Journal of Natural Sciences Research www.iiste.org
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.6, No.6, 2016

Spectrophotometric-Assisted Chemometric Method for the Simultaneous Analysis of Furosemide, Carbamazepine, Diazepam, and Carvedilol in Their Bulk and Marketed Formulation

Sarmad B. Dikran Alaa K. Mohammed Nahla A. Alassaf *
Department of Chemistry, College of Education for Pure Science/Ibn Al-Haitham, Adhamiya, Baghdad University, Baghdad-Iraq

Abstract
Simultaneous determination of Furosemide, Carbamazepine, Diazepam, and Carvedilol in bulk and pharmaceutical formulation using the partial least squares regression (PLS-1 and PLS-2) is described in this study. The two methods were successfully applied to estimate the four drugs in their quaternary mixture using UV spectral data of 84synthetic mixtures in the range of 200-350nm with the intervals $\Delta\lambda$=0.5nm. The linear concentration range were 1-20 µg.mL^{-1} for all, with correlation coefficient (R^2) and root mean squares error for the calibration (RMSE) for FURO, CARB, DIAZ, and CARV were 0.9996, 0.9998, 0.9997, 0.9997, and 0.1128, 0.1292, 0.1868,0.1562 respectively for PLS-1, and for PLS-2 were 0.9995, 0.9999, 0.9997, 0.9998, and 0.1127, 0.1205, 0.1691, and 0.1686 respectively. Satisfactory results were achieved with applying PLS-1 and PLS-2 in the determination of the cited drugs in their pharmaceutical formulations and a good agreement was found between the proposed methods.
Keywords: PLS, spectrophotometry, furosemide, carbamazepine, diazepam, and carvedilol

1. Introduction

Furosemide (FURO), fig.1a; is an anthranilic acid derivative, chemically known as 4-Chloro-2-[(furan-2-ylmethyl) amino]-5-sulfamoylbenzoic acid (British Pharmacopoeia 2013). Furosemide is a potent diuretic drug commonly used in adults, children, and infants for the management of excessive fluid accumulation and edema caused by congestive heart failure, renal disease and cirrhosis of the liver. In adults, oral FURO may be used alone or in combination with different antihypertensive agents for the treatment of hypertension (Şimşek *et al*. 2012).

Carbamazepine (CARB), fig.1b; is a tricyclic highly lipophilic neutral compound, chemically 5*H*-Dibenzo [*b, f*] azepine-5-carboxamide (British Pharmacopoeia 2013). Extensively it is used as antiepileptic, mood stabilizing, and in treatment of bipolar affective disorder like restless leg syndrome, resistant schizophrenia, psychotic behavior associated with dementia, post-traumatic stress disorders, and ethanol withdrawal (Leikin & Paloucek 2008).

Diazepam (DIAZ), fig.1c; is a benzodiazepine derivative drug, chemically defined as 7-Chloro-1-methyl-5-phenyl 1, 3 -dihydro-2*H*-1,4 -benzodiazepin-2-one (British Pharmacopoeia 2013), commonly used in anxiety disorders management, skeletal muscle relaxant, sleep disturbance, seizures including status epilepticus, and in treatment of convulsive disorders (Riss *et al*. 2008).

Carvedilol (CARV), fig.1d; is a racemic lipophilic aryoxypropanolamine, chemically (2*RS*)-1-(9*H*-Carbazol-4-yloxy)-3-[[2-(2-methoxyphenoxy) ethyl] amino] propan-2-ol (British Pharmacopoeia 2013). Carvedilol belongs to a medicines group called β- adrenoceptor antagonist agents which indicated for the treatment of hypertension, angina pectoris, arteriolar vasodilator, and mild or moderate heart failure of ischemic or cardiomyopathic . As compared with other beta-blockers, carvedilol has minimal inverse agonist activity and the morbidity from congestive heart failure has been decreased with its use (Packer *et al*. 2002).

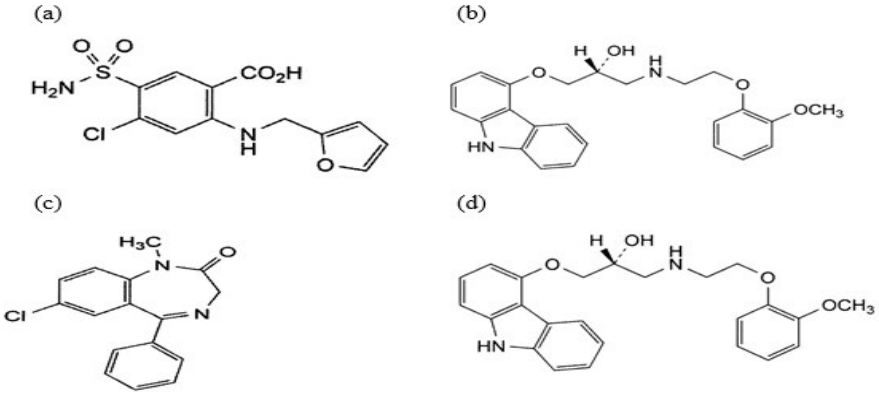

Figure 1. Chemical structures of FURO (a), CARB (b), DIAZ (c), and CARV (d).

Literature survey reveals several conventional analytical techniques including spectrophotometry,

HPLC, flow injection analysis, FT-IR, spectofluorimetry voltammetry, hyphenated techniques such as GC-MS, HPLC-MS and so on for individual or simultaneous quantification of FURO (Semaan *et al*. 2005, Zaporozhets *et al*. 2012, Gallignania *et al*. 2014, Santini *et al*. 2009, Pate & Solanki 2012 and Ram *et al*. 2012), CARB (Jayanna *et al*. 2014, Sultana *et al*. 2013, Zhang *et al*. 2009, Hoehn 2014, Pan *et al*. 2014 and Kumar & Umamaheswari 2011), DIAZ (Mohamed *et al*. 2014, Sruthi *et al*. 2013, Liawruangrath *et al*. 2006, Cordero & Paterson 2007, Moros *et al*. 2007, Metrohm 250/1e and Shweta *et al*. 2013), and CARV (Shariti-Rad *et al*. 2014, Elezovi *et al*. 2015, Silva *et al*. 2008, Yilmaz & Arslan 2011, Jagannathan *et al*. 2010 and Yilmaz & Ekinci 2011) in pure form, pharmaceutical formulation, and biological fluids.

To our knowledge, the published literatures did not cover the simultaneous analysis of the quaternary mixture of FURO, CARB, DIAZ, and CARV in API and pharmaceutical formulation, however there is a few methods (Patil *et al*. 2012, Zhang *et al*. 2011 and Netherton 2011) were reported for the simultaneous determination of two or three of the proposed drugs.

