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Simultaneous non-destructive determination of two components of combined paracetamol and amantadine hydrochloride in tablets and powder by NIR spectroscopy and artificial neural networks

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Abstract

The two components (paracetamol and amantadine hydrochloride) were simultaneously determined in combined paracetamol and amantadine hydrochloride tablets and powder by using near-infrared (NIR) spectroscopy and artificial neural networks (ANNs). The ANN models of three pretreated spectra (first-derivative, second-derivative and standard normal variate (SNV), respectively) were established. The mathematical corrected models of tablets were compared with those of the powder. In the models, the concentrations of paracetamol and amantadine hydrochloride as the active components were determined simultaneously and compared with the results of their separate determination. The parameters that affected the network were studied and the concentrations of the test set samples were predicted. The degree of approximation, a new evaluation criterion of the network was employed to prove the accuracy of the predicted results.

Keywords: Artificial neural networks; NIR spectroscopy; Degree of approximation; Combined paracetamol and amantadine hydrochloride tablets

1. Introduction

Near-infrared (NIR) spectroscopy has proved to be a powerful analytical tool for analyzing a wide variety of samples used in the agricultural, nutritional, petrochemical, textile and pharmaceutical industries [1–12], especially the use of NIR spectroscopy for the quantitative analysis of pharmaceutical samples has been significantly increased during the last decade. According to current quantitative methods, pharmaceutical samples are mainly analyzed in solution after extraction from a dosage form. NIR technique is a non-destructive quantitative analytical technique of the samples with advantages of rapid, simple operation and small samples, particularly the use of solid samples. Compared to conventionally analytical method, NIR spectroscopy not only attracts the attention of researchers in pharmaceutical industry, but also draws more attention of researchers in other research and exploitation areas due to its unparalleled advantages. In quantitative analysis, artificial neural networks are more and more widely applied during the past several years [13–17]. The main advantage of ANNs is their anti-jamming, anti-noise and robust non-linear transfer ability. In the proper model, ANN result in lower calibration inaccuracy and prediction errors.

The quantitative analytical method of State Drug Standard [18] of combined paracetamol and amantadine hydrochloride tablets is very complicated owing to grinding and dissolving tablets. Furthermore, the concentrations of the two components need to be determined. In this work, the applications of ANNs and NIR spectroscopy to the non-destructive quantitative analysis of tablets and powder were explored for

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predicting their concentrations simultaneously. The ANN models of pretreated spectra (SNV, first-derivative and second-derivative) were established. In addition, the concentrations of the two components predicted simultaneously, were compared with those of the two components determined separately. Compared with the tablet models, the ANN models of powder including the three pretreated spectra were established. In the quantitative analysis, the application of derivative spectra showed significant advantages including improved signal-to-noise ratios via signal averaging as well as the ability to resolve mixtures with overlapping spectra [19].

2. Experimental

2.1. Apparatus and reagents

A Shimadzu® UV-3100 spectrophotometer (Tokyo, Japan) with an ISR-3100 integrating sphere was used for the NIR diffuse reflectance spectra measurement. The data were inputted to a microcomputer via a RS-232C interface. The extended deltabar-delta back-propagation training routines contained in the Neural Works Explorer software package were used. Near-infrared spectral analysis software, from the spectrophotometer, enables the recording of spectra and their mathematical processing (SNV, derivation). All the reagents are of analytical-reagent grade and subject to the regulation of the Chinese Pharmacopoeia.

2.2. Preparation of samples

Forty-six batches of combined paracetamol and amantadine hydrochloride tablets (The average concentrations of paracetamol and amantadine hydrochloride were 52.72 and 21.22%, g/g) obtained from Wutai Pharmaceutical Factory (Changchun, China) were divided into three groups: the training set including 24 samples, the monitoring set including 17 samples and the test set including five samples. The 46 tablets contained three concentration levels (viz. the nominal content and concentrations approximately 3% above and 2% below the stated value). In the state standard method ranges, paracetamol accounted for 47.5–58.0% and amantadine hydrochloride 19.0–23.5%. In the extended ranges paracetamol made up 45.5–51.0% and amantadine hydrochloride 17.0–26.5%. All the 46 tablet samples were ground up carefully and turned into powder. The samples were taken successively every few minutes during grinding the tablets with a mortar and pestle. Then they were passed through a 100 μm sieve. The powder was divided into three groups in the same way as the tablets were done. The reference concentrations were measured according to the State Drug Standard.

