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A study on the applicability on multicomponent calibration methods in chemometrics

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Abstract

Twelve multivariate calibration method alternatives are compared to establish the effect of spectral nonlinearity and collinearity on accuracy and precision of determined results. Simulated and real spectral data are used in this research. This study can help us to select an optimum method for determination.

Keywords: Chemometrics; Multicomponent calibration; Collinearity

Contents

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1. Introduction

In recent years, more and more chemometricians have become interested in the multicomponent calibration method. Up to now, many multicomponent calibration methods have been developed, and new methods will be presented in the future. Although it is necessary to introduce some new methods, how to use them is more important. Especially, which method can be used under what condition, and which method cannot be used in a studied object. For this reason, we have investigated the applicability of multicomponent calibration methods and got satisfactory results. We are working on a software package to present these results.

1. A set of standard samples must be arranged in an orthogonal factorial design in order to avoid the collinearity among them. The experimental operation is normalized according to error propagation theory.

2. We simulated two component spectra with equal strength and a different degree of collinearity. A group of optimum criteria was used to represent the collinear feature of the system studied and built the relationship between these criteria and the applicability of calibration methods. On this basis, we suggested a method to simulate an objective system. It enables us to select a suitable multicomponent calibration method with only a few experiments.

3. We use these simulation results in four different systems of compound preparation. We show that the simulation results accord surprisingly well with those obtained from an objective pharmaceutical system.

In our study, we compared the results of classical calibration methods (Multivariate Linear Regression, K-matrix Represent, Target Factor Analysis, Kalman filter, Partial Least Squares), improved calibration methods (Ridge Regression, Modified Target Factor Analysis, Iterative Target Factor Analysis, NonLinear Partial Least Squares), robust regression (Maximum Likelihood Estimator, Robust Partial Least Squares) and the methods of classical calibration method combined with a diagnostic method. These studies gave us some rules about the applicability of these calibration methods.

In order to evaluate these methods, mean recovery was used as the measure of accuracy and RSD as the measure of precision. The concentration of the component in each sample is not the same. In order to calculate the RSD, firstly, the concentration must be expressed as a percentage, and then the RSD is calculated.

The results of P-Matrix Representation were very unsatisfactory. This is probably because the matrix (AA^T), the covariant matrix of absorbance, is commonly large dimensional and singular. Its inverse will give a larger error or not exist. So, the results of P-Matrix Representation is not shown here.

2. Mathematical theory and algorithm

In this study, we compared twelve commonly used multivariate calibration methods: the Multivariate Linear Regression (MLR) [1], K-matrix representation (AKC) [2], Kalman filter (Kalman) [3], Target transformation Factor Analysis (TFA) [4], Modified Target Factor Analysis (MTFA) [5], Iterative Target Factor Analysis (ITFA) [6], Partial Least Squares (PLS) [7], Ridge regression (Ridge) [8], NonLinear Partial Least Squares (NLPLS) [9], Maximum likelihood estimation: Andrew function method (Andrew) and Hampel function method (Hampel) [10], Robust Partial Least Squares (RPLS) [11]. To evaluate the effect of outliers on results of variable methods, three different diagnostic methods are selected and compared. They are Cook square distance (Cook) [12], Hatmatrix (H-matrix) [13] and Robust Diagnosis (RD) [14]. In order to discriminate the quality of the analytical system (main relativity of spectra), we selectively use condition number (Cond) [15], correlation coefficient (R) and net analytical signal of component (NS) [16]. The theory and algorithms can be found in the references mentioned above. So, they will not be given here.

3. Simulation

3.1. Numerical simulated spectra

3.1.1. Simulating collinearity of spectra

A two-component analytical system was simulated. Two Gaussian curves were used as the spectra of two components. From these spectra, the absorptivity matrix was given. In order to avoid the interference of other factors, we assumed that the absorp-

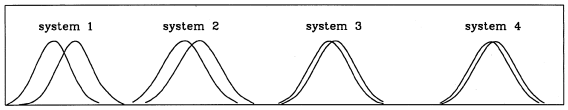

Fig. 1. Simulating spectra: system 1: difference of 1σ between two peak maxima, system 2: difference of 0.62σ between two peak maxima, system 3: difference of 0.23σ between two peak maxima, system 4: difference of 0.20σ between two peak maxima.

tive strength and concentration of the two components are the same. The difference is only the position of maximum absorption in two Gaussian curves. In model sets, the concentrations were 80%, 90%, 100%, 110% and 120% of labeled amount at five levels. We used an orthogonal factorial design to get the matrix of standard concentration of twenty five samples. In test sets, fifty samples were observed by varying 10% to 100% of labeled amount with five levels. The matrices of absorbance of observed samples were obtained by Beer's law and 2% random error was added to the absorbance. Four simulated spectra are shown in Fig. 1. Their net analytical signal of component, condition number and correlation coefficient are given in Table 1. We used a multicomponent calibration method to process these data as stated above. The results are presented in Table 2. From Tables 1 and 2, we can conclude as follows.