The spectrophotometric study of several species in their mixture to discriminate between in them depending on differences in their spectral properties is often suffer from having overlapping spectral characteristics. Recently, such studies in which heavily overlapping responses of the studied components are present were possible to be carry out relying on chemometrics multivariate procedures (Niazi & Yazdanipour 2007).

Several chemometric techniques based on artificial intelligence and factor analysis; including design of experimental (DOE), classical least square (CLS), principle component regression (PCR), artificial neural network (ANN), and partial least squares (PLS-1 and PLS-2 models) have been applied increasingly for multicomponent investigations (Solanki *et al*. 2014, Karaoglan *et al*. 2007, Torrecilla *et al*. 2008, Nguyen & Rocke 2002 and Dinc *et al*. 2006).

PLS is a linear regression method that forms components (factors, or latent variables) as new independent variables (explanatory variables, or predictors) in a regression model. In PLS two matrices are worked: X and Y, where generally X matrix (its dimension N x K) contains the independent variables (predictors) and Y matrix (its dimension N x M) contains dependent variables (response) (Figure 2) (Eriksson *et al*. 20130).

Figure 2. Schematic representation of PLS

In analytical chemistry, where PLS is mainly used in multivariate calibration, X matrix contains the digitized spectral data (N) at wavelengths (K) while Y matrix contains the analyte concentrations of N training set samples.

The PLS regression can be considered as involving of outer relations, for X and Y matrices individually, (equations 1 and 2 respectively) and an inner relation links both matrices together (equation 3).

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

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

$$U = BT + H \quad (3)$$

Where T and U are score matrices, P′ and C′ are loading matrices, E and F are matrices residual of X and Y block respectively (Geladi & Kowalski 1986).

Two PLS models of calculations are available, in the PLS1 regression (PLS univariate regression), there is only one dependent variable, whilst in the PLS2 regression (PLS multivariate regression) there are several dependent variables. In light of the fact that both PLS-1 and PLS-2 enables quick and simultaneous analysis of each species in the mixture, with slightest sample pre-treatments or need for pre-separation (Steele 1989 and Geladi & Kowalski 1986), therefore we have applied both of them in this study.

2. Experimental

2.1 Apparatus

A Cecil CE7200 UV-Visible double beam spectrophotometer (Cambridge-England) equipped with 10 mm quartz cell was used. The Cecil Instrument-DataStream Software Version: 5.1 was used for all data acquisition.

2.2 Software

A Simplex Lattice Mixture Design (create by JMP® 11.0.0 SAS Institute Inc.) was used to prepare a set of calibration mixtures for the simultaneous determination of FURO, CARB, DIAZ, and CARV, while PLS analysis which were carried out by using OriginPro software (internal version 9.2).

2.3 Chemicals

The standard grade powder (furosemide, carbamazepine, diazepam, and carvedilol) used in this work received in pure form (99.99%) as a gift from the State Company for Drug Industries and Medical Appliances Samara-Iraq (SDI). Methanol (99.9 %) for HPLC (Sigma Aldrich, Germany).

Pharmaceutical formulations assayed in this study were procured from local pharmacies; Lasix 40 mg / table (SWI, France), Tegretol® 200 mg / tablet (NOVARTIS, Switzerland), VALIAPAM 2 mg / tablet (SDI, Iraq), and Carvidol® 25 mg /tablet (Pharma International, India).

2.4 Standard Drugs Solution

Standard solutions (1000 µg.mL^{-1}) of FURO, CARB, DIAZ, and CARV (SDI) were prepared by dissolution in methanol. All chemicals and solvents used were of analytical reagent grade.

2.5 Procedure

2.5.1 Procedure for resolving quaternary mixtures

In a 5-ml volumetric flask aliquot of mixture sample solution containing between 5-100 µg of each of the studied drugs was introduced and diluted with methanol. The calibration set composed of eighty-four mixtures combination prepared according to optimal mixture design (Simplex Lattice).

The spectrum for each of the 84-prepared mixtures was recorded in wavelength range 200-350 nm, with a scan speed of 10 nm.sec^{-1}, averaging of 1.0 nm, bandwidth of 1.8 nm, and data interval of 0.5 nm against solvent blank. The optimized conditions used for calibration matrix was applied to analyze the problem samples and calculate the concentrations for the four components in the mixture.

2.5.2 Analysis of FURO, CARB, DIAZ, and CARV in commercial formulations

Ten tablets of each pharmaceutical product were separately weighed to an average weight. After crushing, mixing and homogenizing, a portion equivalent to 0.0399 gm, 0.0128 gm, 0.6014 gm, and 0.1522 gm for Lasix®, Tegretol®, VALIAPAM, and Carvidol® respectively were weighed, dissolved in about 40 mL of methanol and sonicated for at least 10 min with intermittent shaking. The contents of each were transferred quantitatively into a separate 10 mL volumetric flask, shaken well and diluted to mark with methanol to get 1000 µg.mL^{-1}. The solutions were filtered through Whatman No.41 filter paper and stored as the standard stock solutions for further dilution in subsequent use. Aliquots of the standard stock solution were placed in a 5-ml volumetric flask, and then analyzed as described above.

3. Results and discussion

The absorption spectra of FURO, CARB, DIAZ, and CARV were recorded in the wavelength range of 200-350 nm. The linearity of the maximal signals was examined to select an adequate concentration range (for each individual compound) suitable for spectrophotometric measurements. It was found that linear concentration range of 1.0-20.0 µg.mL^{-1}, as absorbance versus drugs concentrations, at 233 nm, 228.5 nm, 215.0 nm, and 242.5 nm for FURO, DIAZ, CARB, and CARV respectively was obtained and statistically evaluated by linear regression.

On the other hand, Figure 3 shows that resolving of such mixture cannot be carried out by univariate analysis methods snice their spectra of seems to be strongly overlapped. Therefore, simultaneous multicomponent analysis of UV-VIS measurements via partial least squares (PLS) was applied for this purpose.