2.3. Recording of NIR spectra

The instrument used permits the recording of diffuse reflection spectra of intact tablets. The tablets were always placed in the central position of the integrating sphere in order to ensure maximal reproducibility and minimal stray light. The entrance slit of the NIR spectrophotometer used was 12 nm and the scan wavelength range was from 1100 to 2500 nm. Each spectrum was the average one of 10 scans performed at 1 nm intervals over the wavelength range 1100–2500 nm.

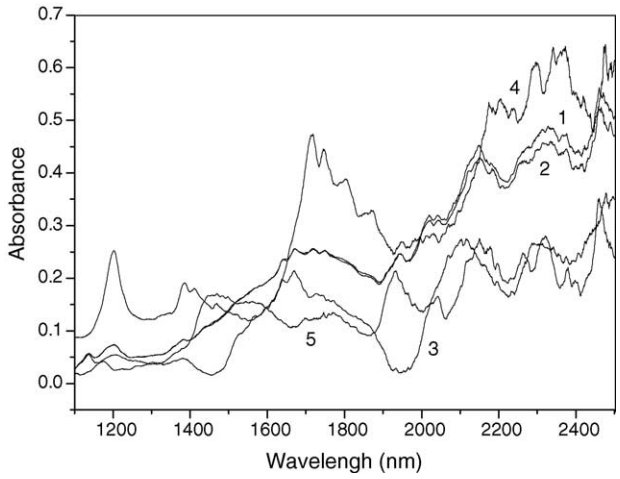

Fig. 1. NIR spectra for (1) trained spectrum, (2) predicted spectrum, (3) paracetamol, (4) amantadine hydrochloride, and (5) starch.

Fig. 1 shows the conventional NIR spectra for the samples with different concentrations, the two active pure components and the main excipient. As can be seen, the quantum yield decreased and the dynamic range of the detector dropped drastically in the region around 1100–1300 nm. Thus, the data evaluation was limited to wavelength range between 1300 and 2500 nm.

2.4. Data processing

Back-propagation (BP) is an ANN algorithm most widely used in Chemometrics practice [20,21]. The present criterion of optimization is to make the error of the training set or the monitoring set the smallest; however, it is easy to choose an over-fitting model, namely, the error of test set is not the smallest. This kind of network is unsteady when it is used to predict an unknown sample. This is an over-fitting phenomenon. It usually appears because of too many numbers of iterations. In order to avoid this kind of situation, a new evaluation criterion of the network, the degree of approximation is employed [22,23]. The definition of this criterion is given by Eqs. (1) and (2).

$$e_a = \left(\frac{n_l}{n}\right) e_l + \left(\frac{n_c}{n}\right) e_c + |e_l - e_c| \quad (1)$$

where e_a is the error of the approximation, e_l and e_c the relative standard errors of training set and monitoring set, n_l and n_c the sample numbers of training set and monitoring set, n the whole number of known samples, and n_l/n and n_c/n the weights contributed to the error of approximation (e_a) by

Table 1
Prime ANN model parameters of the two active components determined simultaneously

Spectra	Input/output nodes	Hidden nodes	Momentum	Learning coefficient	Numbers of iteration
SNV spectra of tablets	30/2	19	0.20	0.010	2000
First-derivative spectra of tablets	40/2	19	0.05	0.009	1300
Second-derivative spectra of tablets	40/2	11	0.10	0.015	1100
SNV spectra of powder	40/2	13	0.15	0.010	1500
First-derivative spectra of powder	30/2	13	0.10	0.007	1100
Second-derivative spectra of powder	40/2	13	0.05	0.009	1100

training set and monitoring set.

$$D_a = \frac{c}{e_a} \quad (2)$$

where D_a is the degree of approximation and c a constant number by which D_a is adjusted to get a good chart. It is very obvious that the smaller e_a or the larger D_a can obtain the better ANN models, which are approaching to the real data. Therefore, the effects of both training set and monitoring set are considered in this evaluation criterion.

The predictive abilities of training set, monitoring set and test set were compared in terms of the relative standard error, R.S.E. (%), defined as

$$\text{R.S.E.}\,(\%) = \sqrt{\frac{\sum_{i=1}^{n}(c_{\text{NIR}_i} - c_{\text{REF}_i})^2}{\sum_{i=1}^{n} c_{\text{REF}_i}^2}} \times 100$$

where n is the number of samples included in the validation set, and c_{REF} and c_{NIR} are the concentrations provided by the State Drug Standard method and NIR method, respectively.