1. In an analyzed system, the collinearity between the spectra is lower, i.e., in system 1, almost every calibration method can get a good result. The accuracy and precision of these methods have no significant difference. The results of every calibration method show a significant difference, accompanied by an increment of the collinearity of spectra of the analyzed system. The accuracy and precision of every method decrease with the increment of collinearity of spectra. The degree and the rate of change differ with the quality of analyzed system. The results of the AKC method and the MLR method are poorer. This is obvious because the AKC method must be inversed two times. So, the error will be enlarged with collinearity of analyzed system. The MLR method is similar to the AKC method. In system 2, other methods can give satisfactory results, but the results of these two methods are worse. The algorithm of Kalman filter has a smoothing function. It can partly filter off noise. Factor analysis, Partial least squares, etc. use a latent vector instead of the observed matrix. Because of the orthogonality among latent vectors, the selectivity is increased and the error is eliminated partly when the collinearity of the analyzed system is further increased, as in systems 3 and 4. The results of every method and the tendency of change are the same as above. In this situation, PLS still gives good results. The results of TFA and MTFA are also better but the fluctuation of results is larger. It is shown that PLS is best at resisting the influence of collinearity of the analyzed system.

2. The criteria of collinearity for spectra of the analyzed system: we selected three criteria in order to elucidate the degree of collinearity of spectra of the analyzed system. From Tables 1 and 2, we can see that the tendency of the quality of the analyzed system to change is the same as that of the applicability of the method following the change of quality of the analyzed system, when the analyzed systems have shown strong or weak collinearity of spectra. The accuracy and precision of methods become lower or higher. The net analytical signal of the component is the most efficient for deciding the capacity of the method over three criteria. It can show not only the situation of every component, but also give high accuracy of judgement. When the net analytical signal of each component is more than ten, the quality of the analyzed system is high. Every method can get good results. When the net analytical signal of components is close to ten, these are ill-conditioned. The effects of every method show obvious differences. Only PLS

Table 1
The criteria of collinearity for four systems

	System 1		System 2		System 3		System 4	
	Component 1	Component 2	Component 1	Component 2	Component 1	Component 2	Component 1	Component 2
NS	36.56	36.56	24.21	24.19	10.10	10.09	9.19	9.19
Cond	5.2928		14.49		96.10		116.28	
R	0.2193		0.5060		0.9022		0.9455	

Table 2
The relationship between collinearity of system and applicability of calibration method

Method	System 1				System 2				System 3				System 4			
	Component 1		Component 2		Component 2		Component 2		Component 1		Component 2		Component 2		Component 2	
	Recovery(%)	RSD. (%)	Recovery(%)	RSD. (%)	Recovery(%)	RSD. (%)	Recovery(%)	RSD. (%)	Recovery(%)	RSD. (%)	Recovery(%)	RSD. (%)	Recovery(%)	RSD. (%)	Recovery(%)	RSD. (%)
MLR	98.95	0.76	99.5	90.72	101.26	1.85	98.18	1.17	103.25	1.96	97.14	2.30	103.56	4.05	95.82	3.42
AKC	98.88	0.85	97.26	0.84	103.02	1.73	97.84	1.05	103.32	1.69	94.17	2.44	107.11	3.66	93.27	3.10
Kalman	99.31	0.71	99.88	0.63	101.30	1.54	98.22	1.17	103.15	2.04	97.07	2.18	103.68	4.02	95.93	3.42
TFA	99.42	0.75	99.80	0.69	101.45	1.51	99.08	1.28	102.35	1.90	97.61	2.29	106.00	5.22	93.30	5.56
MTFA	100.01	0.74	99.99	0.63	101.07	1.51	99.98	0.99	100.08	1.84	99.94	2.13	100.42	4.83	99.98	3.69
ITFA	100.01	0.74	99.98	0.63	100.05	1.51	99.96	1.00	100.03	1.95	99.89	2.23	100.35	4.77	99.66	3.82
PLS	100.00	0.42	100.00	0.47	100.01	0.82	100.00	0.57	100.09	0.89	100.00	0.86	100.03	1.59	100.07	1.34
Ridge	99.31	0.70	99.88	0.60	101.32	1.60	98.21	1.22	103.29	2.29	97.05	2.18	103.07	3.40	96.22	3.28
NLPLS	97.59	0.85	98.81	0.50	99.46	3.09	100.13	2.87	100.04	3.33	98.93	2.96	98.10	2.91	99.37	2.61
Andrew	99.46	0.76	99.72	0.69	100.98	1.49	97.82	1.23	103.72	1.92	97.38	2.22	103.55	4.12	95.44	3.29
Hampel	99.37	0.71	99.84	0.63	101.80	1.56	97.77	1.20	103.77	1.92	97.14	2.24	103.64	4.08	94.98	3.38
RPLS	100.00	0.42	100.00	0.47	100.00	0.42	100.00	0.47	100.00	0.89	100.00	0.86	100.05	1.61	100.02	1.42

and RPLS can give better results in such analyzed systems. When the net analytical signal of a component is less than ten, the condition number and correlation coefficient are also very large. This shows that the analyzed system is seriously ill-conditioned. In such a situation, we must select a method that is good at resisting collinearity of spectra such as Ridge, PLS and RPLS, etc. We can also select the wavelength points so as to decrease collinearity of spectra.

3. For a linear analyzed system, the results of NLPLS are not ideal. In our experience, the regression coefficient of square terms is neither zero nor near zero. Thus if we want to select a model to fit a certain system, it must be combined with our special knowledge and practical experience about the system. A better model leads to better results. Most of the analyzed systems observed by us have a good linearity.

4. Ridge regression shows superiority only when the collinearity of the analyzed system is so strong that ordinary calibration methods can not be used. For a system with good selectivity as in system 1 and 2, its results are similar to that of other methods or worse. So, only when we have satisfactory reasons to show the system analyzed is seriously ill-conditioned can we use this method. So, we suggest that ridge regression is used only when the system almost cannot be estimated.