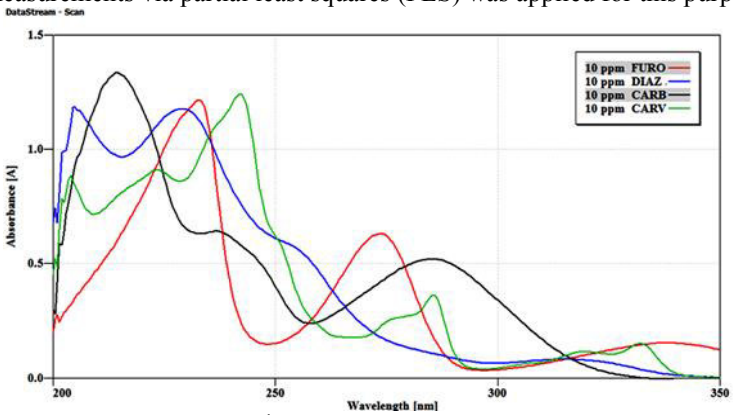

Figure 3. Normal mode spectrum of 10 µg.mL^{-1} of FURO, CARB, DIAZ, and CARV against their solvent blanks (recorded with scan speed of 10 nm.sec^{-1}, averaging of 1.0 nm, bandwidth of 1.8 nm, and data interval of 0.5 nm).

Training set was obtained from the spectrophotometric data by a careful UV-measurements on a set of known samples, composing of 84 mixtures. The composition of samples to be determined have been selected according to Simplex Lattice Mixture Design in order to span all dimensions without correlation between different calibration samples since collinear components in the training set data tends to cause under fitting in the PLS

models (Zolgharnein *et al*. 2015).

This set was used to establish PLS-1 and PLS-2 modes of calibrations after arranging the data as matrix, which organized into pairs i.e. each absorbance matrix is paired with its corresponding concentration matrix. In the first case, PLS-2, the concentrations of all components in the quaternary mixture were correlated to the values of the measured absorbance, while in PLS-1 the concentration of each drug in the mixture was used to build up the models.

The spectral information in the range of 200-350 nm (301 experimental points per spectrum) was used for the calibration. The data were feed to OriginPro 2015 software, after arranging them as row absorbance matrix paired with their corresponding concentration matrix, to build up PLS-1 and PLS-2 regression modes.

3.1 Selection of the optimum number of factors for the partial least-squares methods

Cross-validation technique was used to select the smallest model with fewest numbers of factors in the PLS algorithm. This was carried out by randomly leaving out one sample at a time and letting the software to predict the value of residual error sum of squares (**PRESS** =$\sum(y_i - \hat{y}_i)^2$) and use it as an indicator for appropriateness of models (Afkhami & Sarlak 2005). Figure 4 shows the plot of PRESS against the number of factors for PLS-2 and PLS-1 model for determination of drugs in their pure forms.

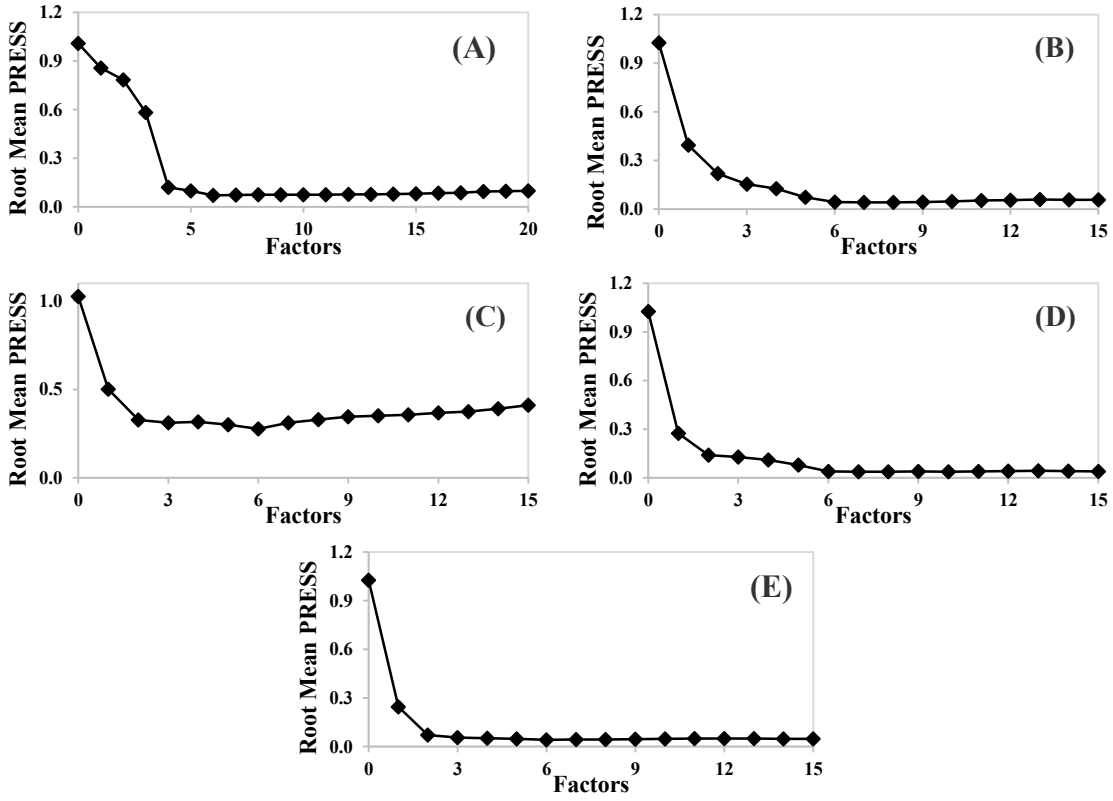

Figure 4. Plots of Root Mean PRESS against number of factors for eighty-four CARV, DIAZ, CARB, and FURO mixtures for (A) PLS-2 model, (B) PLS-1 model for CARV, (C) PLS-1 model (D) for DIAZ, and (E) for CARV PLS-1 model.

3.2 Statistical parameters for the optimized models

The predictive capabilities PLS-1 and PLS-2 models were examined for simultaneous determination of FURO, DIAZ, CARB, and CARV in each mixtures of the train set. Moreover, the prediction error of a single component in the mixture contain the four drugs samples (N) and total prediction error of forty sample mixtures (M) were calculated as the single and total relative standard error (R.S.E %) of the prediction concentration (Niazi & Yazdanipour 2007).

$$\text{R.S.E.(\%) single} = \sqrt{\sum_{i=1}^{N}(\hat{y}_i - y_i)^2 / \sum_{i=1}^{N}(y_i)^2} \times 100 \,,$$

$$\text{R.S.E.(\%) total} = \sqrt{\sum_{j=1}^{M}\sum_{i=1}^{N}(\hat{y}_{ji} - y_{ji})^2 / \sum_{j=1}^{M}\sum_{i=1}^{N}(y_{ji})^2} \times 100.$$

The developed PLS-1 and PLS-2 models were validated by testing their predictive abilities for simultaneous

determination of CARV, DIAZ, CARB, and FURO in twenty quaternary synthetic mixtures set. Tables 1 and 2 show the predicted results and other statistical parameters.

