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Research Article

A Training Set Selection Strategy for a Universal Near-Infrared Quantitative Model

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Abstract. The purpose of this article is to propose an empirical solution to the problem of how many clusters of complex samples should be selected to construct the training set for a universal near infrared quantitative model based on the Næs method. The sample spectra were hierarchically classified into clusters by Ward's algorithm and Euclidean distance. If the sample spectra were classified into two clusters, the 1/50 of the largest Heterogeneity value in the cluster with larger variation was set as the threshold to determine the total number of clusters. One sample was then randomly selected from each cluster to construct the training set, and the number of samples in training set equaled the number of clusters. In this study, 98 batches of rifampicin capsules with API contents ranging from 50.1% to 99.4% were studied with this strategy. The root mean square errors of cross validation and prediction were 2.54% and 2.31% for the model for rifampicin capsules, respectively. Then, we evaluated this model in terms of outlier diagnostics, accuracy, precision, and robustness. We also used the strategy of training set sample selection to revalidate the models for cefradine capsules, roxithromycin tablets, and erythromycin ethylsuccinate tablets, and the results were satisfactory. In conclusion, all results showed that this training set sample selection strategy assisted in the quick and accurate construction of quantitative models using near-infrared spectroscopy.

KEY WORDS: calibration set selection; cluster analysis; near-infrared spectroscopy; quantitative analysis.

INTRODUCTION

Compared with conventional analytical methods, near-infrared spectroscopy (NIR) has clear advantages in that it is fast, environmentally friendly (requires no disposal of samples, solvents, or reagents), and can measure through transparent packaging materials like glass and certain plastics (1). In recent years, a growing number of pharmaceutical manufacturers and experts in pharmaceutical analysis are switching to NIR from the tedious and time-consuming conventional detection methods, and NIR analysis has now gained wide application in the analysis of pharmaceutical products (2–4). Because NIR is a secondary method, its accuracy relies not only on the accuracy of the reference method but also on the careful selection of representative samples to construct the model.

Sample selection has been a persisting bottleneck for the application of NIR. There are many mathematical methods for calibration set selection, such as the uniform mapping algorithm (5), the DUPLEX method (6), D-optimal concept algorithm (7), the Næs method (8–11), the Puchwein method (12), etc. However, except for the Næs method, these methods need large data pool and complex calculation that requires programming calculation by mathematical software such as MATLAB, MATHEMATICA, MAPLE, etc. The unavailability of the corresponding commercial mathematical software has largely limited the application of some methods.

Since 2004, the National Institute for the Control of Pharmaceutical and Biological Products (NICPBP) has attempted to establish the universal quantitative model for the quick inspection of API contents in medicines from different manufacturers. This universal model must accurately identify a given pharmaceutical product made by different manufacturers under the same international nonproprietary name and be executable with different NIR instruments at different locations. We have reported some universal models for the quantification of pharmaceuticals in recent years (13–17) and found that the selection of representative samples is especially important to construct the universal model. Rifampicin is a common anti-tuberculosis (anti-TB) drug recommended by WHO and widely used all over the world. There are many Chinese manufacturers of rifampicin capsules, each having varying prescriptions and drug quality. Hence, it is highly desirable to set up a universal quantitative model for

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the quick inspection of rifampicin capsules to detect counterfeit or sub-standard rifampicin capsules. In this paper, we propose a training set selection strategy to construct a universal model for the analysis of rifampicin capsules from different manufacturers. For further validation of this new training set selection strategy, we also use this strategy to reconstruct the previously published universal models for cefradine capsules, roxithromycin tablets, and erythromycin ethylsuccinate tablets (13,17).

MATERIALS AND METHODS

Samples

A total of 98 batches of rifampicin capsules from 56 different manufacturers were used in the present work. The API contents of the rifampicin capsules were determined by high-performance liquid chromatography (HPLC) according to the Chinese Pharmacopoeia 2005 Edition (18) and ranged from 50.1% to 99.4% mg/mg. The challenge samples for model specificity validation included three batches of rifampin, isoniazid, and pyrazinamide complex capsules (the mixture of the three components), one batch of rifandin capsules, two batches of rifapentine capsules, and six batches of pyrazinamide capsules. All samples were the retention samples at NICPBP.

Recording the NIR Spectra

Spectra acquisition was performed on Matrix-F FT-NIR spectrometers (Bruker Optics Inc., Germany). Fiber optic probe was used to collect the diffuse reflectance spectra with an InGaAs detector. Bruker OPUS software (version 6.5) was used for all data collection and analysis. Diffuse reflection spectra were recorded from the outer surface of each capsule at 8 cm^{-1} resolution with 64 co-added scans over 4,000–12,000 cm^{-1}. For each batch, six random capsules were selected, and their NIR spectra were recorded and averaged to construct the model for data analysis.

