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RESEARCH ARTICLE

Quantitative Analysis of Hydrochlorothiazide And Losartan Potassium in A Binary Mixture by Artificial Neural Network

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Quantitative Analysis of Hydrochlorothiazide And Losartan Potassium in A Binary Mixture by Artificial Neural Network

Summary

A chemometric calibration technique based on the artificial neural network (ANN) was proposed for losartan potassium (LST) and hydrochlorothiazide (HCT) in their mixture without using chemical separation and mathematical graphical treatment. A training set (or a concentration set) of 84 different mixtures containing LST and HCT in large concentration ranges between 0.0-40.0 µg/mL were prepared in methanol. The absorption spectra of the training sets were recorded in the spectral region of 200.0–300.0 nm. The ANN chemometric calibration was computed by using the relationship between the concentration set (x-block) and their corresponding absorption data (y-block). The ability of the proposed ANN calibration was validated by analyzing various synthetic mixtures of the related drugs, and by using standard addition technique. The ANN calibration approach was applied to the simultaneous quantitative evaluation of LST and HCT drugs in tablets and a good agreement was reported.

Key Words: *Artificial neural network, losartan potassium, hydrochlorothiazide, chemometry, quantitative analysis.*

Yapay Sinir Ağları Yöntemi ile İkili Karışımdaki Hidroklorotiazid ve Losartan'ın Kantitatif Analizi

Özet

Losartan potasyum (LST) ve hidroklorotiazid (HCT) içeren karışımdaki etken maddelerin miktar tayini için kimyasal bir ayrım ve grafiksel düzeltmeler gerektirmeyen ve yapay sinir ağları tekniğine dayanan kemometrik bir yöntem geliştirildi. Konsantrasyon seti LST ve HCT içeren 84 farklı karışımdan, 0.0-40.0 µg/mL aralığında metanol içerisinde hazırlandı. Bu konsantrasyon setinin absorpsiyon spektrumu 200.0–300.0 nm aralığında kaydedildi. Yapay sinir ağları kalibrasyonu konsantrasyon seti (x-blok) ve karşılık gelen absorbans dataları (y-blok) arasındaki ilişkiye dayalı olarak hesaplandı. Yöntemin gücü etken maddeleri içeren çeşitli karışımların analizi ve standart ekleme yöntemi kullanılarak valide edildi. Geliştirilen yöntem tabletlerdeki LST ve HCT'in aynı anda miktar tayinine uygulandı ve iyi sonuçlar gözlendi.

Anahtar Kelimeler: *Yapay sinir ağları, losartan potasyum, hidroklorotiazid, kemometri, miktar tayini.*

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INTRODUCTION

The simultaneous spectral determination of two or more compounds in a given complex mixture is one of very interesting topics in chemometrics. In analytical studies, the relationship between dependent and independent variables may be nonlinear. Additionally, the deviation from the Lambert-Beer Law can be observed due to very small and high concentration levels, interaction between compounds and excipiants in samples. Under these conditions, severeal techniques have been used. One of them is the artificial neural network (ANN), to overcome the above mentioned analytical problems. The use of neural networks in chemometrics has increased in the last decades. Applications indicate that ANN provides major advantages for solving non-linear conditions and analysis of complex systems (1-11). For this reason, the neural networks are an alternative to traditional calibration models when the more classical multivariate calibration methods fail.

In the last test, ANN has demonstrated high ability in acquiring useful information from complex systems, in the presence of noise or instrumental fluctuations, providing robust models. Generally, ANN is a computer system able to establish the relationships between the independent and dependent variables directly from raw data and can be used to model complex relationships between inputs and outputs or to find patterns in data. In our previous studies, ANN tool and its combination of other mathematical techniques have been applied to the analysis of the multicomponent pharmaceutical preparations.

The LST-HCT mixture is widely used in the pharmaceutical products as antihypertensive drugs. Several analytical methods have been reported for the quantitative analysis of hydrochlorothiazide in its mixtures with other active compounds (12-16).

In this study, ANN calibration model was proposed for the simultaneous determination of HCT and LST in tablets was developed. This method was tested by analyzing various synthetic mixtures containing HCT and LST in the non-linear experimental condition and in the presence of their strongly overlapping spectra. After that the method was applied to the quantitative analysis of these drugs in tablets and successful results were obtained.