Table 1. Composition of synthetic samples, their predictions by PLS-1 model and statistical parameters for the system.

Mixture (µg.mL^{-1})				**Prediction (µg.mL^{-1})**				**Recovery %**			
CARV	***DIAZ***	***CARB***	***FURO***	***CARV***	***DIAZ***	***CARB***	***FURO***	***CARV***	***DIAZ***	***CARB***	***FURO***
3.3	13.3	0.0	3.3	3.2026	13.13663	0.07597	3.18185	97.0485	98.7717	----	96.4197
10.0	0.0	0.0	10.0	10.12346	0.12174	0.00193	10.15812	101.2346	----	----	101.5812
6.7	0.0	13.3	0.0	6.64987	0.08006	13.35623	----	99.2518	----	100.4228	----
6.7	13.3	0.0	0.0	6.72821	12.93153	0.19586	----	100.4210	97.2295	----	----
3.3	0.0	13.3	3.3	3.31485	0.04508	13.32951	3.34501	100.4500	----	100.2219	101.3639
16.7	3.3	0.0	0.0	17.00413	3.12232	----	0.2181	101.8211	94.6158	----	----
13.3	3.3	0.0	3.3	13.32955	3.33491	0.07932	3.35378	100.2222	101.0579	----	101.6297
3.3	6.7	6.7	3.3	3.21623	6.53646	6.92744	3.22136	97.4615	97.5591	103.3946	97.6170
6.7	0.0	3.3	10.0	6.78976	0.08845	3.3884	10.07961	101.3397	----	102.6788	100.7961
0.0	10.0	10.0	0.0	----	9.67557	10.21714	----	----	96.7557	102.1714	----
0.0	3.3	3.3	13.3	0.02684	3.31828	3.47743	13.45101	----	100.5539	105.3767	101.1354
0.0	0.0	10.0	10.0	0.07424	----	9.99918	10.12232	----	----	99.9918	101.2232
0.0	13.3	6.7	0.0	----	13.18113	6.76265	----	----	99.1062	100.9351	----
0.0	0.0	20.0	0.0	----	0.12764	19.96457	-0.14006	----	----	99.8229	----
0.0	10.0	0.0	10.0	----	10.06795	0.01335	9.80244	----	100.6795	----	98.0244
0.0	20.0	0.0	0.0	----	19.83869	----	----	----	99.1935	----	----
10.0	0.0	10.0	0.0	10.36272	----	9.8465	----	103.6272	----	98.4650	----
0.0	0.0	13.3	6.7	0.03046	----	13.28889	6.83778	----	----	99.9165	102.0564
3.3	10.0	0.0	6.7	3.28586	9.87426	0.0476	6.68036	99.5715	98.7426	----	99.7069
0.0	6.7	10.0	3.3	----	6.52563	10.17081	3.30186	----	97.3975	101.7081	100.0564
Mean recovery								100.2227	98.4719	101.2588	100.1342
RSE (%) single								2.4059	1.8988	1.3259	2.1000
RSE (%) total									1.8828		

Table 2. Composition of synthetic samples, their predictions by PLS-2 model and statistical parameters for the system.

Mixture (µg.mL^{-1})				Prediction (µg.mL^{-1})				Recovery %			
CARV	***DIAZ***	***CARB***	***FURO***	***CARV***	***DIAZ***	***CARB***	***FURO***	***CARV***	***DIAZ***	***CARB***	***FURO***
3.3	13.3	0.0	3.3	3.3481	13.1693	0.1820	3.13599	101.4576	99.0173	----	95.0300
10.0	0.0	0.0	10.0	10.2570	0.0835	0.0261	10.0956	102.5700	----	----	100.9560
6.7	0.0	13.3	0.0	6.5809	0.0699	13.3650	0.00675	98.2224	----	100.4887	----
6.7	13.3	0.0	0.0	6.7602	12.9805	0.1865	----	100.8985	97.5977	----	----
3.3	0.0	13.3	3.3	3.3314	0.0086	13.3641	3.37328	100.9515	----	100.4820	102.2206
16.7	3.3	0.0	0.0	17.0327	3.1270	0.1241	0.24191	101.9922	94.7576	----	----
13.3	3.3	0.0	3.3	13.3979	3.3460	0.0817	3.34953	100.7361	101.3939	----	101.5009
3.3	6.7	6.7	3.3	3.3528	6.5393	6.9267	3.1975	101.6000	97.6015	103.3836	96.8939
6.7	0.0	3.3	10.0	6.8766	0.0705	3.3952	10.01743	102.6358	----	102.8848	100.1743
0.0	10.0	10.0	0.0	----	9.7072	10.1334	----	----	97.0720	101.3340	----
0.0	3.3	3.3	13.3	0.0248	3.3474	3.4425	13.4353	----	101.4364	104.3182	101.0173
0.0	0.0	10.0	10.0	0.0861	----	10.0766	10.11617	----	----	100.7660	101.1617
0.0	13.3	6.7	0.0	----	13.1707	6.8976	----	----	99.0278	102.9493	----
0.0	0.0	20.0	0.0	----	0.1239	20.0435	----	----	----	100.2175	----
0.0	10.0	0.0	10.0	----	10.1378	0.0041	9.81082	----	101.3780	----	98.1082
0.0	20.0	0.0	0.0	----	19.9132	0.1409	----	----	99.5660	----	----
10.0	0.0	10.0	0.0	10.2569	----	9.9736	0.02623	102.5690	----	99.7360	----
0.0	0.0	13.3	6.7	0.0631	----	13.3380	6.84287	----	----	100.2857	102.1324
3.3	10.0	0.0	6.7	3.3726	9.8785	0.0820	6.61038	102.2000	98.7850	----	98.6624
0.0	6.7	10.0	3.3	0.0290	6.5577	10.1353	3.25968	----	97.8761	101.3530	98.7782
Mean recovery								101.4393	98.7924	101.5165	99.7197
RSE (%) single								2.4413	1.7160	1.4215	2.1113
RSE (%) total									1.8620		

The mean squares (MSE =$\frac{\Sigma(y_i-\hat{y}_i)^2}{N}$), the root mean squares error (RMSE = $\sqrt{\frac{\Sigma(y_i-\hat{y}_i)^2}{N}}$), which is an indication of the average error in the analysis, for each component, was determined for the calibration (RMSEC), where y_i and $\hat{y}_i$ are the known and predicted analyte concentrations, respectively, and N is the number of mixtures in the data set (Jahan & Ghasemi 2010),. Moreover, mean standard deviation of the entire calibrated mixture solutions and correlation coefficient (r) between the used and calculated concentration in the synthetic mixture are given in Tables 3 and 4 for PLS-1 and PLS-2 models respectively.