Spectra Pre-Processing

Data pretreatment was performed in OPUS software (version 6.5). The quantitative model was optimized by combination of the Savitzky and Golay (19) first derivation (FD) and the vector normalization (VN) (20, 21) methods (22). The FD pretreatment could eliminate the baseline drift and background noises caused by capsule lining and excipient. The VN method could spherinize the data (23).

RESULTS AND DISCUSSION

Building the Quantitative Model for Rifampicin

The Næs method selects the training set from a large set of samples based on the principle of cluster analysis. In practice, the spectra are first analyzed by cluster analysis and the number of clusters is set according to the expected number of samples in the training set. The spectrum which is farthest from the center point of each cluster is then added to the training set (Fig. 1). Key selection criteria include: (1) select the samples covering the entire variation and avoid the samples of similar spectra, (2) select samples close to the edge of each cluster, (3) select few samples or only one sample from each cluster (24). The underlying rationale is that samples in the same cluster contain similar information. Therefore, to minimize duplication, it is preferable to select only one sample from each cluster, as opposed to using several samples from only a few of clusters or from a limited region. However, the Næs method did not address the issue of how many clusters should be selected to build the training set.

In the OPUS software, the cluster analysis method belongs to hierarchical cluster analysis. The spectral distance indicates the degree of spectral similarity. Two spectra/clusters having a spectral distance of 0 are entirely identical within the frequency ranges used. The heterogeneity value was used to express the distance between two spectra/clusters.

The NIR average spectra of the rifampicin capsules from 56 different manufactures were shown in Fig. 2. We chose the spectral range of 4,000–12,000 cm^{-1}, calculated the Euclidean distances between every two spectra and grouped all spectra by Ward's algorithm (25). As shown in Fig. 3, the NIR spectra of the rifampicin capsules from 56 different manufacturers were clearly grouped into two parts. The *y*-axis was the heterogeneity value, which was used to classify the spectra and determine the number of clusters. The number of clusters was dependent on the value of Heterogeneity. One spectrum from each cluster was randomly selected to build the training

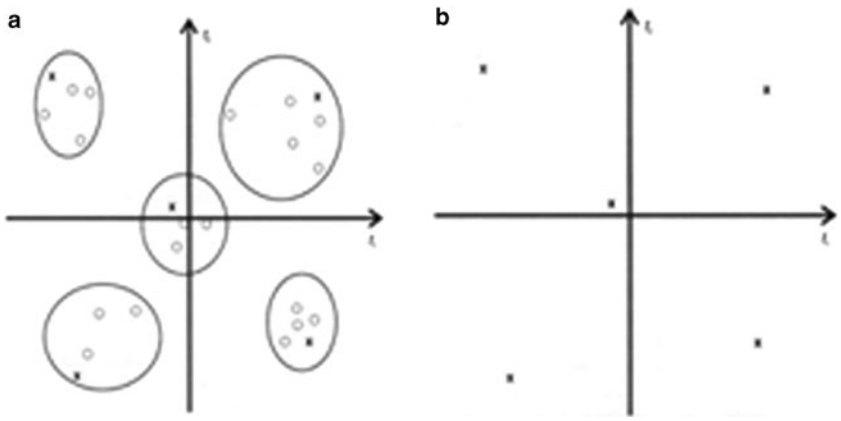

Fig. 1. The Næs algorithm diagram (24). **a** The samples in training set grouped into five clusters, **b** select one samples from each cluster to build the model

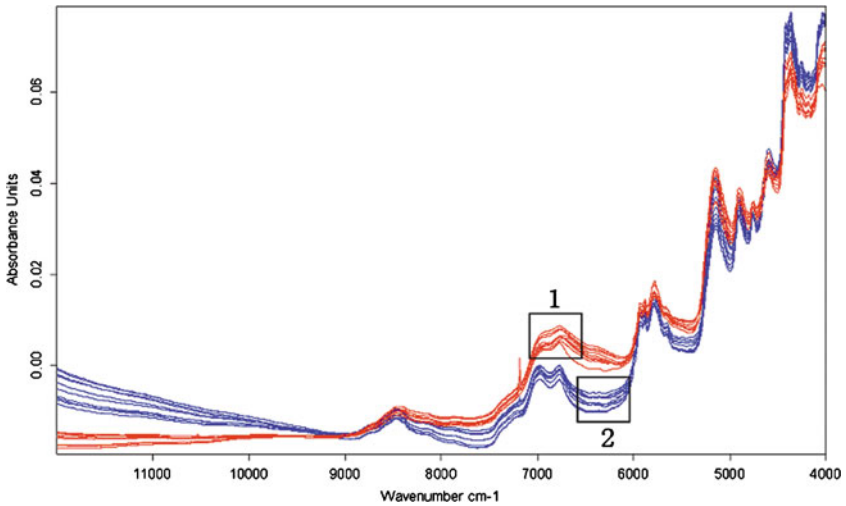

Fig. 2. The average spectra of two clusters (*1* first cluster, *2* second cluster)

set for modeling and the remaining spectra were used as the validation set. Therefore, the number of samples in the training set equaled the number of clusters. The automatic method in OPUS was used to construct the mathematical models (Table I) to investigate the relationship between the number of clusters (the expected number of samples in the training set) and the model performance. Table I shows all optimal calibration models obtained from the automatic optimization routine implemented in the OPUS Quant 2 software using different numbers of calibration samples.

