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Construction of universal quantitative models for determination of roxithromycin and erythromycin ethylsuccinate in tablets from different manufacturers using near infrared reflectance spectroscopy

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Abstract

Universal quantitative models using NIR reflectance spectroscopy were developed for the analysis of API contents (active pharmaceutical ingredient) in roxithromycin and erythromycin ethylsuccinate tablets from different manufacturers in China. The two quantitative models were built from 78 batches of roxithromycin samples from 18 different manufacturers with the API content range from 19.5% to 73.9%, and 66 batches erythromycin ethylsuccinate tablets from 36 manufacturers with the API content range from 28.1% to 70.9%. Three different spectrometers were used for model construction in order to have robust and universal models. The root mean square errors of cross validation (RMSECV) and the root mean square errors of prediction (RMSEP) of the model for roxithromycin tablets were 1.84% and 1.45%, respectively. The values of RMSECV and RMSEP of the model for erythromycin ethylsuccinate tablets were 2.31% and 2.16%, respectively. Based on the ICH guidelines and characteristics of NIR spectroscopy, the quantitative models were then evaluated in terms of specificity, linearity, accuracy, precision, robustness and model transferability. Our study has shown that it is feasible to build a universal quantitative model for quick analysis of pharmaceutical products from different manufacturers. Therefore, the NIR method could be used as an effective method for quick, non-destructive inspection of medicines in the distribution channels or open market.

Keywords: NIR reflectance spectroscopy; Quantitation; Validation; Erythromycin ethylsuccinate; Roxithromycin

1. Introduction

Near infrared (NIR) spectroscopy technology is a quick, non-destructive and environmentally friendly method comparing with traditional analysis methods. In the last two decades, it has experienced great developments and has been increasingly used in pharmaceutical industry for raw material and excipient identification [1,2], particle size measurement [3,4], polymorphic analysis [5] and determination of the active ingredient or moisture contents in various intermediate and finished products [6,7] in both process control and lab quality control. NIR spectroscopy has successfully been applied to pharmaceutical samples in many different forms such as solutions [8], powders [9,10] and intact tablets and capsules [11–13]. Recently feasibility studies of using NIR spectroscopy in the identification of counterfeit drugs of a specific brand have been reported [14,15]. In response to these advancements, several pharmacopoeia such as EP 5th edition, USP 28, BP2004 and Chinese Phamacopia 2005 version had adopted the NIR method either in an official chapter or in the appendix.

One of the critical challenges of NIR spectroscopy was the model transfer when implementing a NIR method over many different NIR instruments. Since a small instrumental difference between NIR spectrometers could produce a very different result, a calibration model usually could be used only with the spectra collected on the same instrument. To deal with this problem, a number of standardization approaches and mathematical treatments were proposed [16–19]; on the other hand,

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there are considerable improvements in the instrument hardware in the term of wavelength accuracy and photometric linearity in the past 10 years. For example, dedicated FT-NIR (Fourier-transform NIR) spectrometers with a high wavelength accuracy and precision (better than 0.1 cm^{-1} or 0.02 nm) are commercially available. These improvements make it feasible that the models can be directly transferred between different spectrometers without any mathematical treatments [20].

From 1999, China has been implementing a crackdown on the manufacture and sale of counterfeit and substandard drugs, a problem, which has been widespread in the mainland, especially in the countryside [21]. However, finding counterfeit drugs is becoming a growing challenge in stopping the manufacture and marketing of counterfeit medicines. For the purpose of quick inspection of medicines in the countryside and fighting against fake and substandard drugs, a "fast drug identification system" which includes a NIR prescreening system and a fast chemical identification system equipped in a mobile vehicle is being developed. The NIR prescreening system contains two functions: firstly, it is used to identify if a product is a fake or counterfeit drug; then, it is used as a quantitative tool to verify the labeled claimed API contents in the product quickly. When NIR is to be used as a fast screening tool, a method based on a universal model which can be used in different NIR instruments and determine a given pharmaceutical product with the same INN (international non-proprietary names) but from different manufacturers is far more preferred than a method based on individual quantitative models for the products from every individual manufacturers. The universal model, in theory, should be achievable for a given product from all manufacturers. But so far, almost all of these reported NIR applications in pharmaceutical field are used as internal quality control in individual pharmaceutical companies. In this study, roxithromycin tablets and erythromycin ethylsuccinate tablets from different manufacturers were chosen as examples for the feasibility of building such universal quantitative models used in our NIR prescreening system. Direct transfer of the developed quantitative models to multiple FT-NIR instruments that were not used in the model building was also investigated in this study.