Table 3. Goodness of fit statistics for synthetic samples set analyses by PLS-1.

	Variable			
Parameter	**CARV**	**DIAZ**	**CARB**	**FURO**
Observations	11	12	12	12
Sum of weights	11	12	12	12
Correlation coefficient (r)	0.9997	0.9997	0.9998	0.9996
Std. deviation	0.1493	0.1314	0.1140	0.1141
MSE	0.0244	0.0349	0.0167	0.0127
RMSEC	0.1562	0.1868	0.1292	0.1128

Table 4. Goodness of fit statistics for synthetic samples set analyses by PLS-2.

	Variable			
Parameter	**CARV**	**DIAZ**	**CARB**	**FURO**
Observations	11	12	12	12
Sum of weights	11	12	12	12
Correlation coefficient (r)	0.9998	0.9997	0.9999	0.9995
Std. deviation	0.1291	0.1338	0.0712	0.1176
MSE	0.0284	0.0286	0.0145	0.0127
RMSEC	0.1686	0.1691	0.1205	0.1127

The predicted concentrations of synthetic mixture samples via PLS-1 and PLS2 models for the four drugs in their quaternary synthetic mixtures were plotted versus the true concentrations, Figures 5 and 6. The plots show a large agreement in the results.

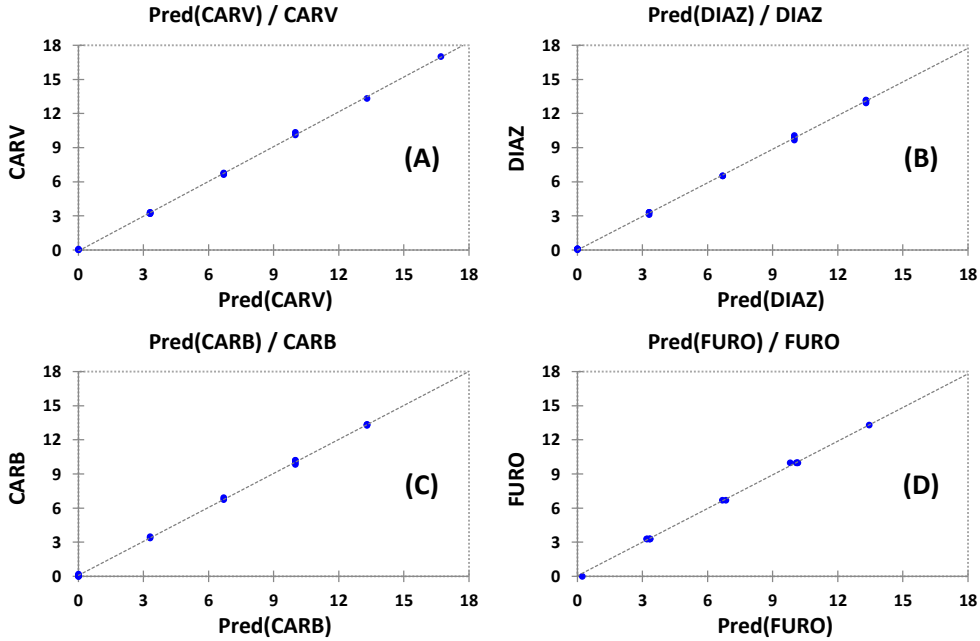

Figure 5. Plots of PLS-1 predicated vs true drugs concentrations in synthetic quaternary mixtures; (A) CARV, (B) DIAZ, (C) CARB, and (D) FURO.

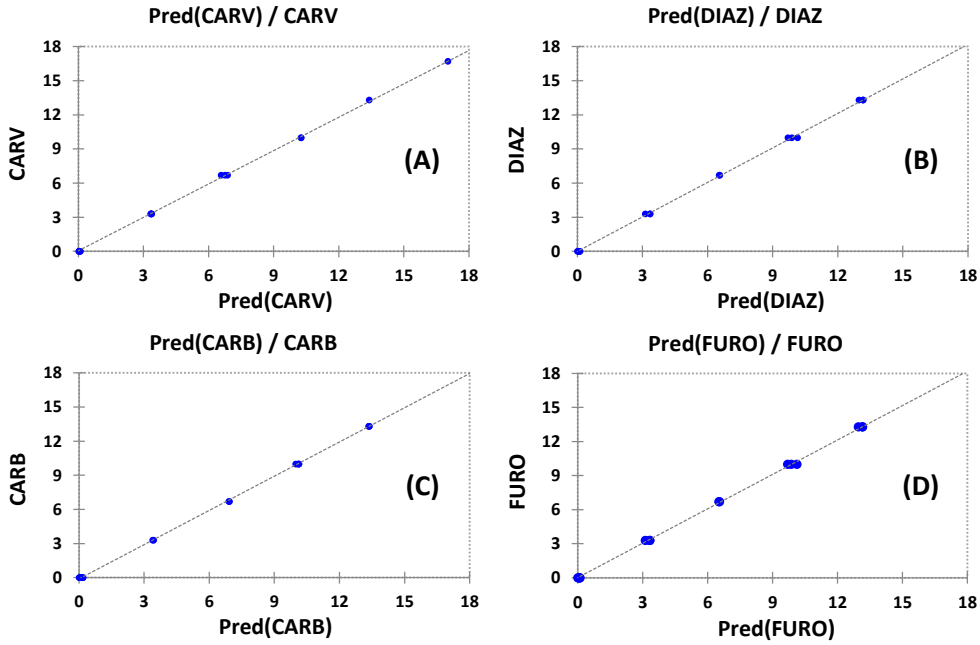

Figure 6. Plots of PLS-2 predicated vs true drugs concentrations in synthetic quaternary mixtures; (A) CARV, (B) DIAZ, (C) CARB, and (D) FURO.

Figures 7 and 8 represent the plots of the standardized concentration residuals vs the predicted concentrations of the tested mixtures. The residuals for CARV, DIAZ, CARB, and FURO in all samples appear to be randomly distributed around zero.