As can be seen above, different modified methods were presented with problems of the analyzed system. Only when these problems really exist, can these methods be used.

In the situation without outliers, the results of robust methods are similar to that of ordinary calibration methods. According to theory, the base of maximum likelihood is still least square estimated. It only improves the target function so as to decrease its sensitivity to outliers. When a system does not have an outlier, the results of the M-estimator are no better than that of ITFA and PLS. The principle of RPLS is the same as PLS, it only adds a weighing procedure so as to decrease or eliminate the effect of outliers. Replicating weights are complex and the amount of computation is increased by a factor of ten or more. Also, its results are related to the parameters selected. The RPLS is the best in all situations.

3.1.2. Simulation of nonlinearity of spectra

In order to observe the effect of an added outlier in the system on the applicability of the calibration method, we first constructed four groups of data by adding outliers differing in number and magnitude following Tables 3 and 4 into the simulation data shown in Fig. 1. They are denoted as database 1, 2, 3 and 4, respectively.

We used each method to calculate every database. The results are presented in Table 4. (The results of database 3 are already changed to so bad. The results of database 4 are very ugly. So they are not to be listed here).

1. As can be seen in Table 4, the effect of the outlier on the applicability of multivariate calibration methods was related to the number and magnitude of the outlier (i.e., nonlinearity) and to the collinearity of the system.

In systems 1 and 2, the collinearity of spectra is not so strong. When outliers are small and fewer as in database 1, their effect on the results is small. Same outliers give a greater effect with strongly collinear systems such as 3 and 4. The fluctuation of results becomes large.

When the outlier is greater as in database 3, the results change for the worse and fluctuation obviously becomes larger. In strongly collinear systems, some methods even give negative results. When more outliers are included as in database 2, even if they are

Table 3
Design for perturbation studies with outliers

Database	System 1		System 2		System 3		System 4	
	Number	Magnitude	Number	Magnitude	Number	Magnitude	Number	Magnitude
1	3	5σ	3	5σ	3	5σ	3	5σ
2	6	5σ	6	5σ	6	5σ	6	5σ
3	1	10σ	1	10σ	1	10σ	1	10σ
4	3	10σ	3	10σ	3	10σ	3	10σ

Table 4
a. The relationship between outlier in system and applicability of calibration methods

Method	Database 1															
	System 1				System 2				System 3				System 4			
	Component 1		Component 2		Component 2		Component 2		Component 1		Component 2		Component 2		Component 2	
	Recovery(%)	RSD.(%)	Recovery(%)	RSD.(%)	Recovery(%)	RSD.(%)	Recovery(%)	RSD.(%)	Recovery(%)	RSD.(%)	Recovery(%)	RSD.(%)	Recovery(%)	RSD.(%)	Recovery(%)	RSD.(%)
MLR	98.96	0.82	99.63	0.95	101.17	1.87	98.12	0.95	103.17	2.04	97.21	2.54	103.64	4.93	95.35	3.31
AKC	98.90	0.95	97.29	1.03	102.89	1.72	97.80	1.00	103.26	1.62	94.27	2.44	107.18	3.56	92.88	2.96
Kalman	99.31	0.74	99.89	0.63	101.41	1.58	98.20	1.34	103.03	2.27	97.12	2.29	104.00	3.70	95.59	3.31
TFA	99.42	0.74	99.79	0.68	101.51	1.62	98.01	1.48	102.29	5.78	97.30	5.51	107.16	9.22	93.80	8.20
MTFA	100.01	0.73	99.98	0.62	100.13	1.62	99.92	1.12	100.03	5.74	99.56	5.16	101.16	5.50	99.10	4.72
ITFA	100.01	0.76	99.97	0.64	100.13	1.50	99.93	0.94	99.78	2.41	99.93	2.36	100.63	4.83	99.23	4.10
PLS	99.77	5.80	100.24	3.07	100.77	3.27	99.76	2.12	99.59	3.93	100.26	2.93	103.61	6.82	96.91	5.73
NLPLS	98.18	0.80	98.82	0.63	98.36	2.62	98.80	1.15	98.00	2.56	98.82	1.26	98.07	2.97	99.37	2.29
Andrew	99.34	0.71	99.88	0.63	101.33	1.56	98.20	1.18	103.15	1.91	97.32	2.25	103.64	4.12	95.63	3.35
Hampel	99.37	0.83	99.85	0.72	101.91	1.59	97.89	1.23	103.85	1.94	97.25	2.31	103.61	4.15	95.65	3.44
RPLS	99.93	0.69	99.67	0.67	100.97	0.95	100.73	0.79	101.15	1.22	101.72	1.02	100.23	1.88	100.11	1.96

Table 4 (continued)
b. The relationship between outlier in system and applicability of calibration methods