2. Experimental

2.1. Apparatus and software

Eight FT-NIR spectrometers, EQUINOX 55, MPA (1), MPA (2), MATRIX-F (2I011104), MATRIX-F (2I007903), MATRIX-F (2I008003), MATRIX-F (2I002403) and MATRIX-F (2I015904), of three different models from one manufacturer (Bruker Optik GmbH, Ettlingen, Germany) were used in this experiment. All spectrometers are equipped with a 1.5 m fiberoptic diffuse reflectance probe and an extended TE-cooled indium gallium arsenide (InGaAs) detector. The two instrument models, Matrix and MPA, use the same interferometer and similar types of optics to launch NIR light to or collect NIR light from a fiber optic probe. The model EQUINOX is based on an interferometer of the same principle and similar design but larger throughput. The EQUINOX uses an external coupling box to launch NIR light to and collect NIR light from a fiber optic probe. The optics inside the coupling box uses all reflecting optics, which is similar to the optics used in the spectrometers mentioned above. Bruker OPUS software version 4.2 was used for all data collections and analysis.

2.2. Samples

A total of 78 batches of roxithromycin tablets from 18 different manufacturers and 66 batches erythromycin ethylsuccinate tablets from 36 different manufacturers were collected from Chinese market from 2000 to 2004 by National Institute for the Control of Pharmaceutical and Biological Products (NICPBP). The API contents of roxithromycin tablets were determined by HPLC [22] and expressed as % (mg/mg) while the API contents of erythromycin ethylsuccinate tablets were analyzed by biological assay [23] and expressed as % (U/μg).

2.2.1. Calibration set

Forty-six batches of roxithromycin samples from 15 different manufacturers with an API content range from 19.5% to 73.9%, 44 batches erythromycin ethylsuccinate samples from 28 manufacturers with an API content range from 28.1% to 70.9% were chosen as the calibration sets.

2.2.2. Validation set

The validation sets contained samples from batches that were not used for the calibration sets. The validation set for roxithromycin tablets consisted of samples from 32 batches from 12 different manufacturers with an API content range from 22.4% to 71.6%. Seven of the 32 validation batches for roxithromycin tablets were from the manufacturers not included in the calibration set. Twenty-two batches erythromycin ethylsuccinate samples from 18 manufacturers with an API content range from 43.3% to 65.7% were used as the validation set for erythromycin ethylsuccinate tablets. Twelve of the 22 validation batches were from the manufacturers not used in the calibration set.

2.3. Recoding of NIR spectra

Diffuse reflectance spectra were recorded using a 1.5 m fiberoptic probe from one surface randomly of each tablet at 8 cm^{-1} resolution with 64 co-added scans over the spectral range 4000–12,000 cm^{-1}. Six tablets were randomly selected per batch. NIR spectra from six tablets were recorded and averaged. The average spectrum from the six tablets per batch was used for the model construction or the analysis. All the samples were recorded from three spectrometers by three operators over several days using the method described above. The three spectrometers MPA(1), EQUINOX55 and MATRIX-F(2I011104) were used to collect NIR spectra of roxithromycin tablets, MATRIX-F (2I008003), EQUINOX55 and MATRIX-F (2I011104) were used to collect NIR spectra of erythromycin ethylsuccinate tablets.

2.4. Development of quantitative models

The initial calibration models were developed using the PLS-1 algorithm available in the Quant 2 package of Bruker OPUS software, version 4.2. The number of PLS factors in the models was determined based on *F*-test on PRESS (sum of square error of prediction) values in the cross validation. The overall predictability of each calibration model was expressed in terms of RMSECV and RMSEP.

3. Results and discussion

3.1. Selection of calibration samples and validation samples

The selection of representative samples is very important to the PLS modeling. It was illustrated in the initial model building stage. When using a model of roxithromycin with an even API content distribution but random manufacturer distribution to predict seven batches of unknown samples from three new manufacturers, the mean accuracy (Eq. 1) was found to be 21% and three batches were considered as outliers. It was obvious that the initial model did not cover all the sample matrix of the validation samples. Although the API in the roxithromycin tablets is the same, the types and amounts of excipients in their formulations can be very different in different manufacturers' products. The variations in the formulations could impose quite a challenge to development of the universal model. However, the variations of the formulation for a given product are limited. If careful considerations are made in selection of a representative calibration sample set that will cover these variations, the universal model should be achievable.