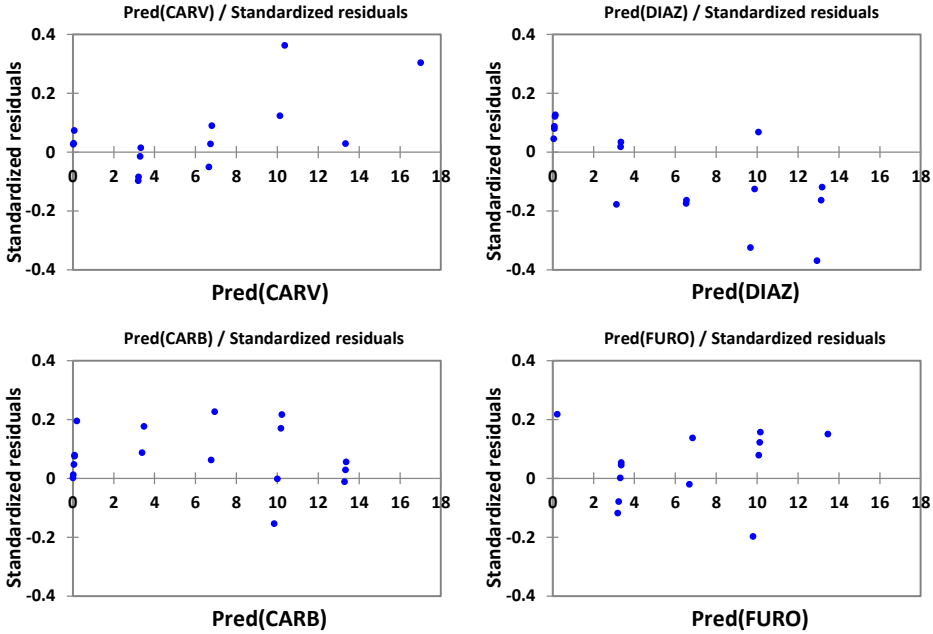

Figure 7. Plots of standardized concentration residuals vs predicted drugs concentrations for the quaternary synthetic mixture samples, by PLS-1 model.

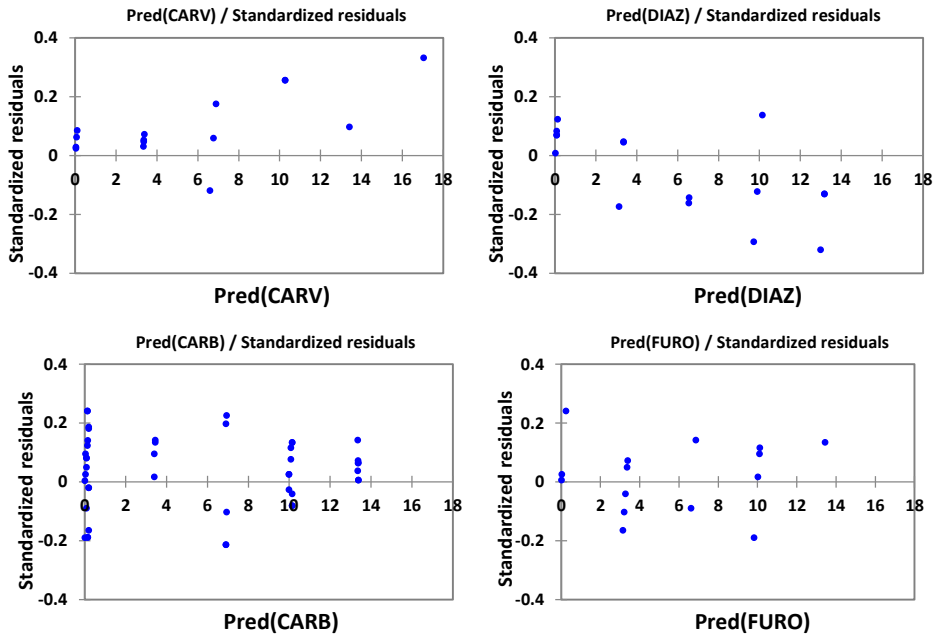

Figure 8. Plots of standardized concentration residuals vs predicted drugs concentrations for the quaternary synthetic mixture samples, by PLS-2 model.

3.3 Accuracy and Precision

Two different levels of concentration of each drug were used to check the accuracies and precisions of the two proposed PLS models in the simultaneously determination of CARV, DIAZ, CARB, and FURO in quaternary synthetic mixtures. Results are depicted in Table 5 indicate excellent accuracy and precision values of the method at each concentration level.

Table 5. Accuracy and precision of the proposed method.

		(µg.mL^{-1})							
Drug	**Model**	**Taken**	**Found**				**Mean**	**RE %**	**RSD%**
CARV	PLS-1	3.3	3.2026	3.3149	3.2162	3.2859	3.2549	-1.3671	1.6624
	PLS-2		3.3481	3.3314	3.3528	3.3726	3.3512	1.5523	0.5059
	PLS-1	6.7	6.6499	6.7282	6.7898	---	6.7226	0.3378	1.0430
	PLS-2		6.5809	6.7602	6.8766	---	6.7392	0.5856	2.2104
DIAZ	PLS-1	3.3	3.1223	3.3349	3.3183	---	3.2585	-1.2575	3.6284
	PLS-2		3.1270	3.3460	3.3474	---	3.2735	-0.8040	3.8750
	PLS-1	13.3	13.1366	12.9315	13.1811	---	13.0831	-1.6311	1.0176
	PLS-2		13.1693	12.9805	13.1707	---	13.1068	-1.4524	0.8348
CARB	PLS-1	10.0	10.2171	9.99918	9.8465	10.17081	10.0584	0.5841	1.6857
	PLS-2		10.1334	10.0766	9.9736	10.1353	10.0797	0.7972	0.7521
	PLS-1	13.3	13.3562	13.32951	13.28889	---	13.3249	0.1870	0.2545
	PLS-2		13.3650	13.3641	13.338	---	13.3557	0.4188	0.1148
FURO	PLS-1	3.3	3.1819	3.3450	3.3538	3.2214	3.2755	-0.7424	2.6534
	PLS-2		3.1360	3.3733	3.3495	3.1975	3.2641	-1.0886	3.5405
	PLS-1	10.0	10.1581	10.0796	10.1223	9.8024	10.0406	0.4062	1.6134
	PLS-2		10.0956	10.0174	10.1162	9.8108	10.0100	0.1000	1.3930

3.4 Application

The proposed methods were successfully applied to several real samples for determination of these drugs in tablet formulations, five replicate measurements were made and the results are shown in Tables 6. The results show that there is a respectable agreement between the calculated values and the label claims indicates the applicability of the proposed PLS models for the simultaneous determination of CARV, DIAZ, CARB, and FURO in real sample.