Method	Database 2															
	System 1				System 2				System 3				System 4			
	Component 1		Component 2		Component 2		Component 2		Component 1		Component 2		Component 2		Component 2	
	Recovery(%)	RSD.(%)	Recovery(%)	RSD.(%)	Recovery(%)	RSD.(%)	Recovery(%)	RSD.(%)	Recovery(%)	RSD.(%)	Recovery(%)	RSD.(%)	Recovery(%)	RSD.(%)	Recovery(%)	RSD.(%)
MLR	98.89	0.89	99.60	1.37	101.03	2.02	97.93	1.80	103.21	3.29	97.17	4.47	103.64	3.93	95.35	3.31
AKC	98.83	1.00	97.27	1.39	102.77	1.96	97.59	1.86	103.27	3.11	94.22	4.75	107.18	3.56	93.88	2.96
Kalman	99.31	0.83	99.89	0.72	101.37	1.58	98.14	1.39	103.18	3.84	97.15	3.91	104.00	3.72	95.59	3.31
TFA	99.40	0.83	99.81	0.76	101.39	1.58	97.90	1.64	101.95	7.54	97.83	8.47	107.80	15.90	90.95	19.62
MTFA	100.00	0.84	99.98	0.70	100.23	1.58	99.80	1.23	100.31	7.20	99.55	7.87	101.38	13.26	98.62	13.20
ITFA	99.99	0.84	99.98	0.73	100.09	1.63	99.85	1.23	99.89	4.04	99.90	4.40	101.37	5.37	98.35	4.70
PLS	100.62	7.00	99.87	3.71	101.21	3.81	99.11	2.53	100.64	5.39	99.67	3.80	101.79	10.27	99.16	9.04
NLPLS	97.55	0.83	98.87	0.60	98.18	2.62	98.86	1.22	97.96	2.26	98.34	1.04	98.57	3.75	99.44	2.54
Andrew	99.31	0.82	99.87	0.63	101.94	1.56	97.52	1.33	103.79	1.91	97.88	2.34	103.72	4.17	95.40	3.41
Hampel	99.37	0.81	99.77	0.72	101.82	1.59	97.69	1.38	103.82	1.88	97.79	2.31	103.12	4.21	95.62	3.48
RPLS	99.93	0.69	99.67	0.67	100.97	0.95	100.73	0.79	101.55	1.22	101.72	1.02	100.13	1.93	99.67	2.12

Table 4 (continued)
c. The relationship between outlier in system and applicability of calibration methods

Method	Database 3															
	System 1				System 2				System 3				System 4			
	Component 1		Component 2		Component 2		Component 2		Component 1		Component 2		Component 2		Component 2	
	Recovery(%)	RSD.(%)	Recovery(%)	RSD.(%)	Recovery(%)	RSD.(%)	Recovery(%)	RSD.(%)	Recovery(%)	RSD.(%)	Recovery(%)	RSD.(%)	Recovery(%)	RSD.(%)	Recovery(%)	RSD.(%)
MLR	98.80	0.81	99.52	1.25	101.26	4.90	98.17	4.27	102.51	3.46	97.77	4.97	103.71	4.41	95.61	4.73
AKC	98.73	1.06	97.18	1.29	103.03	1.78	97.84	2.06	102.52	3.83	94.85	6.04	107.28	3.72	93.08	3.99
Kalman	99.35	0.83	99.87	0.66	101.28	1.74	98.21	1.55	102.23	4.18	97.59	4.19	104.00	4.90	95.76	4.27
TFA	99.21	1.15	99.89	1.55	101.13	4.59	98.27	4.56	86.39	74.7	107.98	60.6	(in Chinese)	(in Chinese)		
MTFA	99.79	1.22	100.09	1.40	99.94	4.60	99.97	4.11	86.14	71.7	108.62	58.3	(in Chinese)	(in Chinese)		
ITFA	100.07	0.72	99.92	0.68	99.88	1.81	100.01	1.47	99.05	12.7	99.44	12.3	101.61	8.80	98.64	9.39
PLS	101.20	6.27	99.74	3.40	99.90	4.11	100.00	2.53	98.32	7.51	100.78	5.39	101.50	12.1	99.38	9.86
NLPLS	97.55	0.83	98.87	0.60	98.17	2.63	98.86	1.22	97.57	1.48	98.69	1.06	98.52	3.65	99.33	2.43
Andrew	99.32	0.69	99.86	0.64	101.78	1.52	97.81	1.22	103.74	1.98	97.64	2.28	103.58	4.15	96.95	3.55
Hampel	99.37	0.71	99.84	0.62	100.85	1.43	98.77	1.31	103.82	2.03	97.61	2.31	103.43	4.03	96.01	3.53
RPLS	99.93	0.69	99.67	0.67	100.97	0.95	100.73	0.79	99.84	1.22	99.02	0.98	100.13	2.03	99.67	2.02

small, the results of each method are obviously worse than that of database 1. We can see from this that firstly, the effect of the outlier on the results is closely related to the quality of the system. More collinearity of the system shows more effect of the outlier. Secondly, the effect of the outlier on the results is related to the number and magnitude of the outlier. It should be pointed out specially that the effect of the outlier on the results of the Kalman filter is weak because the filtering process of this method is from one wavelength to another. Small amounts of outlier only change the results of some points, but its effect on the final results is small.

2. It can be seen from Table 4 that when the collinearity of a system is not strong (system 1) and the number of outliers is less, the magnitude is small (database 1), and the robust regression methods have no obvious superiority. When the collinearity of the system is strengthened (system 3, 4 of database 1), the robust methods are better than classical methods in resisting the effect of outliers. The results are close to those of systems 3 and 4 without outliers. When large outliers are added (database 3), the results of robust methods with every system are obviously superior to those of classical methods. When the number of outliers are increased (database 2), robust methods still get good results in the system with good quality (systems 1 and 2 with lower collinearity). When the collinearity of a system is strong and the number and magnitude of the outliers reach a certain degree, the results of Andrew and Hampel methods become worse but RPLS is superior. The change of collinearity of the system has no obvious effect on results of RPLS. So, RPLS is the best method when the collinearity of the system is very strong.