According to Table I, when the heterogeneity value in the cluster analysis ranged from 0.0185 to 0.0195 and the training set sample number was 60 (model 4), the root mean square errors of cross validation (RMSECV) and prediction (RMSEP) obtained by the model were optimal. Comparing the PCA score plots of PC1 against PC2 for the training set and the validation set of rifampicin capsules, the distribution of the training set in model 4 was even and similar to that of the validation set, which implied that this calibration set was representative (Fig. 4a). As for other models, the distribution of the training set in model 1 in PCA did not cover all samples in the validation set (Fig. 4b), whereas the distribution of the training set in model 7 in PCA was partly overlapping (Fig. 4c). The superiority of model 4 was further evaluated through a validation set made from 18 batches of rifampicin capsules not used in the calibration procedure. The methodology from reference (26) was used to compare the performance between each two models. At 95% confidence interval, the ratios of true standard deviations were from 1.03 to 1.60 between model 1 and model 4 and from 1.08 to 1.95 between model 4 and model 7. The ratios of the standard deviations (models 1 and 4, models 4 and 7) were always

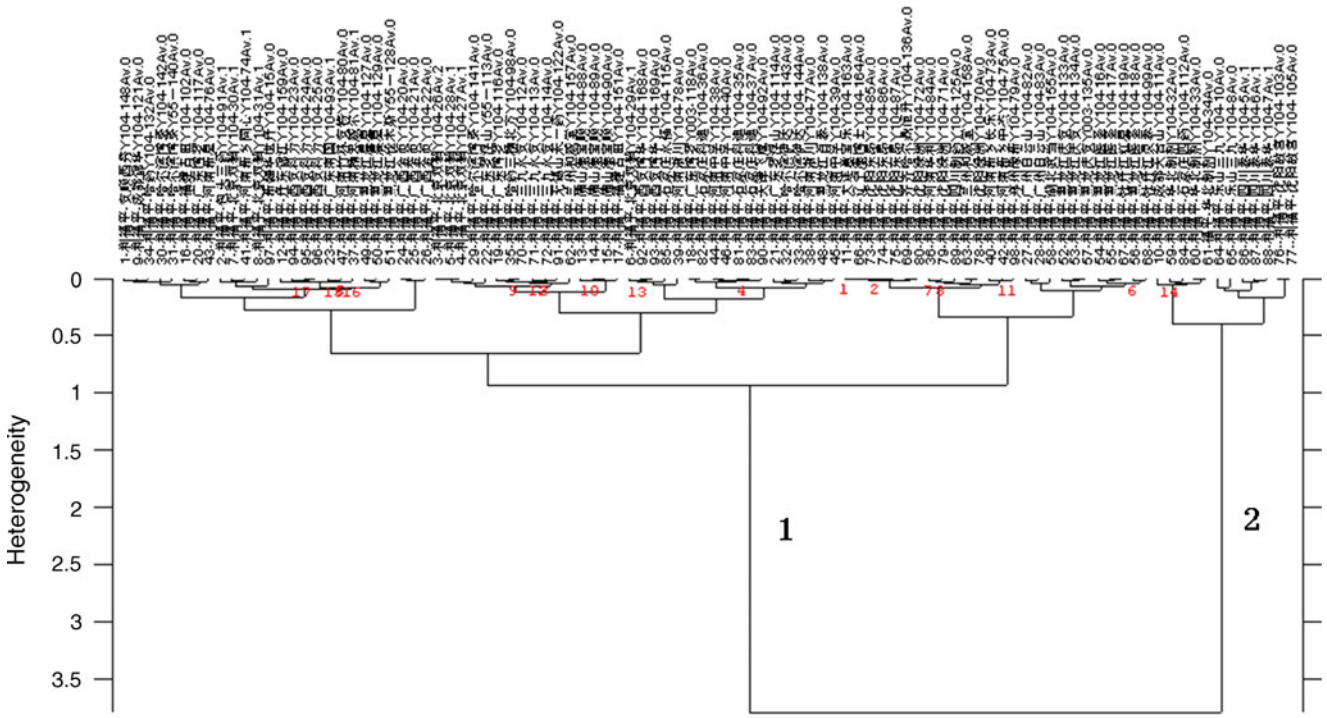

Fig. 3. The hierarchical cluster analysis result for rifampicin capsules. (*1* first cluster, *2* second cluster)