2.5. Training and optimization of ANN models

2.5.1. The establishment of ANN models

The properties of the training set data determine the number of input and output neurons. The pretreated spectral data (SNV, derivative) of the tablets and powder were regarded as input nodes. The different number of input nodes, namely, the different interval of wavelength was changed in order to scan the data. Because there were two kinds of active ingredients in combined paracetamol and amantadine hydrochloride tablets, the output layer contained two neurons.

Neural networks were trained with different numbers of hidden neurons (5–25) and training cycles (100–3000). At the start of a training run, both momentum and learning coefficient were initialized with random values. During training, the modifications of the network input nodes, hidden nodes, momentum, learning coefficient and iteration numbers were made by the back-propagation of the error and degree of approximation. The training set was used to train the network, the monitoring set was used to avoid over-fitting and the maximal degree of approximation was used to determine the network topology parameters (number of input, hidden, iterations, momentum and learning coefficient). All the optimal parameters are shown in Table 1. While the network was optimized, the testing data were fed into the network to evaluate the trained network.

2.5.2. Evaluation of the analytical accuracy of ANN models

In order to evaluate the ANN models, the regression equation of the reference concentration values and NIR concentration values were established. The intercept, slope of regression equation and R (correlation coefficient) are shown in Table 2. The intercept and slope represented the linearity degree of the reference concentration values and NIR concentration values. The relative standard error of training set, monitoring set and test set are shown, too.

In order to compare the two component models, the separate calibration models of the two components including SNV spectra, first-derivative spectra and second-derivative spectra were designed. Because there was one kind of active ingredient in established models, the all output layer contained one neuron. The optimum ANN models are shown in Table 3. The evaluation of the analytical accuracy of models is listed in Table 4.

3. Results and discussion

3.1. Two components determined simultaneously

The network topology parameters selection of the two component SNV spectra of the tablets are shown in Figs. 2–6.

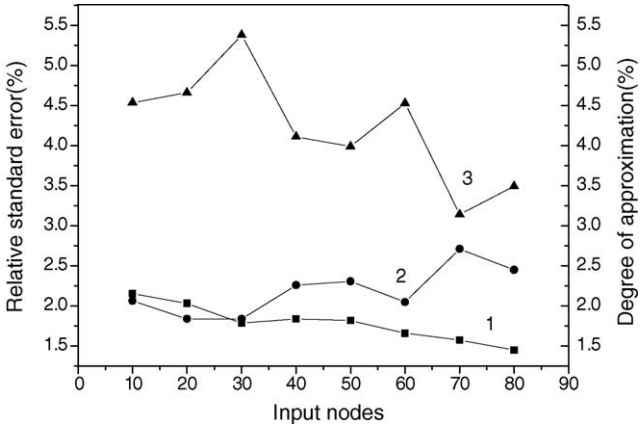

Fig. 2. Effect of input nodes: (1) relative standard error of training set; (2) relative standard error of monitoring set; (3) the degree of approximation.

Table 2
Linear regression parameters and errors of the two active components determined simultaneously

Spectra	Intercept	Slope	*R*	R.S.E. (%)
SNV spectra of tablets				
Paracetamol				
Training set	−0.0017	1.0142	0.9898	1.471
Monitoring set	0.0133	0.9872	0.9896	1.478
Test set	0.0179	0.9780	0.9809	1.479
Amantadine hydrochloride				
Training set	−0.0038	1.0285	0.9909	1.458
Monitoring set	0.0006	1.0093	0.9907	1.488
Test set	0.0150	0.9437	0.9865	1.521
First-derivative spectra of tablets				
Paracetamol				
Training set	−0.0135	1.0353	0.9923	1.311
Monitoring set	0.0091	0.9942	0.9913	1.367
Test set	0.0442	0.9278	0.9909	1.368
Amantadine hydrochloride				
Training set	0.0063	0.9794	0.9929	1.225
Monitoring set	0.0038	0.9918	0.9923	1.245
Test set	0.0269	0.9271	0.9895	1.398
Second-derivative spectra of tablets				
Paracetamol				
Training set	−0.0010	1.0094	0.9951	0.995
Monitoring set	0.0145	0.9824	0.9930	1.194
Test set	0.0269	0.9427	0.9911	1.210
Amantadine hydrochloride				
Training set	−0.0002	1.0065	0.9958	0.989
Monitoring set	0.0072	0.9741	0.9956	0.993
Test set	−0.0077	1.0468	0.9928	1.261
SNV spectra of powder				
Paracetamol				
Training set	0.0169	0.9784	0.9902	1.408
Monitoring set	−0.0119	1.0334	0.9900	1.414
Test set	0.0276	0.9601	0.9858	1.478
Amantadine hydrochloride				
Training set	0.0005	1.0085	0.9914	1.435
Monitoring set	−0.0046	1.0334	0.9909	1.462
Test set	0.0210	0.9154	0.9904	1.472
First-derivative spectra of powder				
Paracetamol				
Training set	0.0084	0.9942	0.9926	1.306
Monitoring set	0.0277	0.9593	0.9922	1.359
Test set	0.0309	0.9533	0.9911	1.366
Amantadine hydrochloride				
Training set	0.0044	0.9885	0.9939	1.220
Monitoring set	0.0032	0.9952	0.9931	1.256
Test set	0.0247	0.9270	0.9935	1.347
Second-derivative spectra of powder				
Paracetamol				
Training set	−0.0060	1.0183	0.9950	0.980
Monitoring set	0.0069	0.9949	0.9949	0.985
Test set	−0.0010	1.0112	0.9922	1.070
Amantadine hydrochloride				
Training set	0.0054	0.9824	0.9968	0.965
Monitoring set	0.0052	0.9837	0.9960	0.980
Test set	−0.0069	1.0430	0.9931	1.222