3. The Robust methods were compared with a combined diagnostic and classical calibration method. In order to combine the diagnostic method and classical calibration method, we firstly studied and compared three diagnostic methods, the Cook, the H-maxtrix and the Robust Diagnosis (RD). In practice, the RD diagnosis is the best. So, we used this diagnostic method combined with classical calibration methods.

Robust regression is the same in nature as the diagnostic combined method. Their difference is only the deleting order of outliers. The object and effect deleting outlier are the same. Database 2 was calculated with Robust methods and the combined method of diagnostic and classical calibration methods. The results are presented in Table 5. It is shown that the results of robust methods have no obvious differences from those of combined diagnostic methods. (Andrew and Hampel compared with the combined method of diagnosis based on LS estimator, RPLS with the combination of diagnosis-PLS).

3.2. Simulating real spectra

In order to predict the effect of collinearity of a system on the applicability of calibration methods, we simulated real spectra.

Under the experimental condition selected, the pure components are determined and are assigned the E (absorptivity) matrix. In model sets, the concentration of each component is divided into five levels by the proportion of prescription giving a suitable range of absorbance. A standard concentration matrix was constructed with twenty five standard mixtures by orthogonal factorial design. The standard absorbance matrix for these concentrations was given by Beer's law. In test sets, the labeled amount around 100% is divided into five levels with a span of 10% giving us the concentration matrix observed. From these concentrations, the absorbance matrix of the sample was calculated by Beer's law. To all absorbance matrices we randomly added 2% as error.

The aim of simulating real spectra is to predict the applicability of calibration methods from the collinearity of the system. We only need to determine the absorbance of pure components and to get the E matrix for simulating. First, we can use the criteria of the quality of the system to analyze the collinearity of the system and then we can decide which method can be used, and which method cannot be used from the results simulated. Thus, we can gain knowledge about the system analyzed in advance.

3.2.1. The simulation of two components in compound Dong-Mian-Ling tablets

The spectra of chloropromazine and promethazine were determined. The matrix of standard concentration, standard absorbance and the absorbance of the observed system were constructed by the method

Table 5
The results of robust method compared with those of a combined diagnostic and classical method

Method	Database 2							
	System 1				System 2			
	Component 1		Component 2		Component 1		Component 2	
	Recovery(%)	RSD.(%)	Recovery(%)	RSD.(%)	Recovery(%)	RSD.(%)	Recovery(%)	RSD.(%)
MLR	98.86	0.76	99.49	0.80	101.22	1.86	98.00	1.29
AKC	98.92	0.89	97.88	0.79	102.98	1.76	97.49	1.06
Kalman	99.30	0.72	99.89	0.63	101.31	1.51	98.34	1.14
TFA	99.40	0.76	99.80	0.69	101.51	1.64	98.03	1.35
MITFA	99.99	0.74	99.95	0.61	100.05	1.57	99.95	1.05
ITFA	100.01	0.75	99.98	0.64	100.06	1.63	99.95	1.06
PLS	100.00	0.49	100.00	0.52	100.00	1.02	100.01	0.74
NLPLS	97.58	0.84	98.85	0.68	99.43	3.23	99.06	2.87
RPLS	99.93	0.69	99.67	0.67	100.97	0.95	100.73	0.79
Method	System 3				System 4			
	Component 1		Component 2		Component 1		Component 2	
	Recovery(%)	RSD.(%)	Recovery(%)	RSD.(%)	Recovery(%)	RSD.(%)	Recovery(%)	RSD.(%)
MLR	103.20	2.02	102.11	2.28	103.57	4.10	95.71	3.56
AKC	103.55	1.88	94.25	2.79	107.09	3.51	94.03	2.88
Kalman	103.43	2.19	96.82	2.30	103.61	3.72	95.59	3.31
TFA	102.55	2.03	97.41	2.42	106.10	5.31	93.21	5.67
MITFA	100.02	1.82	99.93	2.23	100.22	4.22	99.91	3.66
ITFA	100.02	2.07	99.90	0.83	100.37	4.85	99.66	3.89
PLS	99.9	0.92	100.11	0.83	100.00	1.71	100.00	1.47
RPLS	101.55	1.22	101.72	1.02	100.13	1.93	99.67	2.02

shown above. The outliers of observed data were eliminated by RD diagnosis. The quality criteria of the observed system were calculated in Table 6.

From Table 6 we can predict that the net analytical signal of components is more than 10. Cond = 39.32, $R = 0.8417$. This system has some ill-conditioning. We can conclude by numerical simulation of results that every method has different applicability. ITFA, PLS and RPLS will give better results. The data simulated real spectra were calculated by every calibration method. The applicability of calibration methods were evaluated by mean recovery and RSD. The results are listed in Table 7.