Tables 6. Application of the PLS methods to the CARV, DIAZ, CARB, and FURO concentration measurements in drugs tablet formulation samples.

Sample	Weight (mg/tablet)						Mean	Recovery %	C.V. %
	Labeled	Found							
				PLS-1					
Carvidol®	25	26.164	26.315	26.123	25.909	25.418	25.986	103.943	1.344
Valiapam	2	1.995	2.000	1.899	1.911	1.888	1.939	96.943	2.809
Tegretol®	200	188.682	195.633	191.006	191.290	197.030	192.728	96.364	1.804
Lasix	40	42.397	41.303	39.755	39.927	39.744	40.625	101.563	2.918
				PLS-2					
Carvidol®	25	26.290	26.297	26.035	25.772	25.402	25.959	103.836	1.460
Valiapam	2	1.967	1.964	1.870	1.862	1.836	1.900	94.989	3.225
Tegretol®	200	184.976	193.910	189.946	190.664	196.109	191.121	95.560	2.220
Lasix	40	40.495	40.362	39.294	39.826	40.912	40.178	100.445	1.563

4. Conclusion

The present work proved that UV spectrophotometric-assisted chemometric methods (PLS-1 and PLS-2) can be successfully used in the resolving of multicomponent mixtures without previous treatment. The obtained results of analysis of correlation parameters for the cited drugs were shown high precision that allow simple, rapid, economical, and accurate simultaneous determination of the cited drugs in their pure form and commercial dosage preparations.

References

Afkhami, A. & Sarlak, N. (2005), "Simultaneous Determination of Salicylamide and Paracetamol by Spectrophotometric H-Point Standard Addition Method and Partial Least Squares Regression", *Acta Chim. Slov*, 52, 98–103.

British Pharmacopoeia (2013), volume I & II, p716, 267, 494, 287.

Cordero, R. & Paterson, S. (2007), "Simultaneous Quantification of Opiates, Amphetamines, Cocaine, and Metabolites and Diazepam and Metabolite in A Single Hair Sample Using GC–MS", *J. Chromatogr. B*, 850, 423–431.

Dinc, E., Ozdemir, A., Aksoy, H., Ustundag, O. & Baleanud, D. (2006), "Chemometric Determination of Naproxen Sodium and Pseudoephedrine Hydrochloride in Tablets by HPLC", *Chemical and Pharmaceutical Bulletin*, 54 (4), 415-421.

Elezovi, A., Pilipovi, S., Elezovi, A. & Uzunovi, A. (2015), "Development and Comparison of Two HPLC Methods, Chiral and Achiral, for Determination of Carvedilol Content in Tablets", *Pharmacia*, 18 (1), 30-35.

Eriksson, L., Byrne, T., Johansson, E., Trygg, J. & Vikström, C. (2013), "Multi- and Megavariate Data Analysis Basic Principles and Applications", Umetrics Academy, 3rd edition, chapter 4: 55-88.

Gallignania, M., Rondón, R. A., Ovalles, J. F. & Brunetto, M. R. (2014), "Transmission FTIR Derivative Spectroscopy for Estimation of Furosemide in Raw Material and Tablet Dosage Form", *Acta Pharmaceutica Sinica B*, 4 (5), 376–383.

Geladi, P. & Kowalski, B. (1986), "Partial Least-Squares Regression: A Tutorial", *Analytica Chimica Acta*, 185, 1–17.

Hoehn, E. (2014), "Detection of the Pharmaceuticals Carbamazepine and Diphenhydramine in Tissue Extracts Using Gas Chromatography-Mass Spectrometry (GC-MS)", *An undergraduate thesis University of Nebraska-Lincoln*.

Jagannathan, L., Meenakshi, R., Gunasekaran, S. & Srinivasan, S. (2010), "FTIR, FT-Raman and UV–VIS Spectra and Quantum Chemical Investigation of Carvedilol", *NRC Cnd. Sci. Lib*, 36 (4), 283-290.

Jahan, B. & Ghasemi, E. Z. (2010), "Simultaneous Spectrophotometric Determination of Trace Amounts of Uranium, Thorium, and Zirconium Using the Partial Least Squares Method After their Preconcentration by Benzoin Oxime Modified Amberlite XAD-2000 Resin", *Talanta*, 80, 1191–1197.

Jayanna, B. K., Devaraj, T. D. & Gowda, N. (2014), "A Facile Spectrophotometric Method for the Determination of Carbamazepine in Tablets", *Indian Journal of Drugs*, 2(3), 132-135.

Karaoglan, G. K., Gumrukcu, G., Ozgur, M. U., Bozdogan, A. & Asci, B. (2007), "Abilities of Partial Least-Squares (PLS-2) Multivariate Calibration in the Analysis of Quaternary Mixture of Food Colors (E-110, E-122, E-124, E-131)", *Analytical Letters*, 40, 1893-1903.

Kumar, T. & Umamaheswari, S. (2011), "FTIR, FTR and UV-Vis Analysis of Carbamazepine", *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 2(4), 685-693.

Leikin, J.B. & Paloucek, F.P. (2008), "Poisoning and Toxicology", 4th edition, *Taylor & Francis*, 167.

Liawruangrath, S., Makchit, J. & Liawruangrath, B. (2006), "A Simple Flow Injection Spectrophotometric Procedure for the Determination of Diazepam in Pharmaceutical Formulation", *Anal Sci.*, 22(1), 127-130.

Metrohm, "Polarographic Determination of Diazepam in Body Fluids and Pharmaceutical Preparations", *Application Bulletin No*. 250/1 e.

Mohamed, G. G., Frag, E. Y. Z., Zayed, M. A., Omar, M. M. & Elashery, S. E. A. (2014), "Spectrophotometric Determination of Diazepam via Charge Transfer Complex Formation Reaction", *Journal of Pharmacy Research*, 8(10), 1503-1509.

Moros, J., Salvador arrigues, S. & de la Guardia, M. (2007), "Quality Control Fourier Transform Infrared Determination of Diazepam in Pharmaceuticals", *J. Pham. Biomed Anal.*, 43, 1277–1282.

Netherton, M. J. (2011), "Uptake and Metabolism of Pharmaceuticals in Aquatic Invertebrates", *Ph.D. Thesis in Chemistry, University of York*.

Nguyen, D.V. & Rocke, D.M. (2002), "Tumor Classification by Partial Least Squares Using Microscopy Gene Expression Data", *Bioinforatics*, 18(1), 39-50.

