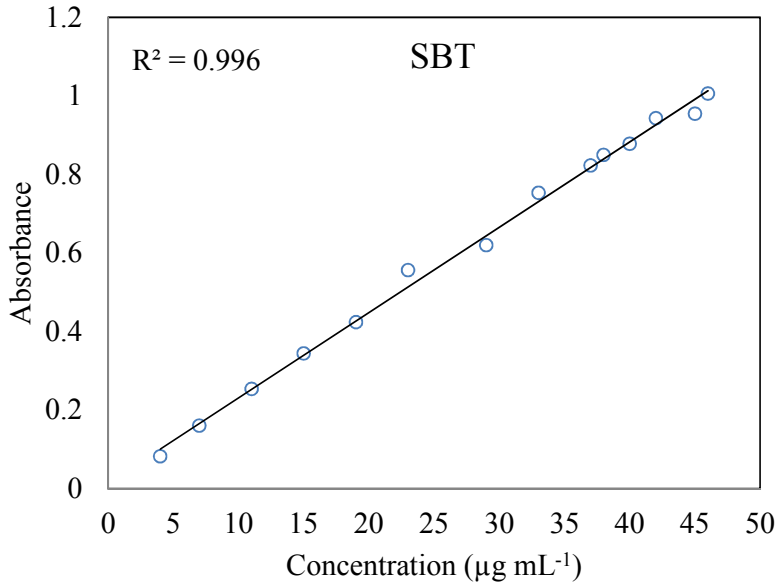


Fig. 1.