From Table 7, we can see that the conclusion predicted by the quality criteria of the observed system is basically identical to that predicted by numerically

Table 6
The qualitative analysis of two component systems in the compound Dong-Mian-Ling tablet

	NS	Cond	*R*
Chloropromazine	15.56	39.32	0.8417
Promathazine	15.55		0.8417

Table 7
The applicability of simulating the compound Dong-Mian-Ling tablets system

Method	Chloropronazine		Promethazine	
	Recovery(%)	RSD(%)	RSD(%)	Recovery(%)
MLR	100.46	1.78	99.45	1.62
AKC	93.91	1.92	104.06	1.34
Kalman	100.46	1.79	99.46	1.46
TFA	100.39	1.99	99.46	1.66
MTFA	99.92	1.59	100.10	1.26
ITFA	99.92	1.59	100.07	1.25
PLS	100.00	0.74	100.00	0.62
Ridge	100.52	1.76	100.11	1.58

Table 8
The qualitative analysis of a three-component system in Su-Xiao capsule

	NS	Cond	*R*		
PAR.	31.12	15.93	1.0000	−0.1622	0.4534
CAF.	34.25		−0.1622	1.0000	0.2432
CHL.	31.90		0.4534	0.2432	1.0000

simulated spectra. PLS, ITFA and RPLS gave the best results. AKC gave the worst results. The performance of other methods lies in between them.

3.2.2. The simulation of three components in compound Su-Xiao capsules

The spectra of paracetamol, chlorophenamine and caffeine were determined. The matrix of standard concentration, standard absorbance and absorbance of observed system were constructed by the method as indicated above, The outliers of observed data were eliminated by RD diagnosis. The quality criteria of the observed system were calculated as shown in Table 8. From Table 8, we can predict that the net analytical signal of components is more than 10. Cond = 15.93, *R* is small. The quality of this system is good. Every calibration method will give good results if the observed data are good. The performances of these calibration methods show no significant difference.

The data of simulated real spectra were calculated by every calibration method. The applicability of calibration methods were evaluated by mean recovery and RSD. The results are listed in Table 9. From Table 9, we can see that the results were similar to those predicted with numerically simulated spectra.

Table 10
The qualitative analysis of four-components system in Qu-Tong tablets

	NS	Cond	*R*			
Phein	9.58		1.0000	−0.1521	−0.8924	0.1054
Phenl	8.96		−0.1521	1.0000	−0.6112	0.4172
Caffe	8.48	619.66	−0.8924	−0.6112	1.0000	−0.9082
Amino	6.68		0.1054	0.4172	−0.9082	1.0000

3.2.3. The simulation of four components in Qu-Tong tablets

The spectra of phenacetin, phenobarbital, caffeine and aminophenazone were determined. The matrix of standard concentration, standard absorbance and the absorbance of the observed system were constructed by the method as indicated above. The outliers of observed data were eliminated by RD diagnosis.

The quality criteria of the observed system were calculated in Table 10. From Table 10 the prediction can be made. The net analytical signal of the phenacetin component is near 10. There are large amounts in a prescription and with a big absorptivity. So, better results are obtained by PLS, ITFA and RPLS and worse results by other methods. Under the quality of this system (Cond = 619.66, serious ill-condition) most methods will not give very satisfactory results with other components.

The data of simulated real spectra were calculated by each calibration method. The applicability of calibration methods was evaluated by mean recovery and RSD. The results are listed in Table 11. We can see from Table 11 that the results agree with prediction.

Table 9
The applicability of simulating the Su-Xiao capsule system

Method	Paracetamol		Caffeine		Chlorophenamine	
	Recovery(%)	RSD(%)	Recovery(%)	RSD(%)	Recovery(%)	RSD(%)
MLR	100.22	0.72	100.09	0.96	102.25	2.02
AKC	99.52	0.52	101.31	1.26	95.79	2.68
Kalman	100.24	0.49	99.22	0.78	101.97	2.39
TFA	100.36	0.54	98.06	1.21	102.90	3.02
MTFA	99.47	0.58	99.72	1.13	103.45	2.88
ITFA	99.61	0.51	100.05	0.84	102.23	2.19
PLS	99.34	0.40	99.55	0.62	105.04	1.93
Ridge	100.19	0.66	99.84	0.64	98.76	4.51

Table 11
The applicability of simulating the Qu-Tong tablets system

Method	Phenacetin		Phenobarbitol		Caffeiene		Aminophenazone	
	Recovery (5)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
MLR	99.33	1.80	103.11	5.14	97.24	6.41	102.74	6.91
AKC	73.85	17.7	95.73	17.72	−53.90	−0.43	−51.70	−3.36
Kalman	99.82	1.57	104.71	4.87	99.86	4.53	99.86	3.38
TFA	98.93	3.21	102.25	4.72	92.60	8.20	101.96	5.40
MTFA	99.89	2.11	100.41	4.53	100.58	6.67	100.86	5.24
ITFA	100.02	1.81	100.22	3.64	100.84	5.61	100.42	4.63
PLS	100.01	0.97	100.15	2.68	100.29	3.54	100.01	2.86
RIDGE	102.55	8.31	99.53	1.79	98.22	7.00	101.32	4.11

The simulated results of three real systems have shown that the quality criteria of a system can give some direction before experiment.

4. Determination of real samples

4.1. Instrument and chemicals

A Shimadzu UV–Vis 265 spectrophotometer was used to obtain the experimental data. An IBM 586 microcomputer was used in the simulation and calculation of experimental data. All programs were written in BASIC and FORTRAN. Chloropromazine, promethazine, paracetamol, caffeine, chlorophenamine, phenacetin, phenobarbital and aminophenazone are of pharmacopoeia (1995, P.R. China) quality. Analytical reagent grade chemicals and deionized water were used.