A cluster analysis was then performed to learn more about the sample variance using Ward's Algorithm implemented in the Cluster Analysis package of Bruker OPUS software. Based on the hierarchical cluster analysis results and the API contents of the sample, the distribution of manufacturers was then optimized. The cluster analysis was carried out with the average spectra of each batch of roxithromycin. Once we select one batch as a calibration sample, we will use the average spectra of this batch from each instrument for PLS model construction. As shown in Fig. 1, the representative samples from each sub-cluster and each content range were chosen to be the calibration set and the validation set. When the final model constructed using this sample selection method was applied to predict the same seven batches of the unknown samples mentioned above, the mean accuracy was reduced to 3% without any sample flagged as an outlier. Therefore, the selection of representative calibration samples and validation samples is really important to the PLS model building. In this study, the samples used to develop a model came from different manufacturers. Since different manufacturers may use different or same formulations of excipients to produce the same product, the corresponding NIR spectra of the same pharmaceutical product can be either very similar or quite different. The average spectra of roxithromycin tablets from different manufacturers were shown in Fig. 2. Therefore the distribution of manufacturers was taken into careful consideration as well in this study, and the predictability and the robustness of the universal model were also improved

$$\text{Mean accuracy}\,(\%) = \frac{\sum_{i=1}^{n} \frac{|\text{NIR value} - \text{REFERENCE value}|}{\text{REFERENCE value}}}{n} \times 100 \quad (1)$$

n is the number of samples used for calculating the mean accuracy.

3.2. Calibration models

Based on the initial calibration models obtained from the automatic optimization routine implemented in OPUS Quant 2 software, some further adjustments about the spectral range were made to get more reliable models for the prediction of the drugs being analyzed. The detailed parameters for final models of roxithromycin and erythromycin ethylsuccinate were shown in Table 1. The external validation results of the final models for roxithromycin and erythromycin ethylsuccinate were plotted in Figs. 3 and 4. The values of R^2 and RMSECV in the final model for the roxithromycin were 98.84% and 1.84% (mg/mg), respectively. The number of PLS factors used in the final model for roxithromycin was 11. The values of R^2 and RMSECV in the final model for erythromycin ethylsuccinate were 95.13% and 2.31% (U/μg), respectively. The number of PLS factors used in the final model of erythromycin ethylsuccinate was 6. For the external validation results, the values of R^2 and RMSEP in the final model for the roxithromycin were 99.00% and 1.45% (mg/mg), respectively. The values of R^2 and RMSEP in the final model for erythromycin ethylsuccinate were 85.53% and 2.16% (U/μg), respectively.

3.3. Validation of the proposed method

The proposed methods were evaluated according to the ICH guidelines [24], which involved assessing the specificity, linearity, accuracy, precision and robustness of the method.

3.3.1. Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components, which may be expected to be present. In addition to the common practice of comparing the PLS loading factors with a spectrum of the pure API, the Mahalanobis distance was used to test the specificity of the models. Three times of the average Mahalanobis distance of the calibration spectra was used as a threshold to identify the outliers. When the roxithromycin model was applied to an unknown sample, and if the corresponding Mahalanobis distance of the unknown sample was bigger than the threshold of the model, the unknown sample was considered as an outlier. The specificity of erythromycin ethylsuccinate model was tested in the same way.

At first, the comparisons between the PLS loading factors with the spectrum of the API for roxithromycin and erythromycin ethylsuccinate were made, respectively. All were shown good correlation. The PLS loading 1 and the spectrum of