Table I. Characteristics of the Calibration Models with Different Sample Numbers in the Training Set

Model number	Range of heterogeneity in cluster analysis	Cluster numbers	Spectral pretreatment	Wavelength range (cm^{-1})	Calibration			Validation	
					Batches	R^2	RMSECV	Batches	RMSEP
1	0.029–0.030	40	First derivative+vector normalization(17)	9002.5–7498.2 6101.9–4246.7	40	92.7	3.26	58	3.22
2	0.024–0.025	50	First derivative+vector normalization(17)	9002.5–8250.3 6101.9–4246.7	50	94.5	2.86	48	2.48
3	0.0215–0.0225	55	First derivative+vector normalization(17)	9002.5–8250.3 6101.9–4246.7	55	94.5	2.79	43	2.71
4	0.0185–0.0195	60	First derivative+vector normalization(17)	8840.5–8258 5939.9–4196.5	60	95.3	2.54	38	2.31
5	0.016–0.018	65	First derivative+vector normalization(17)	7502–4246.7	65	93.9	2.86	33	2.83
6	0.0135–0.0145	70	First derivative+vector normalization(17)	7502–4246.7	70	94.6	2.70	28	2.03
7	0.011–0.012	80	First derivative+vector normalization(17)	9002.5–7498.2 6101.9–4246.7	80	94.7	2.75	18	2.46

greater than 1; thus, the standard deviations were significantly different at the 5% level. Clearly, the number of samples in the training set should be neither too few nor too many: the model with too few samples cannot adequately represent the sample pool, whereas too many samples in the training set will increase interference and obscure the useful information, thus reduce the model performance.

There was no significant difference in the prediction between models built from average spectra and those built from original spectra. We replaced the spectra for the optimal model for rifampicin capsules with the original spectra without changing other parameters. The RMSEP was 2.45% for the model from original spectra and the RMSEP was 2.31% for the model from average spectra. At 95% confidence interval, the ratio of the standard deviations was from 0.86 to 1.01. Since the range of the ratio of standard deviations included 1, the standard deviations were not significantly different at the 5% level, and the average spectra were thus selected to establish the models.

Validation of the Optimal Model for Rifampicin Capsules

We further evaluated the proposed method (model 4) according to the characteristics of NIR spectroscopy and the ICH guidelines (27) with regard to its outlier diagnostics, accuracy, precision, and robustness.

Outlier Diagnostics

We selected a total of 12 batches of samples to determine how effectively the calibration model could detect the outliers. Our study included three batches of rifampin, isoniazid, and pyrazinamide complex capsules (the mixture of the three components), one batch of rifandin capsules, two batches of rifapentine capsules, and six batches of pyrazinamide capsules. These were chosen as the challenge samples because the API of rifampin, isoniazid, and pyrazinamide capsules contained rifampicin, the chemical structures of rifandin and rifapentine were very similar to rifampicin, and pyrazinamide was an anti-TB drug. The Mahalanobis distance is used to detect the outlier and can be calculated in the relevant subspace spanned by the calibration PLS vectors (28). The Mahalanobis distance limit in the Quant 2 package of the OPUS software is defined as follows:

$$\text{MahDist Limit} = \frac{X \times \text{Rank}}{M} \tag{1}$$

$$\text{PRESS} = \sum_{i=1}^{M} (\text{Differ}_i)^2 \tag{2}$$

where Rank is the number of PLS latent variables which is determined by one-sided F test on Prediction Error Sum of Squares (PRESS, Eq. 2) in LOOCV at α=0.05, M is the total number of calibration set samples, and X is the coefficient that was set to 2 in our experiment. The Rank identified the model with fewest factors whose PRESS was not significantly different from the lowest one within the first 10 factors. The Mahalanobis distance limit (threshold) of the model was set to 0.23 to identify the outliers. When the optimized model for rifampicin capsules was applied to identify the drugs listed above, all Mahalanobis distances exceeded the threshold. Therefore, the universal model developed for rifampicin capsules could effectively detect outliers.

Accuracy

The accuracy of the proposed method was assessed by comparing the results of NIR prediction and standard reference method for several validation batches. We used a validation set consisting of 38 batches from 28 manufacturers with the API content ranging from 51.3–99.0% to test the accuracy of the model. The average deviation of the NIR predictions to the HPLC values was 1.91%. The average relative deviation was 2.1% and the value of RMSEP was 2.31%. A paired t test was also performed to check whether the NIR values and the reference value varied significantly. The t value (1.02) was smaller than $t_{(0.05,37)}$ (2.03), suggesting that the NIR predicted values and reference results were not significantly different.