Niazi, A. & Yazdanipour, A. (2007), "Spectrophotometric Simultaneous Determination of Nitrophenol Isomers by Orthogonal Signal Correction and Partial Least Squares", *Journal of Hazardous Materials*, 146, 421-427.

Packer, M., Fowler, M. B. & Roecker, E. B. (2002), "Circulation106", 2194–2199.

Pan, M. L., Lin, W. Y., Wang, H. Y., Tsai, S. C., Hsieh, P. F., Su, Y. L. O. & Huang, P. W. (2014), "Determination of Carbamazepine: A Comparison of the Differential Pulse Voltammetry (DPV) Method and the Immunoassay Method in A Clinical Trial", *Journal of Analytical Chemistry*, 69(1), 57-61.

Pate, H. & Solanki, S. (2012), "Development and Validation of Spectrophotometric Methods for Simultaneous Estimation of Furosemide and Spironolactone in Combined Tablet Dosage Form", *Inter J Phar Pharma Sci*, 4, 383-386.

Patil, S. R., Kumar, L., Kohli, G. & Bansal, A. K. (2012), "Validated HPLC Method for Concurrent Determination of Antipyrine, Carbamazepine, Furosemide and Phenytoin and its Application in Assessment of Drug Permeability through Caco-2 Cell Monolayers", *Sci Pharm.*, 80, 89–100.

Ram, V. R., Dave, P. N. & Joshi, H. S. (2012), "Development and Validation of a Stability-Indicating HPLC Assay Method for Simultaneous Determination of Spironolactone and Furosemide in Tablet Formulation", *Journal of Chromatographic Science*, 50, 721-726.

Riss, J., Cloyd, J., Gates, J. & Collins, S. (2008), "Benzodiazepines in Epilepsy: Pharmacology and Pharmacokinetics", *Acta Neurol. Scand.* 118, 69–86.

Santini, A. O., Pezza, H. R., Sequine, R., Rufino, J. L. & Pezza, L. (2009), "Potentiometric Sensor for Furosemide Determination in Pharmaceuticals, Urine, Blood Serum and Bovine Milk", *J. Braz. Chem. Soc.*,20(1), 64-73.

Semaan, F. S., de Sousa, R. A. & Cavalheiro, É. T. (2005), "Flow Injection Spectrophotometric Determination of Furosemide in Pharmaceuticals by the Bleaching of a Permanganate Carrier Solution", *J. Flow Injection Anal.*, 22(1), 34-37.

Shariti-Rad, M., Irandoust, M. & Sheikhi, S. (2014), "Development of Highly Sensitive and Selective Spectrophotometric Method for the Determination of Carvedilol in Pharmaceutical and Urine Samples", *RSC Adv.*, 4, 40816-40823.

Shweta, B., Paresh, P. & Hiral, M. (2013), "Development and Validation of High Performance Thin-Layer Chromatography and Derivative Spectrophotometry methods for determination of Diazepam and Propranolol Hydrochloride in Combined Dosage Form", *International Journal of Drug Development & Research*, 5(1), 91-98.

Silva, R. A., Wang, C. C., Ferna´ndez, L. P. & Masi, A. N. (2008), "Flow Injection Spectrofluorimetric Determination of Carvedilol Mediated by Micelles", *Talanta*, 76, 166–171.

Şimşek, F. Ö., Kaynak, M. S., Şanli, N. & Şahin, S. (2012), "Determination of Amlodipine and Furosemide with Newly Developed and Validated RP-HPLC Method in Commercially Available Tablet Dosage Forms", *Hacettepe University Journal of the Faculty of Pharmacy*, 32(2), 145-158.

Solanki, T. B., Shah, P. A. & Patel, K. G. "Central Composite Design for Validation of HPTLC Method for Simultaneous Estimation of Olmesartan Medoxomil, Amilodipine Besylate and Hydrochlorothiazide in Tablets", *Indian Journal of Pharmaceutical Sciences*, 76 (3), (2014), 179-187.

Sruthi, A., Tejaswi, P., Thanuja, N., Kumar, D.S. & Sagar, P. V. (2013), "Simple RPHPLC Method for Estimation of Diazepam in Tablet Dosage Form", *J Pharmacy Res*, 6, 140-144.

Steele, D. (1989), "Multivariate calibration", *Sprectrochim Acta A Mol Biomol Sprectosc*, 46, 419.

Sultana, N., Arayne, M. S. & Ali, S. N. (2013), "An Ultra-Sensitive LC Method for the Simultaneous Determination of Paracetamol, Carbamazepine, Losartan and Ciprofloxacin in Bulk Drug,

Pharmaceutical Formulation and Human Serum by Programming the Detector", *American J. Anal. Chem.*, 4, 24-33.

Torrecilla, J. S., Mena, M. L., Yáñez-Sedeño, P. & García, J. (2008), "Field Determination of Phenolic Compounds in Olive Oil Mill Wastewater by Artificial Neural Network", *Biochem Eng J*, 38 (2), 171-179.

Yilmaz, B. & Arslan, S. (2011), "Determination of Carvedilol in Human Plasma by Gas Chromatography–Mass Spectrometry Method", *Journal of Chromatographic Science*, 49, 35-39.

Yilmaz, B. & Ekinci, D. (2011), "Voltammetric Behavior of Carvedilol in Non- Aqueous Media and its Analytical Determination in Pharmaceutical Preparations", *Rev. Anal. Chem*, 30, 187–193.

Zaporozhets, O., Tsyrulneva, I. & Ischenko, M. (2012), "Determination of 8 Diuretics and Probenecid in Human Urine by Gas Chromatography-Mass Spectrometry: Confirmation Procedure", *American Journal of Analytical Chemistry*, 3, 320-327.

Zhang, X. X., Xiong, F. Q. & Tang, Y. (2009), "Determination of Carbamazepine in Pharmaceuticals by Flow Injection Chemiluminescence Method", *Fenxi. Shiyanshi*, 28, 56- 59.

Zhang, Y., Xie, W., Chen, C. & Lin, L. (2011), "Rapid Screening for 61 Central Nervous System Drugs in Plasma Using Weak Cation Exchange Solid-Phase Extraction and High Performance Liquid Chromatography With Diode Array Detection", *Afr. J. Pharm. Pharmacol.*, 5(6), 706-720.

Zolgharnein, J., Asanjarani, N., Azimi, G. & Ghasemi, J. (2015), "Simultaneous Spectrophotometric Determination of Ga(III) And Tl(III) by Using Genetic Algorithm Based on Wavelength Selection-Partial Least Squares Regression", *Journal of Analytical Chemistry*, 70(2), 148-153.