4.2. Experiments

4.2.1. The determination of components in Dong-Mian-Ling tablets

Standard solutions of chloropromazine and promethazine and the solutions of ten artificial samples with the proportion of prescription were prepared with 0.1 mol l^{-1} HCl. The solutions of artificial samples serve as model sets. The concentration of chloropromazine and promethazine was 5 μg ml^{-1}. These concentrations described above were used as 100% of labelled amount. The artificial samples were given by changing 10% around 100% of labelled amount with five levels according to orthogonal factorial design. In test sets, ten artificial samples were prepared as follows: Suitable amounts of each component and excipient in tablet with the same proportions as in the prescriptions were weighed accurately, ground and mixed in mortar. The spectra of pure components were recorded from 200 to 280 nm and are shown in Fig. 2. The stability and linear range of each solution were observed. The absorbance of each solution of model sets and test sets were determined every two nm from 220 to 280 nm. The outliers were deleted by RD diagnosis. The experimen-

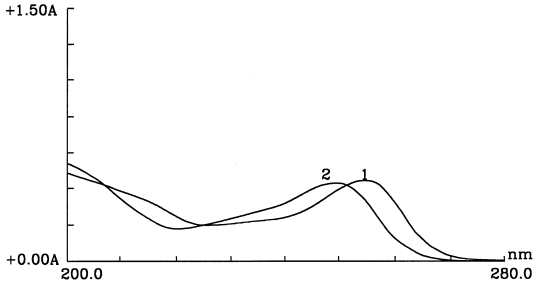

Fig. 2. Spectra of chloropromazine and promethazine. 1: Chlorpromazin (5 μg ml^{-1}); 2: Promethazine (5 μg ml^{-1}).

Table 12
Comparison of results of combined and robust methods with an artificial sample of Dong-Mian-Ling tablets

Method	Chloropronazine		Promethazine	
	Recovery(%)	RSD(%)	RSD(%)	Recovery(%)
MLR	101.19	1.84	98.25	2.01
AKC	92.15	2.24	103.25	2.23
Kalman	101.23	1.63	98.59	1.87
TFA	100.88	2.04	98.95	2.53
MTFA	100.39	1.58	99.15	1.51
ITFA	99.97	1.52	99.26	1.39
PLS	100.75	1.02	100.49	1.15
Ridge	101.35	1.42	101.74	1.77
Hampel	101.17	1.56	98.44	1.51
RPLS	99.97	0.92	100.76	0.93

tal data were calculated by each calibration method. The results of these and Robust regression methods are presented in Table 12.

From Table 12, we can see that the results are almost identical with those predicted. RPLS, PLS, ITFA and MTFA gave good results. The results of AKC were the worst.

4.2.2. The determination of components in Su-Xiao capsule

Standard solutions of paracetamol, caffeine and chlorophenamine and the solutions of ten artificial samples in the proportion of prescription were prepared with 0.01 mol l^{-1} NaOH. The solutions of artificial samples served as model sets. The concentrations of paracetamol, caffeine and chlorophenamine were 10 μg ml^{-1}, 2 μg ml^{-1} and 1.8 μg ml^{-1}, respectively. These concentrations were used as 100% of labelled amount. The artificial samples were given by changing 10% around 100% of labelled amount with five levels according to orthogonal factorial design. In test sets, ten artificial samples were prepared as follows: Suitable amounts of each component and excipient in tablet with the same proportions as in the prescriptions were weighed accurately, ground and mixed in mortar. The spectra of pure components were recorded from 200 to 350 nm and are shown in Fig. 3.

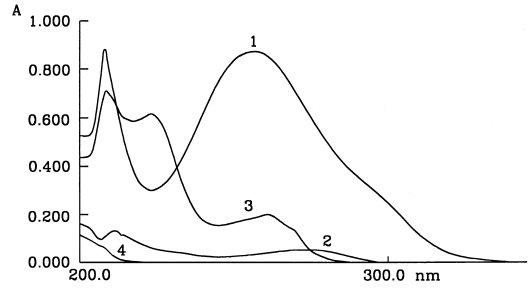

Fig. 3. Spectra of paracetamol, caffeine and chlorophenamine. 1 Paracetamol; 2 Caffeine; 3 Chlorophenamine; 4 Excipient.

The stability and linear range of each solution were observed. The absorbance of each solution of model sets and test sets was determined every two nm from 220 to 300 nm. The outliers were deleted by RD diagnosis. The experimental data were calculated by each calibration method. The results of these and Robust regression methods are presented in Table 13. From Table 13, we can see that the results are almost identical with those predicted. The quality of this system is fine. Each calibration method can give us good results. The results for chlorophenamine are slightly different from those predicted. This is probably due to low amounts in the prescription and less absorptivity. So, the RSD is slightly larger than that of prediction.

Table 13
The results of artificial samples of Su-Xiao capsule

Method	Paracetamol		Caffeine		Chlorophenamine	
	Recovery(%)	RSD(%)	Recovery(%)	RSD(%)	Recovery(%)	RSD(%)
MLR	100.89	0.50	100.26	1.40	96.51	3.53
AKC	101.84	1.85	101.59	2.27	89.83	4.37
Kalman	101.13	0.74	101.99	1.21	95.75	2.26
TFA	100.85	0.78	101.30	1.46	92.94	3.42
MTFA	99.92	0.85	102.22	1.46	94.16	2.35
ITFA	100.56	0.72	100.33	1.52	96.07	2.31
PLS	99.97	1.01	99.72	1.16	102.1	2.11
Ridge	101.10	0.76	97.85	1.14	96.06	2.27
NLPLS	100.24	5.32	95.91	2.79	100.94	3.35
Hampel	101.12	1.71	99.10	0.99	96.88	2.72
RPLS	99.54	1.09	100.43	1.02	101.74	2.15

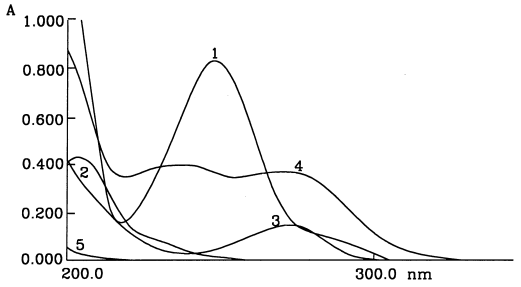

Fig. 4. Spectra of phenacetin, phenobarbitol, caffeine and aminophenazone. 1 Phenacetin; 2 Phenobarbitol; 3 Caffeine; 4 Aminophenazone; 5 Excipient.