MATERIAL and METHOD

Instruments

A Shimadzu UV-1601 double beam UV–Vis spectrophotometer possessing a fixed slit width (2 nm) connected to a computer loaded with Shimadzu UVPC software and a LEXMARK E-320 printer were used to record the absorption spectra. Neural networks were implemented in Matlab version 7.0 (The Math Works, USA) written with a special M-file algorithm. The other mathematical data were processed using the Microsoft *EXCEL.*

Commercial tablet product

A commercial tablet product (HYZAAR® tablet, Merck Sharp&Dohme Ind., Turkey, Batch no: 4010423) was assayed. Its declared content was as follows: 50 mg LST, 12.5 mg HCT per tablet. The LST and HCT were obtained as a donation from Merck Sharp&Dohme Ind.

Standard solutions

Stock solutions of 25 mg/100 ml LST and HCT were prepared in methanol. A concentration set of 84 mixtures consisting of HCT and LST in their non-linear concentration range of 1–40 μg/mL was obtained from the above stock solutions. The synthetic mixture solutions were prepared as a validation set in the above working concentration range.

Sample solutions preparation

Ten tablets were accurately weighed and powdered in a mortar. A sample containing LST and HCT equivalent to one tablet content was dissolved in methanol and made up in 100 ml calibrated flasks. The content of the flask was mechanically shaken for 20 min and filtrated through a 0.45 μm membrane filter. The resulting solution was diluted to the working concentration range for the application of the methods.

RESULTS and DISCUSSION

As it is known, analytical chemists prefer to use the linear calibration systems. However, the use of non-linear calibration models can be required for the spectral quantitative analysis of complex pharmaceutical samples due to small deviations from linearity, some interactions or excipients, and other complexity. In addition, simultaneous determination obligates the usage of large concentration range of analytes owing to multi-component combination containing active compounds at high and low levels.

In our case of the simultaneous determination of HCT and LST in tablets, the possible larger concentration ranges 1.0-40.0 µg/mL for each drug were prepared. Figure 1 shows the absorption spectra of HCT and LST in the spectral range of 200-300 nm. It is clear that the individual spectra of both drugs overlap in the same spectral region.

Polynomial equations for HCT and LST were individually obtained by plotting the concentration versus absorption values at 220 nm, as shown in Figure 2 and 3, respectively. It can be seen from Figures 2 and 3, the simultaneous analysis of such system consisting of two drugs is not possible by classical linear multivariate calibration techniques, such as PCR and PLS, which tend to give large prediction error in presence of substantial non-linearity.