Fig. 2 shows the effect of the different number of input nodes. As can be seen, the relative standard errors of training set was reduced and those of monitoring set increased gradually while the number of nodes increased. When the number of input nodes was 30 (the interval of wavelength was 40 nm), the network had the highest degree of approximation. When the number of nodes exceeded 30, the degree of approximation was reduced evidently. This result demonstrates that in the network an over-fitting phenomenon exists.

The number of hidden nodes has a great effect on the predictive result. Fig. 3 shows the effect of hidden nodes. Curve 2 jumped seriously, and it was difficult to determine the optimum hidden nodes from it. Curve 3 was the curve of degree of approximation. The constant number in the definition of degree of approximation was embodied through which the degree of approximation was adjusted for optimization. Therefore, the degree of approximation had enlarging function. We can determine the optimum hidden neurons to be 19 via the largest degree of approximation.

Momentum and learning coefficient affect the convergence and the stability of the network model. In general, too high momentum and coefficient lead to network instability. The effect of momentum on the network did not change obviously in Fig. 4 in the beginning. But from the degree of approximation curve we can draw a conclusion easily. When momentum arrived at 0.20, the network had the highest degree of approximation. The effect of it on the network had regularity with the change of learning coefficient. The network had highest degree of approximation at which learning coefficient was 0.010. After 0.010 the degree of approximation dropped rapidly due to over-fitting.

The selection of learning periods was made by means of the error curves and degree of approximation shown in Fig. 6. With the increase of number of iterations, the R.S.E. of training set and monitoring set was reduced gradually, and the network reached a maximal degree of approximation at 2000 iterations. The degree of approximation curve decreased distinctly after 2000 periods. The curve of the degree of approximation displayed its advantage again; it could enlarge the error of the network clearly.

In the paper first-derivative and second-derivative spectra of tablets and powder were also made, and the optimal ANN models were established. The selection of corresponding parameters is shown in Table 1. Compared with SNV spectra, the derivative models have smaller hidden nodes and iteration numbers. Of all the spectra, the momentum and learning coefficient of tablets and powder have little difference in Table 1, thus they were not the primary influence factors when the network was established.

When all the adjustable parameters of the neural networks were optimized, the neural networks were established and showed a high ability of generalization. The basic regression parameters of the reference concentration with the predicted ones of training set and monitoring set by the optimized models are listed in Table 2. In Table 2 smaller R.S.E. occurs in the quantitative prediction by means of optimum network model set up by derivative spectra, which shows derivative spectra have the priority over SNV spectra. The models of the second-derivative spectra have smaller R.S.E. than those of the first-derivative spectra. Therefore, the ANN model es-

Table 3
Prime ANN model parameters of the two active components determined respectively