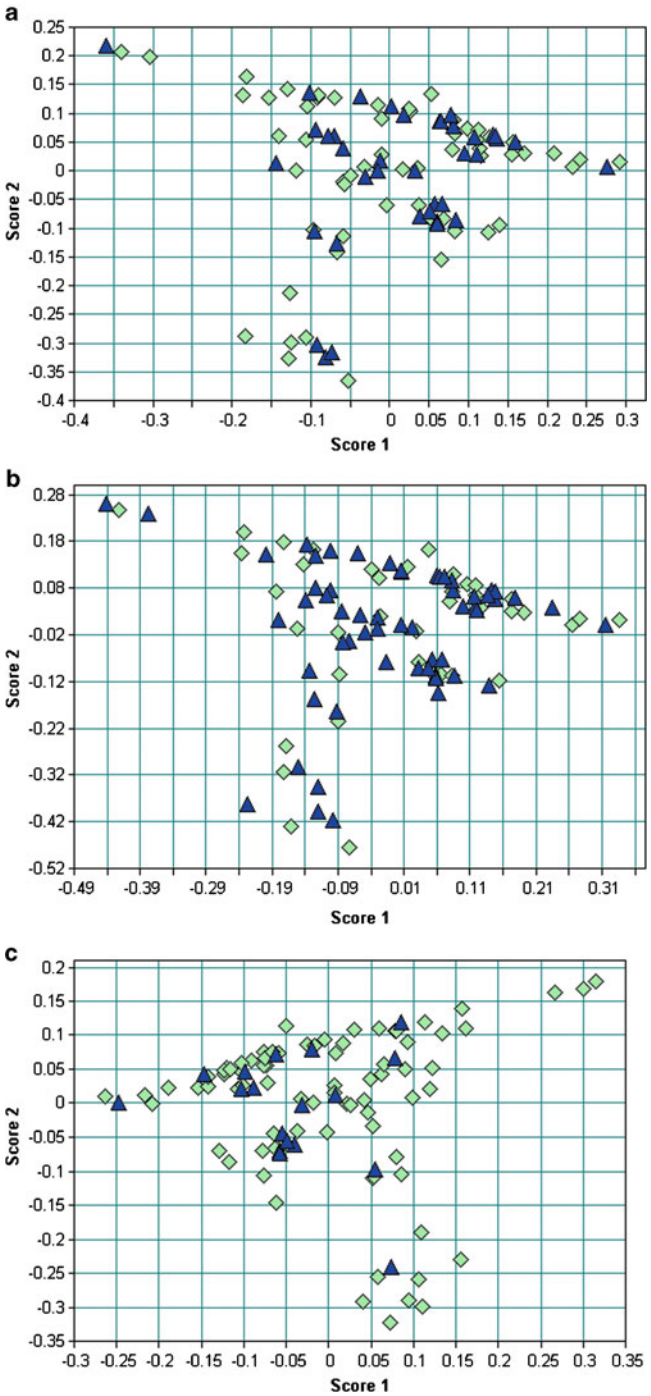

Fig. 4. **a** Model 4 Score2 *vs*. Score1 (*diamonds* training set, *triangles* validation set). **b** Model 1 Score 2 *vs*. Score1 (*diamonds* training set, *triangles* validation set). **c** Model 7 Score 2 *vs*. Score1 (*diamonds* training set, *triangles* validation set)

Precision

The precision of an analytical procedure is expressed as the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under prescribed conditions. In this study, we considered two aspects of precision: repeatability and intermediate precision. Repeatability was determined by having the same operator make ten measurements on the same day. The result of RSD was 0.012. The intermediate precision was determined by having two operators test the same single batch over three consecutive days. The t value (0.55) was smaller than $t_{(0.05,5)}$ (2.57), indicating that time and operator had no significant influence on the model.

Robustness

The robustness of an analytical method measures its capacity to remain unaffected by small but deliberate variation in method parameters and indicates its reliability during normal usage. In this study, the variations in NIR spectra of the same product mainly come from the difference of the manufacturers. Therefore, the model's ability to predict samples from manufacturers not included in the calibration set must be assessed. Fourteen batches from 11 new manufacturers were analyzed by the quantitative model of rifampicin capsules. The RMSEP was 2.49. A paired t test was performed to check whether the NIR value and the corresponding reference value were significantly different. The t value (1.19) of rifampicin capsules model was smaller than $t_{(0.05,13)}$ (2.16); therefore, the NIR and reference results were not significantly different.