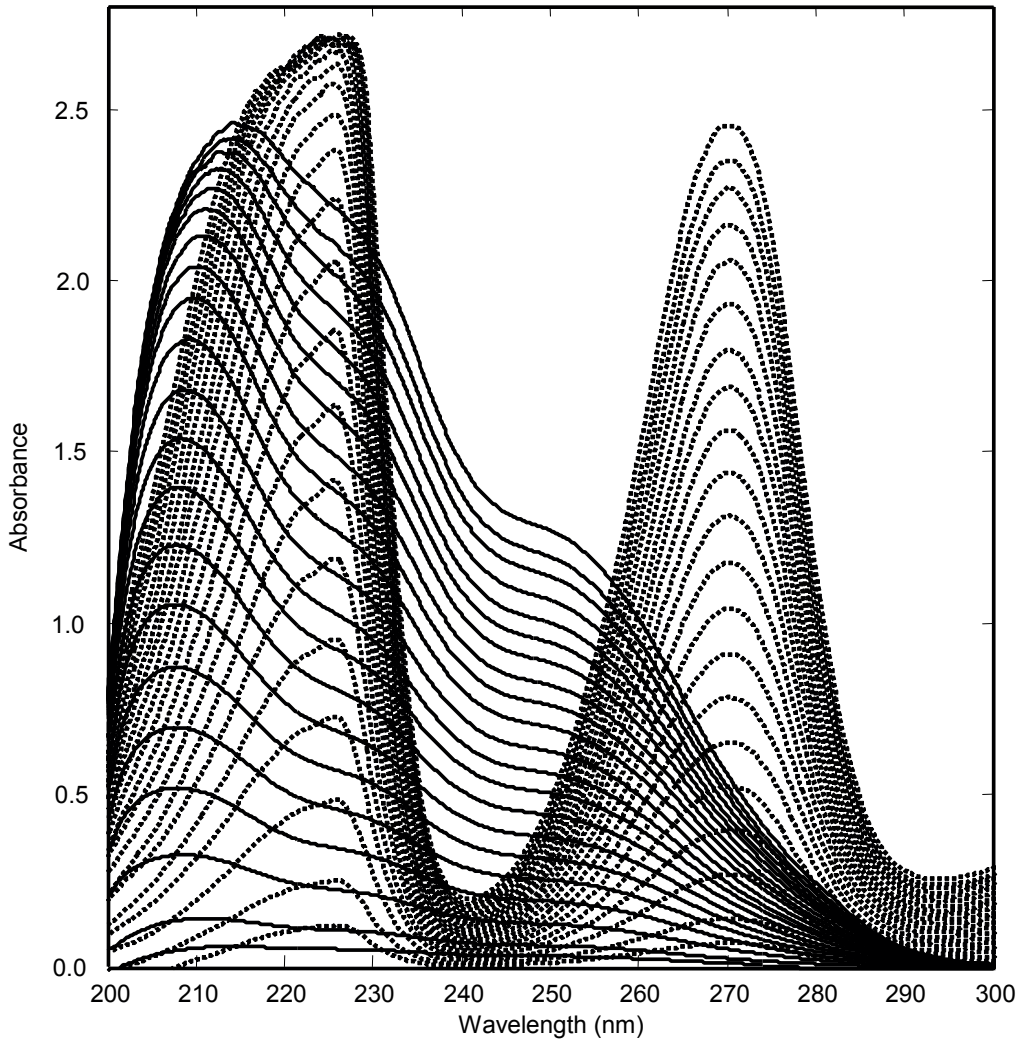

Figure 1. The absorption spectra of 1,2,4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30,32,34,36,38 and 40 µg/mL LST (¾) and HCT (——) in methanol.

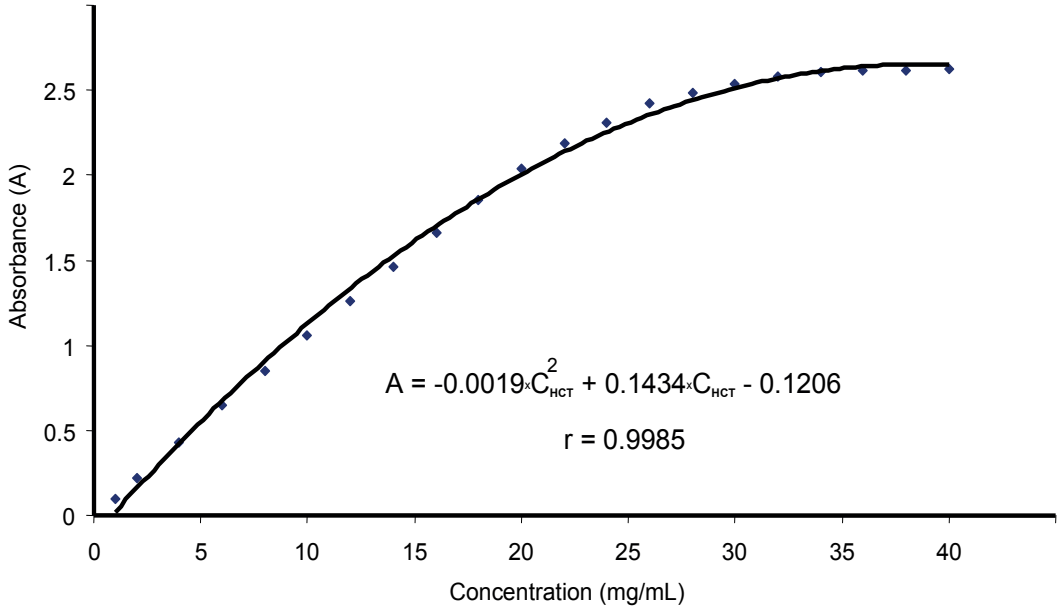

Figure 2. Polynomial equation of HCT in the concentration range of 1.0-40.0 µg/mL