4.2.3. The determination of components in Qu-Tong tablet

Standard solutions of phenacetin, phenobarbital, caffeine and aminophenazone and the solutions of ten artificial samples with the proportion of prescription were prepared with 75% ethanol. The solutions of artificial samples served as model sets. The concentrations of phenacetin, aminophenazone, phenobarbital and caffeine are 15 $\mu g\ ml^{-1}$, 15 $\mu g\ ml^{-1}$, 1.5 $\mu g\ ml^{-1}$ and 5 $\mu g\ ml^{-1}$, respectively. These concentrations were used as 100% of labelled amount. The artificial samples were given by varying 10% around 100% of labelled amount with five levels according to orthogonal factorial design. In test sets, ten artificial samples were prepared as follows: Suitable amounts of each component and excipient in tablet with the same proportions as in the prescriptions were weighed accurately, ground and mixed in mortar. The spectra of pure components were recorded from 200 to 300 nm and are shown in Fig. 4. The stability and linear range of each solution were observed. The absorbance of each solution of model sets and test sets were determined every two nm from 220 to 280 nm. The outliers were deleted by RD diagnosis. The experimental data were calculated by each calibration method. The results of these and Robust regression methods are presented in Table 14.

Table 14
The results of artificial sample of Qu-Tong tablets

Method	Phenacetin		Phenobarbitol		Caffeiene		Aminophenazone	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
MLR	98.29	2.54	98.58	8.61	96.94	5.30	98.41	5.70
AKC	87.38	3.07	67.85	8.57	负值		负值	
Kalman	100.28	2.10	98.77	7.42	97.76	8.60	98.79	6.38
TFA	100.08	3.57	98.80	5.40	91.63	9.24	97.92	4.71
MTFA	99.89	2.55	98.88	4.77	98.23	6.64	96.58	5.36
ITFA	100.01	2.21	100.04	3.87	101.6	3.82	100.15	3.51
PLS	100.00	1.91	100.03	2.04	101.9	3.07	100.08	2.99
Ridge	96.27	2.29	92.79	7.38	88.44	9.02	108.31	4.77
NLPLS	98.42	5.32	95.91	2.79	97.44	3.30	95.26	4.37
Hampel	100.11	3.53	98.73	3.20	96.67	5.49	98.10	3.77
RPLS	100.00	1.52	100.04	2.05	102.88	3.23	100.09	3.02

From Table 14, we can see that the results are identical with that of simulation of numerical and real spectra. The results of PLS, ITFA and RPLS are better than those of other methods.

5. Conclusion

From the results of simulation of numerical and real spectra and that of a real analytical system, it can be seen that the simulated results are almost identical to the determined results of a real analytical system. So, we can predict an observed system with only a few experiments before determination by simulation. This can direct us to select a method for the real determination.

The quality criteria of an observed system are very useful, especially the net analytical signal of components (NS). When the NS is more than ten, most calibration methods can give us good results. If the NS is less than ten we must carefully select the calibration methods for the system observed.

We suggest that the outliers are deleted before treatment with nonrobust methods to improve the results.

References

[1] R.H. Liu, R. Ramesh, J.Y. Liu, Analytical Chemistry 56 (1984) 1808–1816.
[2] C.W. Brown, P.F. Lynch, R.J. Obremski, Analytical Chemistry 54 (1982) 1472–1479.
[3] H.N. Poulisse, Analytica Chimica Acta 112 (1979) 361–374.
[4] E. Malinowski, Factor Analysis in Chemistry, Wiley Interscience (1980).
[5] X.W. He, Chinese Journal of Analytical Chemistry 14 (1986) 1109–1113.
[6] G. Bernard, M. Vandeginste, Analytica Chimica Acta 173 (1985) 253–264.
[7] P. Geladi, Analytical Chemistry 57 (1986) 1–17.
[8] P. Liu, Chinese Journal of Analytical Chemistry 23 (1995) 1447–1450.
[9] I.E. Frank, Chemometrics and Intelligent Laboratory System 8 (1990) 109–119.
[10] Y.L. Xie, Analytica Chimica Acta 313 (1995) 185–196.
[11] I.V. Wakeling, Journal of Chemometrics 6 (1992) 189–198.
[12] R.D. Cook, Technometrics 19 (1977) 15–18.
[13] R.R. Hocking, Technometrics 25 (1983) 219–249.
[14] W.C. Chen, H. Cui, Y.Z. Liang, Chemical Journal of Chinese University 16 (1995) 1349–1354.
[15] M. Otto, Analytica Chimica Acta 180 (1986) 445–456.
[16] A. Lober, Z. Goldbart, Analytical Chemistry 56 (1984) 37–43.