Further Validation of the Training Set Selection Strategy Using Cluster Analysis

The number of clusters selected by cluster analysis in the training set selection strategy determined the effectiveness of model construction, as the number of samples in training set equaled the number of clusters. It is difficult to determine the number of clusters according to the sample characteristics and samples numbers, because the sample complexity and availability vary for different NIR methods. In the preceding case of rifampicin capsules, the spectra of the samples were divided into two clusters. The variation in cluster 1 was larger. The biggest value of Heterogeneity in the cluster 1 is 0.931, the one fiftieth value of which is about 0.0186 and set as the Heterogeneity value to determine the sample number in the optimal model for rifampicin capsules. We drew a line at 0.0186 in the dendrogram to determine the number of clusters, and the model constructed accordingly was optimal. Some published models (13,17) were used to validate the universality of our method to determine the heterogeneity threshold value, i.e., after cluster analysis, if the spectra of samples are classified into two clusters, the 1/50 of the largest heterogeneity value in the cluster with larger variation is set to select the number of training sets.

As shown in Fig. 5, a total of 150 batches of Cefradine capsules from 83 different manufacturers were clearly classified into two clusters. The biggest heterogeneity value in the cluster with larger variation was 2.80, and the spectra were classified into 70 clusters according to the one fiftieth values of 2.80. We randomly selected one spectrum from each of the 70 clusters to build the training set and kept other parameters such as spectral range and spectral preprocessing methods the same as in the initial published model. The results of sample selection were found to be superior to the original model (Table II).

We also revalidated the models of roxithromycin tablets and erythromycin ethylsuccinate tablets using the same idea.

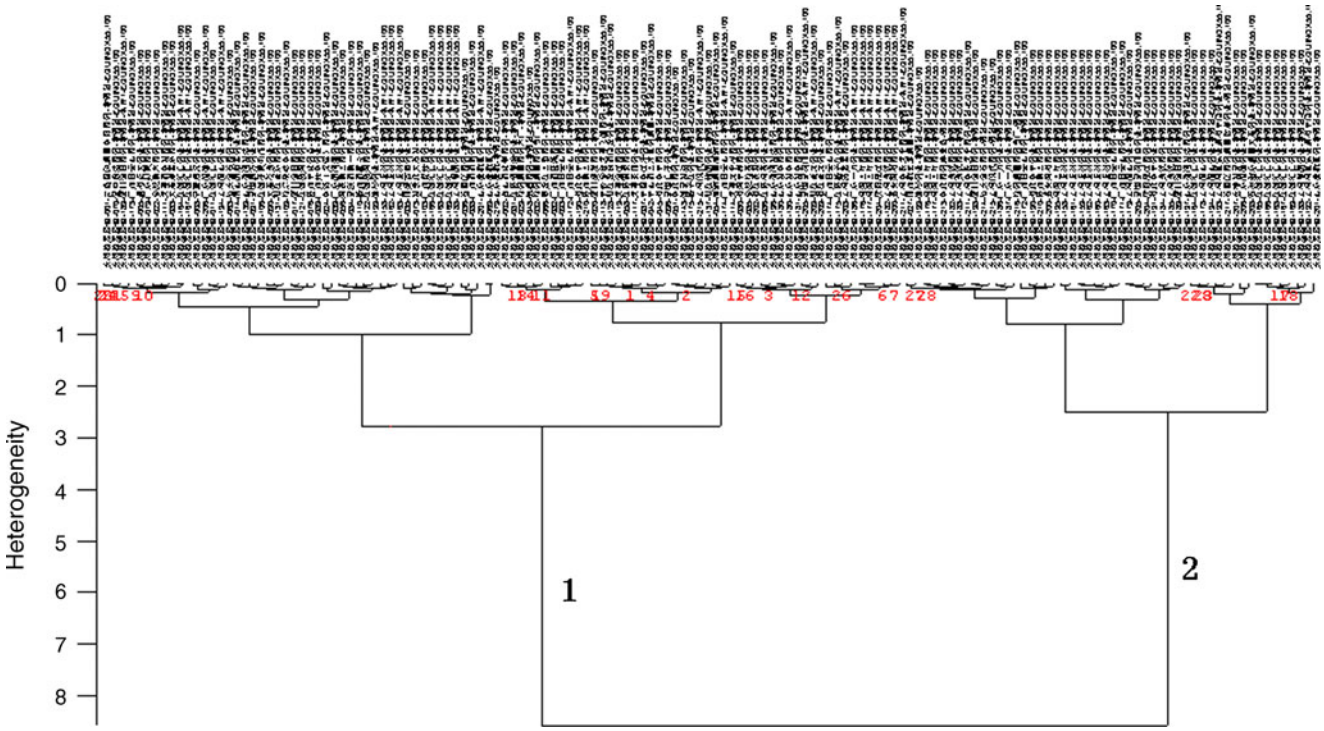

Fig. 5. The hierarchical cluster analysis result for cefradine capsules (*1* first cluster, *2* second cluster)

Spectra of a total of 78 batches of roxithromycin tablets from 18 different manufacturers were clearly classified into two clusters (Fig. 6). Cluster 2 had bigger variation and the biggest heterogeneity value in this cluster was 2.10. The one fiftieth of 2.10 was set as the basis for cluster analysis to classify the spectra of roxithromycin tablets into 40 clusters. We randomly selected one spectrum from each cluster to build the training set and kept other parameters such as spectral range and spectral preprocessing methods unchanged. We found that the results based on this sample selection method were equivalent to the original model (Table II). The spectra of a total of 66 batches of erythromycin ethylsuccinate tablets from 36 different manufacturers were classified into two clusters (Fig. 7). We found that the model based on this sample selection method had improved result compared with the original model (Table II).