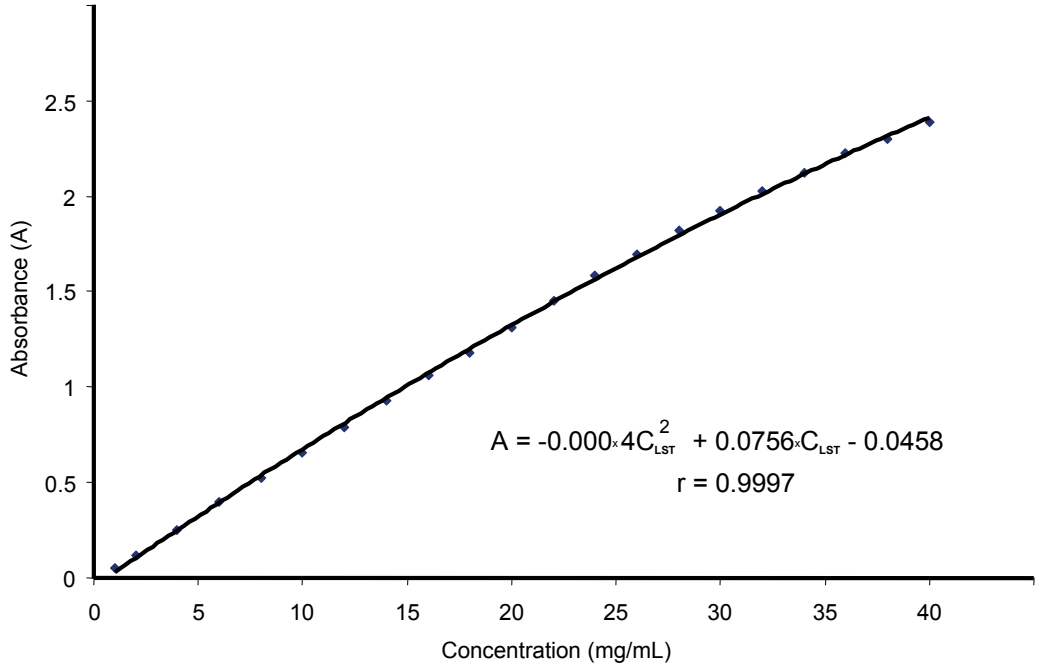

Figure 3. Polynomial equation of LST in the concentration range of 1.0-40.0 µg/mL

ANN approach is an effective chemometric tool to overcome the analytical problem mentioned above.

ANN application

In the ANN application, the concentration set of 84 mixtures containing HCT and LST in the concentration range of 0-40 µg/mL within methanol were prepared and then the absorption spectra of the concentration set were plotted in the spectral range of 200-300 nm.

The absorption values (x-block) corresponding to the concentration set (y-block) were obtained by using the measurements at the 40-wavelenght points in the above spectral range. In this case, the ANN chemometric calibration was computed by using

Table 1. A concentration set of the binary mixtures containing of HCT and LST drugs