Spectra	Input/output nodes	Hidden nodes	Momentum	Learning coefficient	Numbers of iteration
SNV spectra of tablets					
Paracetamol	40/1	21	0.05	0.005	1500
Amantadine hydrochloride	40/1	21	0.05	0.100	1500
First-derivative spectra of tablets					
Paracetamol	40/1	19	0.10	0.011	1100
Amantadine hydrochloride	50/1	17	0.10	0.011	1100
Second-derivative spectra of tablets					
Paracetamol	40/1	17	0.10	0.011	900
Amantadine hydrochloride	30/1	11	0.10	0.013	900
SNV spectra of powder					
Paracetamol	40/1	13	0.10	0.005	1300
Amantadine hydrochloride	40/1	15	0.25	0.100	1000
First-derivative spectra of powder					
Paracetamol	30/1	15	0.05	0.009	900
Amantadine hydrochloride	30/1	17	0.10	0.010	1100
Second-derivative spectra of powder					
Paracetamol	30/1	13	0.05	0.009	900
Amantadine hydrochloride	30/1	13	0.10	0.003	900

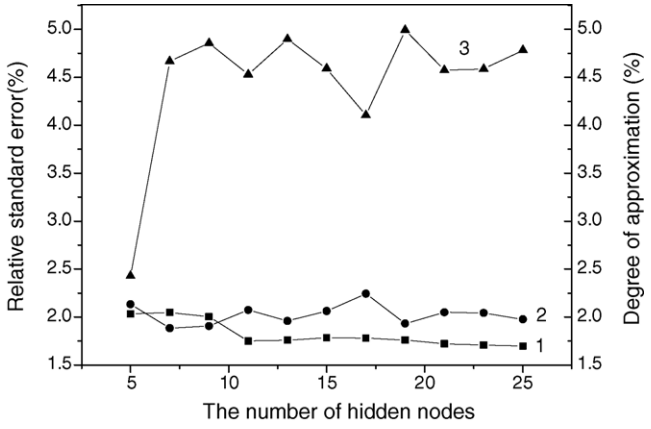

Fig. 3. Effect of hidden nodes.

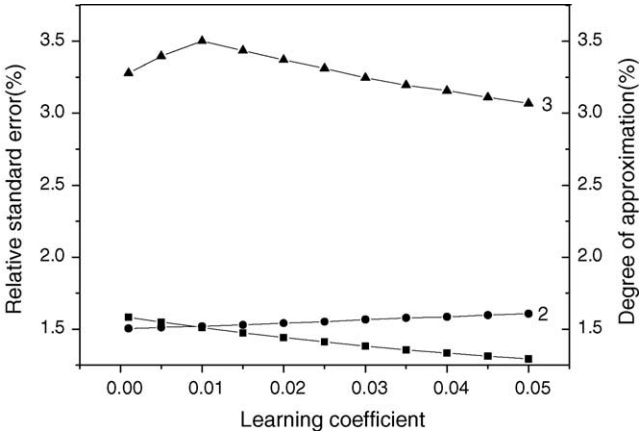

Fig. 5. Effect of learning coefficient.

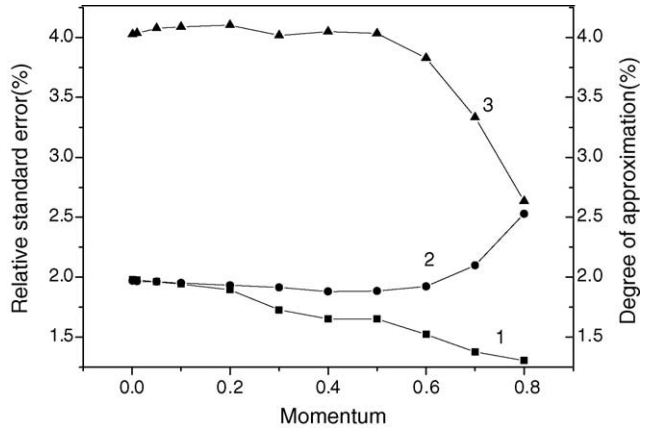

Fig. 4. Effect of momentum.

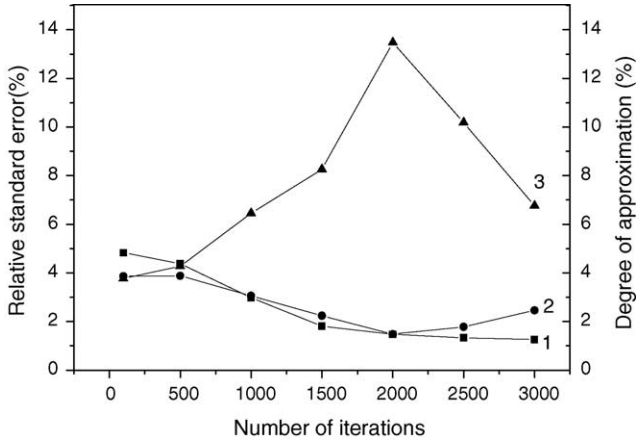

Fig. 6. Effect of numbers of iterations.