We also validated the models (29,30) for cephalosporin powders and got the essentially similar results.

Therefore, our training set selection strategy to construct the universal models for analysis of pharmaceuticals got very great success. The versatility of the method for other complex samples will be explored in future studies.

CONCLUSIONS

On the basis of the Næs method, we proposed a strategy for sample selection to compose the training set for construction of NIR models. For samples clearly classified into

Table II. Validation Results of the Clustering Method for Training Set Sample Selection of Cefradine Capsules, Roxithromycin Tablets, and Erythromycin Ethylsuccinate Tablets

Model number	Spectral pretreatment and wavelength range (cm^{-1})	Number for training set	Calibration		Validation	
			R^2	RMSECV	Number for validation set	RMSEP
Original model for Cefradine capsules (17)	1st derivative, vector normalization 9751–7498.4	100	93.93	1.70	50	2.24
New model for Cefradine capsules		70	95.09	1.51	85	2.26
Original model for Roxithromycin tablets (13)	1st derivative, vector normalization 5581.2–6962.0 8057.4–8971.6	46	98.84	1.84	32	1.45
New model for Roxithromycin tablets		40	98.79	1.72	38	1.42
Original model for Erythromycin Ethylsuccinate tablets (13)	1st derivative, vector normalization 6248.4–5446.2	44	95.4	2.31	22	2.16
New model for Erythromycin Ethylsuccinate tablets		58	95.5	2.00	6	2.15