	Concentration (µg/mL)			Concentration (µg/mL)			Concentration (µg/mL)	
Set no.	**HCT**	**LST**	Set no.	**HCT**	**LST**	Set no.	**HCT**	**LST**
1	6.0	1.0	29	6.0	28.0	57	14.0	24.0
2	1.0	0.0	30	28.0	0.0	58	0.0	14.0
3	6.0	2.0	31	6.0	30.0	59	16.0	24.0
4	2.0	0.0	32	30.0	0.0	60	0.0	16.0
5	6.0	4.0	33	6.0	32.0	61	18.0	24.0
6	4.0	0.0	34	32.0	0.0	62	0.0	18.0
7	6.0	6.0	35	6.0	34.0	63	20.0	24.0
8	6.0	0.0	36	34.0	0.0	64	0.0	20.0
9	6.0	8.0	37	6.0	36.0	65	22.0	24.0
10	8.0	0.0	38	36.0	0.0	66	0.0	22.0
11	6.0	10.0	39	6.0	38.0	67	24.0	24.0
12	10.0	0.0	40	38.0	0.0	68	0.0	24.0
13	6.0	12.0	41	6.0	40.0	69	26.0	24.0
14	12.0	0.0	42	40.0	0.0	70	0.0	26.0
15	6.0	14.0	43	1.0	24.0	71	28.0	24.0
16	14.0	0.0	44	0.0	1.0	72	0.0	28.0
17	6.0	16.0	45	2.0	24.0	73	30.0	24.0
18	16.0	0.0	46	0.0	2.0	74	0.0	30.0
19	6.0	18.0	47	4.0	24.0	75	32.0	24.0
20	18.0	0.0	48	0.0	4.0	76	0.0	32.0
21	6.0	20.0	49	6.0	24.0	77	34.0	24.0
22	20.0	0.0	50	0.0	6.0	78	0.0	34.0
23	6.0	22.0	51	8.0	24.0	79	36.0	24.0
24	22.0	0.0	52	0.0	8.0	80	0.0	36.0
25	6.0	24.0	53	10.0	24.0	81	38.0	24.0
26	24.0	0.0	54	0.0	10.0	82	0.0	38.0
27	6.0	26.0	55	12.0	24.0	83	40.0	24.0
28	26.0	0.0	56	0.0	12.0	84	0.0	40.0

the non-linear relationship between concentration set (x-block) and their corresponding absorption data (y-block). The details of this application are explained below.

To identify the optimal ANN calibration model with different neuron sizes, various topological networks were tested and finally a training network 40 neurons in the input layer, 20 neurons in hidden layers and two output for the calibration and prediction steps were found to be suitable for the construction of ANN calibration for the simultaneous quantitative prediction of HCT and LST in commercial tablet formulation.

During a back propagation ANN algorithm application the relationship between the mean square error of the networks and epochs for the training set was carried out. A difference between the calculated MSE and predefined MSE ($1.0x10^{-8}$) values was not reported for the calculated ANN calibration. The obtained ANN calibration was successfully applied to the simultaneous determination of two subject drugs in samples.

Method validation

The non-linear ANN approach was validated by analyzing the synthetic mixtures of two analyzed drugs. Mean recovery results and relative standard deviations were found between 100.0% and 0.26 for HCT and 100.1% and 0.23 for LST. Table 2 indicates the recovery results. The ANN approach was applied to real samples and the experimental results obtained were observed in a good agreement with the literature HPLC method.

Table 2. Recovery results obtained in synthetic mixtures by ANN approach

	Concentration (µg/mL)				Recovery	
	Actual		Predicted		(%)	
No.	**HCT**	**LST**	**HCT**	**LST**	**HCT**	**LST**
1	6.00	1.00	6.00	1.00	100.0	99.8
2	6.00	2.00	5.99	2.01	99.8	100.6
3	6.00	4.00	6.00	4.00	100.1	100.0
4	6.00	6.00	5.99	6.00	99.9	100.0
5	6.00	8.00	6.01	8.03	100.1	100.4
6	6.00	10.00	6.00	10.00	100.0	100.0
7	6.00	12.00	6.00	12.01	100.0	100.1
8	6.00	14.00	6.00	14.00	100.0	100.0
9	6.00	16.00	6.00	16.00	100.1	100.0
10	6.00	18.00	5.99	18.00	99.9	100.0
11	6.00	20.00	6.00	20.00	100.1	100.0
12	6.00	22.00	6.00	22.00	100.0	100.0
13	6.00	24.00	6.01	23.99	100.1	100.0
14	6.00	26.00	6.00	26.00	99.9	100.0
15	6.00	28.00	6.00	28.00	100.0	100.0
16	6.00	30.00	6.00	30.00	100.0	100.0
17	6.00	32.00	6.00	32.14	100.0	100.4
18	6.00	34.00	6.00	34.00	100.0	100.0
19	6.00	36.00	6.00	36.30	100.0	100.8
20	6.00	38.00	5.97	38.12	99.5	100.3
21	6.00	40.00	6.00	39.99	100.0	100.0
22	1.00	24.00	0.99	24.00	99.1	100.0
23	2.00	24.00	2.01	24.00	100.5	100.0
24	4.00	24.00	3.99	24.00	99.8	100.0
25	6.00	24.00	6.00	24.01	100.1	100.0
26	8.00	24.00	8.00	23.97	99.9	99.9
27	10.00	24.00	10.00	24.00	100.0	100.0
28	12.00	24.00	12.00	24.00	100.0	100.0
29	14.00	24.00	14.00	24.01	100.0	100.0
30	16.00	24.00	16.03	23.99	100.2	100.0
31	18.00	24.00	18.00	24.00	100.0	100.0
32	20.00	24.00	20.00	24.20	100.0	100.8
33	22.00	24.00	22.00	24.00	100.0	100.0
34	24.00	24.00	23.96	24.00	99.8	100.0
35	26.00	24.00	26.00	23.96	100.0	99.8
36	28.00	24.00	28.23	24.00	100.8	100.0
37	30.00	24.00	30.00	24.10	100.0	100.4
38	32.00	24.00	32.10	24.00	100.3	100.0
39	34.00	24.00	34.15	24.00	100.4	100.0
40	36.00	24.00	36.00	24.00	100.0	100.0
41	38.00	24.00	38.00	24.00	100.0	100.0
42	40.00	24.00	39.90	24.00	99.8	100.0
				Mean	100.0	100.1
				RSD	0.26	0.23