1

2

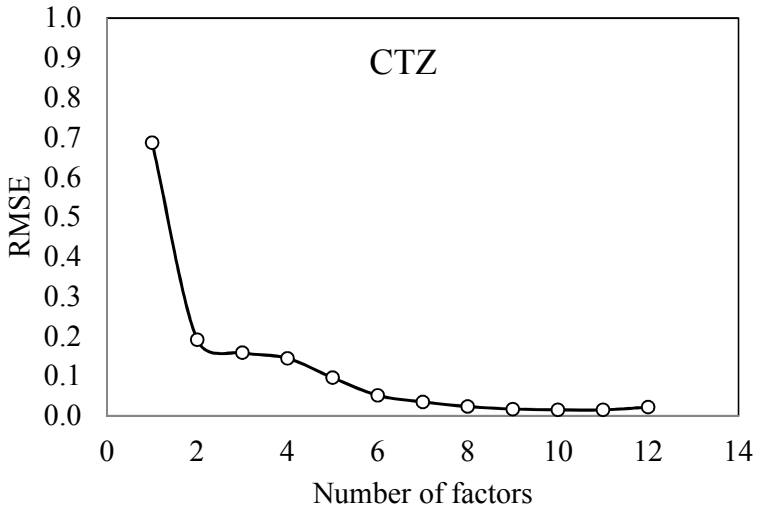


3

4

5 Fig.2.

6



z

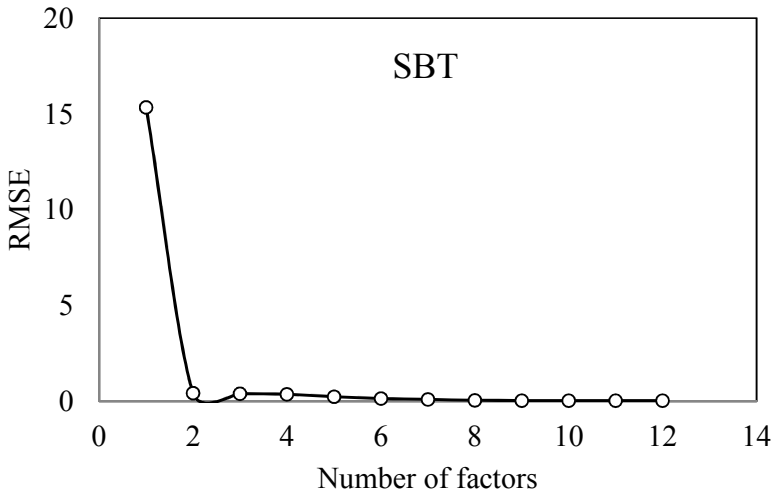
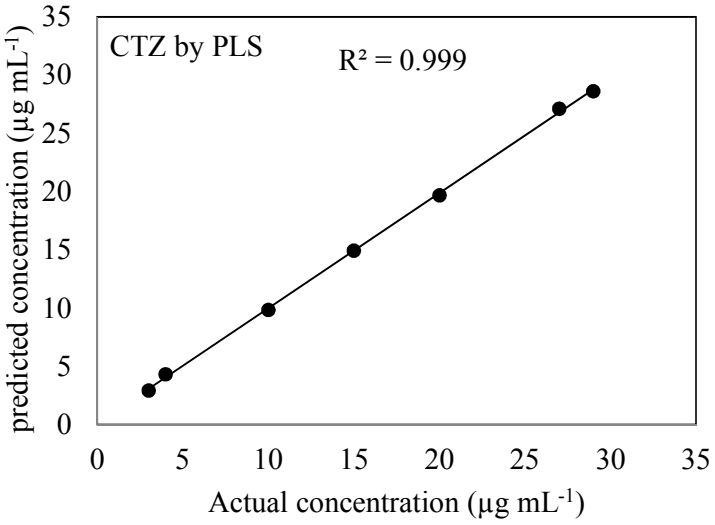
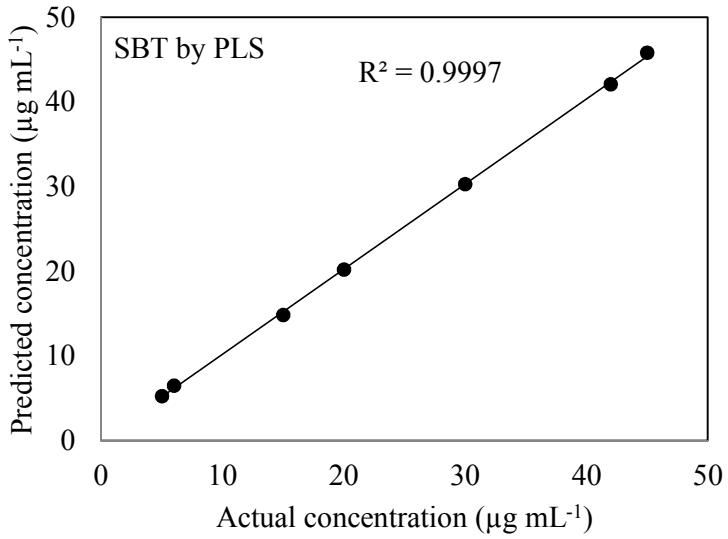


Fig.3.

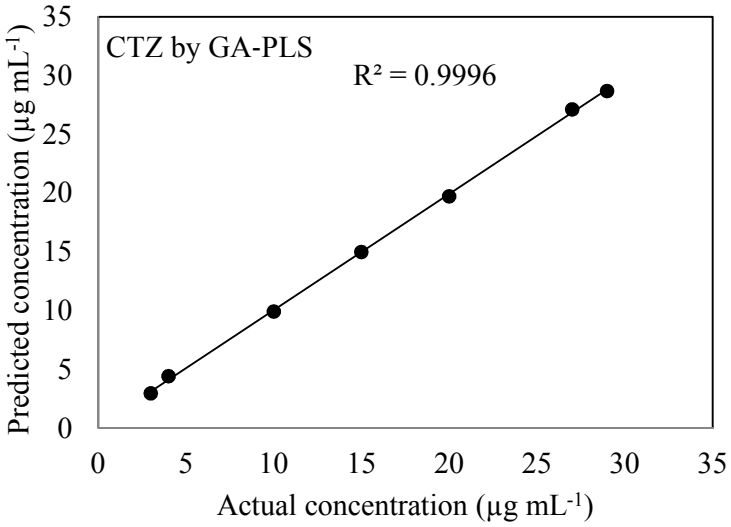


1
2



3

4



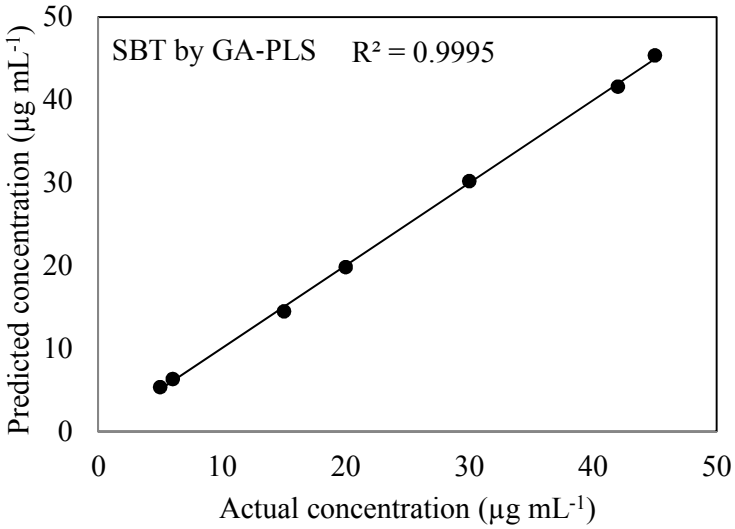


Fig.4.