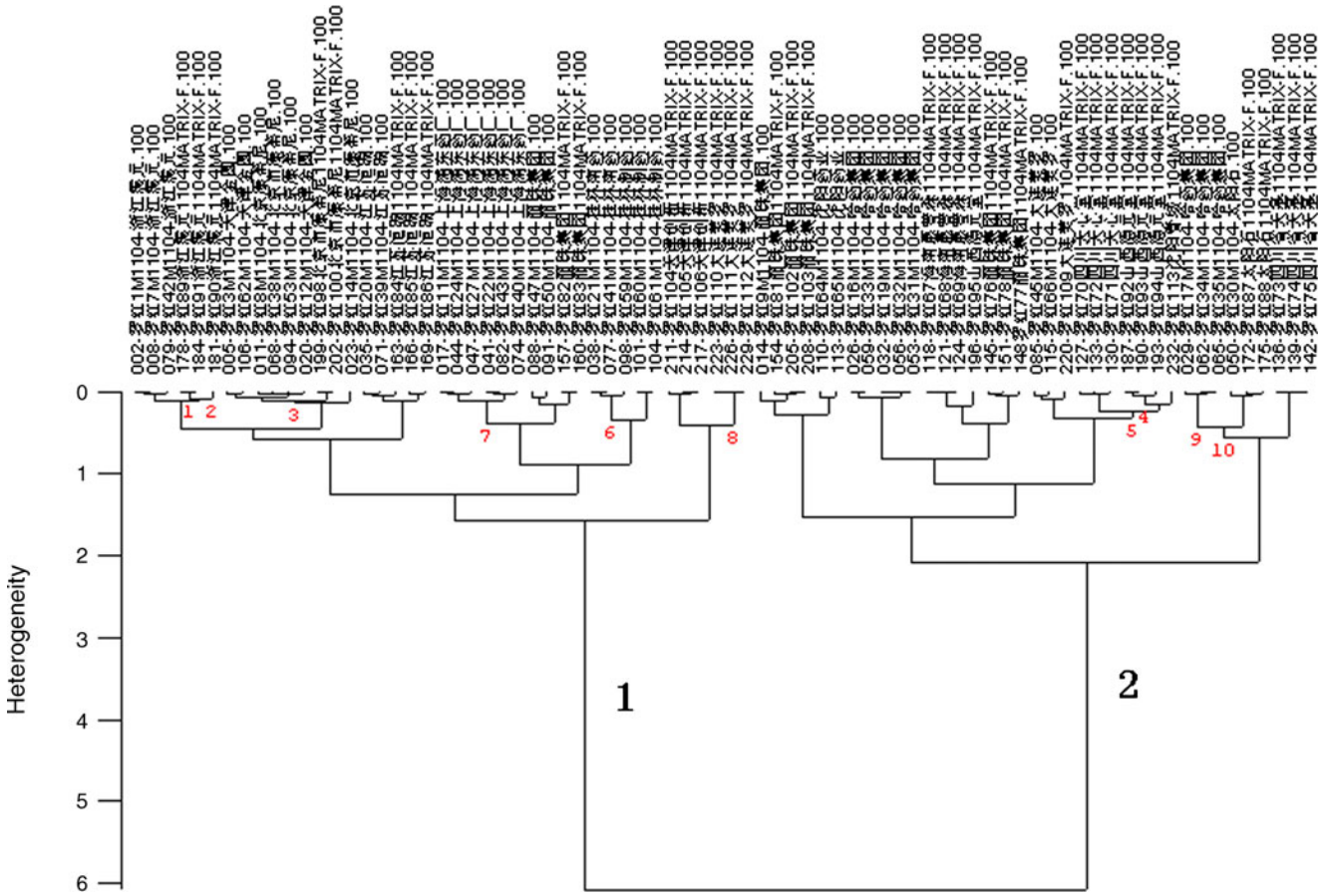

Fig. 6. The hierarchical cluster analysis result for roxithromycin tablets (*1* first cluster, *2* second cluster)

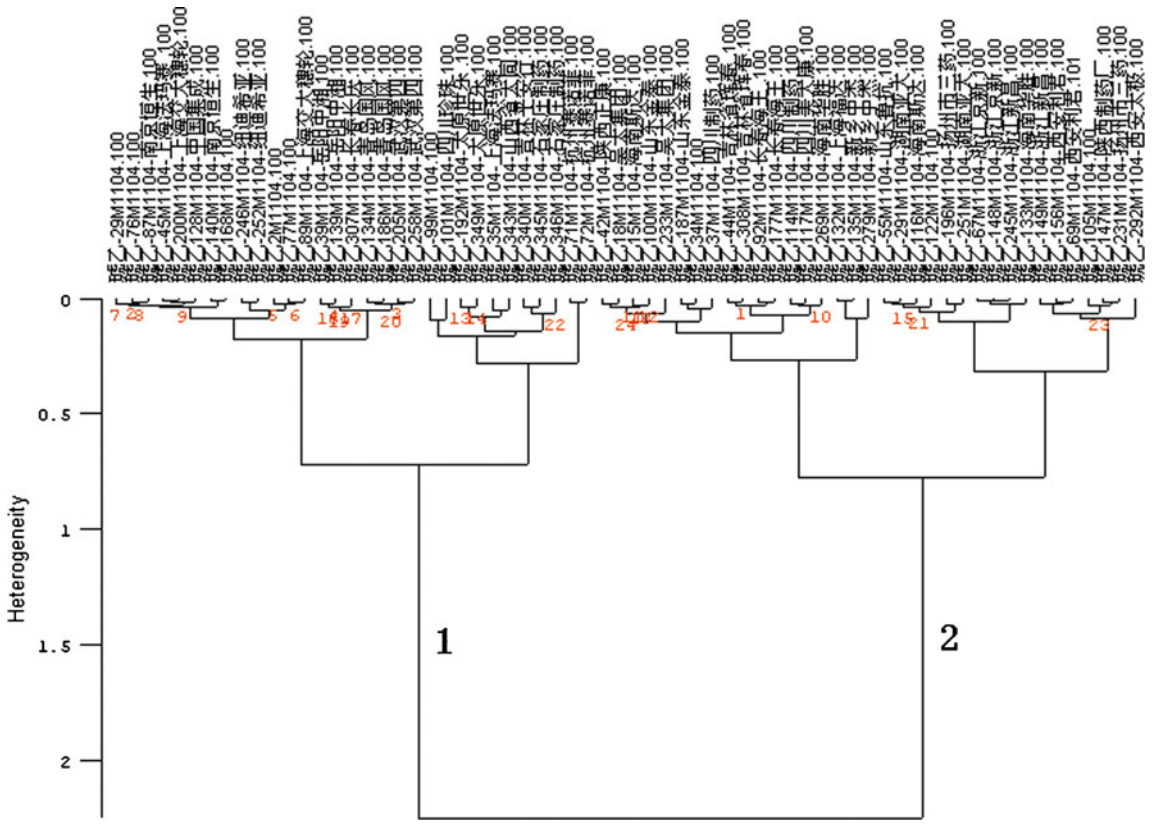

Fig. 7. The hierarchical cluster analysis result for erythromycin ethylsuccinate tablets (*1* first cluster, *2* second cluster)

two clusters, we got better results for NIR quantitative models by (1) setting the threshold at 1/50 of the biggest heterogeneity value in the cluster with larger variation to determine the number of clusters and (2) randomly selecting one sample in each cluster to build the training set. Further validations for this strategy are in progress.

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