Table 4
Linear regression parameters and errors of the two active components determined respectively

Spectra	Intercept	Slope	*R*	R.S.E. (%)
SNV spectra of tablets				
Paracetamol				
Training set	0.01357	0.9849	0.9895	1.439
Monitoring set	0.0213	0.9718	0.9894	1.465
Test set	0.0218	0.9711	0.9842	1.477
Amantadine hydrochloride				
Training set	0.0075	0.9751	0.9927	1.419
Monitoring set	−0.0022	1.0218	0.9908	1.424
Test set	−0.0002	1.0136	0.9886	1.429
First-derivative spectra of tablets				
Paracetamol				
Training set	0.0116	0.9881	0.9926	1.304
Monitoring set	0.0225	0.9692	0.9923	1.352
Test set	0.0337	0.9478	0.9907	1.365
Amantadine hydrochloride				
Training set	−0.0027	1.0219	0.9944	1.219
Monitoring set	0.0018	1.0018	0.9932	1.254
Test set	0.0130	0.9522	0.9932	1.354
Second-derivative spectra of tablets				
Paracetamol				
Training set	−0.0061	1.0188	0.9953	0.984
Monitoring set	0.0138	0.9826	0.9943	1.068
Test set	0.0094	0.9921	0.9942	1.104
Amantadine hydrochloride				
Training set	−0.0008	1.0115	0.9966	0.964
Monitoring set	0.0104	0.9586	0.9964	0.970
Test set	0.0044	0.9905	0.9940	1.164
SNV spectra of powder				
Paracetamol				
Training set	0.0078	0.9963	0.9919	1.396
Monitoring set	0.0045	1.0029	0.9905	1.402
Test set	0.0207	0.9728	0.9867	1.408
Amantadine hydrochloride				
Training set	−0.0004	1.0127	0.9931	1.352
Monitoring set	0.0064	0.9811	0.9919	1.373
Test set	0.0336	0.9053	0.9888	1.421
First-derivative spectra of powder				
Paracetamol				
Training set	0.0139	0.9837	0.9925	1.304
Monitoring set	0.0045	1.0027	0.9924	1.339
Test set	0.0259	0.9628	0.9924	1.354
Amantadine hydrochloride				
Training set	0.0029	0.9951	0.9949	1.192
Monitoring set	−0.0002	1.0114	0.9947	1.246
Test set	0.0081	0.9751	0.9933	1.349
Second-derivative spectra of powder				
Paracetamol				
Training set	0.0046	0.9990	0.9960	0.975
Monitoring set	0.0050	0.9989	0.9960	0.978
Test set	−0.0065	1.0216	0.9955	1.008
Amantadine hydrochloride				
Training set	0.0049	0.9852	0.9976	0.963
Monitoring set	0.0059	0.9806	0.9969	0.968
Test set	−0.017	1.0890	0.9864	1.013

tablished by the second-derivative spectra is better. To verify the reliability of the network models further, five test tablets and powder were predicted. Because the test set did not join in training networks, the R.S.E. was higher than that of training set and *R* was lower than that of training set. The results of test set were similar to those of monitoring set, thus the method using the maximum value of the degree of approximation corresponding to the minimum value of the error of prediction in the test set is feasible.

3.2. Two components determined respectively

In order to compare them with the previous models, the separate calibration models of the two components including SNV, first-derivative and second-derivative spectra were designed. The topology parameters in Table 3 are similar to the corresponding ones in Table 1 except the number of cycles. Furthermore, the regression parameters and R.S.E. in Table 4 are similar to those in Table 2. The results indicate that the two components determined synchronously and determined separately have the same results. The two components determined simultaneously are feasible and they have the virtue of rapider velocity.

4. Conclusions

The methods of ANN and NIR are suitable for non-destructive quantitative analysis of two components of solid pharmaceutical samples. It is feasible to obtain quantitative information by processing NIR spectroscopy of the two components of tablets and powder with ANN models. The two components determined simultaneously and the models determined separately have the similar R.S.E.; therefore, the two components determined simultaneously can be used. Of all the three pretreated spectra, the second-derivative spectra have the smallest R.S.E. and the best R, thus using the second-derivative spectra could obtain the best results.

The tablets are compared with their powder; either their iterations or R.S.E. are similar. Therefore, satisfactory ANN model has been likely established by directly using tablet samples other than by using ground powder samples.

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