RSD = Relative standart deviation

Table 3. Recovery data of standard addition technique by ANN approach

	Concentration (µg/mL)				Recovery	
	Actual		Predicted		(%)	
No.	**HCT**	**LST**	**HCT**	**LST**	**HCT**	**LST**
1	6.00	24.00	6.09	23.43	101.5	97.6
2	6.00	24.00	5.96	23.50	99.3	97.9
3	6.00	24.00	5.98	23.40	99.6	97.5
4	6.00	24.00	6.08	23.44	101.3	97.7
5	6.00	24.00	6.05	23.46	100.8	97.7
6	6.00	24.00	6.18	23.58	103.0	98.3
				Mean	100.9	97.8
				SD	1.34	0.28
				RSD	1.33	0.28

SD = Standard deviation

To test the effect of excipients of commercial samples on the analysis, the standard addition method was applied to the samples obtained by adding the known amounts of synthetic stokes to the pharmaceutical formulation. As a result of this test, the effect of excipients in the commercial pharmaceutical formulation was not reported on the analysis by using one proposed methods. These recovery results obtained from the standard addition technique were depicted in Table 3.

Tablet analysis

Commercial tablet formulation analysis, the ANN method was successfully applied to the quantitative determination of LST and HCT in commercial tablet formulation. The obtained experimental results for the commercial preparation are presented in Table 4.

Statistical values: mean value, SD (standard deviation), RSD (relative standard deviation), SE (statistical value), CL (P = 0.05) (confidential limit)

Table 4. Experimental results obtained by applying the ANN approach to commercial pharmaceutical tablets

no.	mg/tablet	
	HCT	**LST**
1	12.4	47.8
2	12.5	47.9
3	12.3	49.6
4	12.4	47.8
5	12.3	47.7
6	12.4	49.8
Mean	12.4	48.4
SD	0.06	1.00
RSD	0.50	2.06
SE	0.03	0.41
CL (0.05)	0.05	0.80

CL: Confidence limit

Claim label: 50 mg LST and 12.5 mg HCT per tablet.

Mean values is the average of six replicate.

are summarized in Table 4. As it can be seen a good coincidence observed in between the experimental results and the claimed label in application of all the wavelet methods to the tablet formulation.

CONCLUSIONS

In spite of the non-linear condition and the overlapping spectra of HCT and LST in the spectral region of 200-300 nm (see Figure 1) HCT and LST in tablets were successfully determined by using the ANN approach. We observed that the proposed ANN chemometric calibration provide more accurate prediction and is quite useful in the simultaneous determination of two subjected drugs in samples without any separation step.

We conclude that the ANN approach can be applied successfully to the quality control and the routine analysis of the commercial tablet formulation containing LST and HCT in tablets.

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