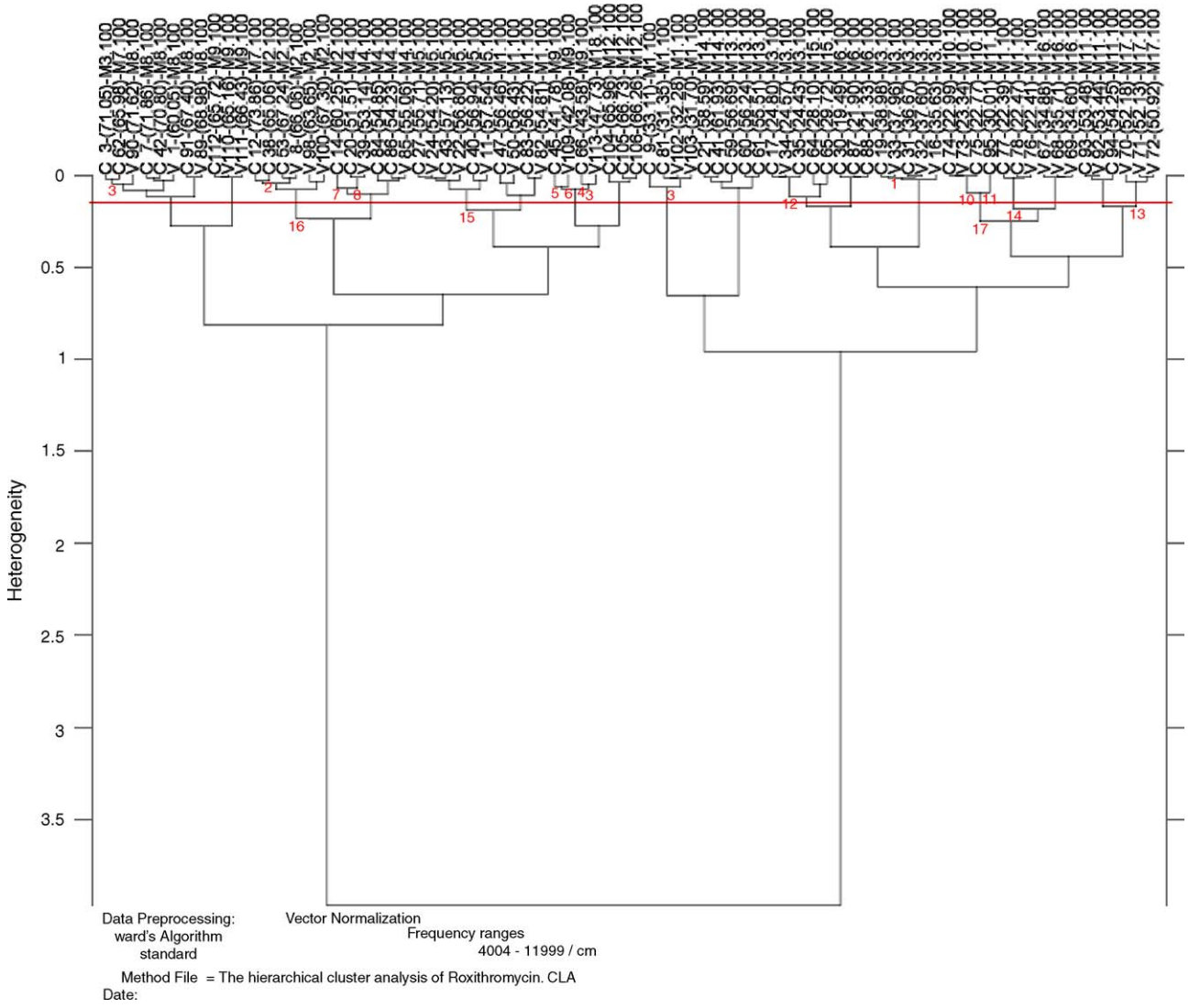

Fig. 1. The hierarchical cluster analysis result for roxithromycin. The file names were constructed as follow: the letters C, V and M represent calibration sample, validation sample and manufacturer, respectively. Each letter was followed by the number. The number in bracket is the HPLC content (%, mg/mg) of each sample. For example the file name V 90-(71.62)-M8 represents the spectrum of the validation sample no. 90 from manufacturer no. 8. Its API content measured by HPLC is 71.62%.

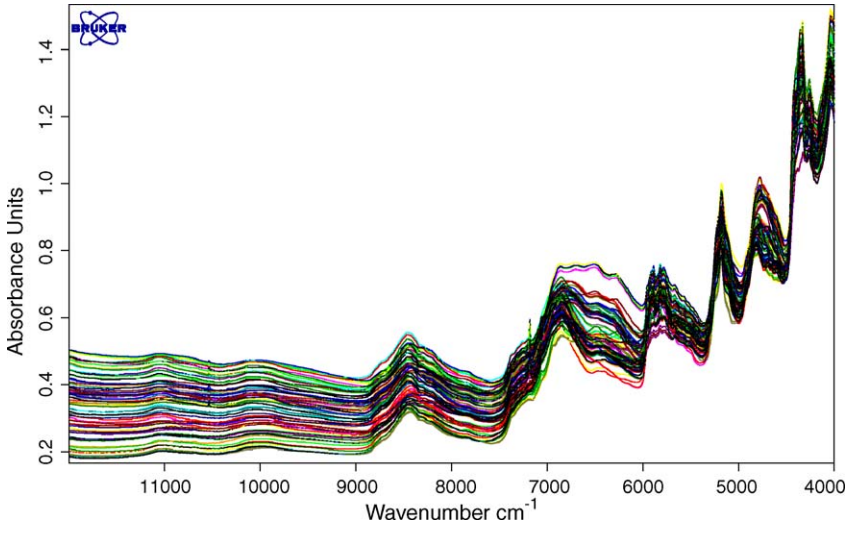

Fig. 2. The average spectra of roxithromycin tablets from different manufacturers.

Table 1
Characteristics of the final calibration models

Parameter	Roxithromycin		Erythromycin ethylsuccinate	
	Calibration	Validation	Calibration	Validation
Batches	46	32	44	22
Samples	276	192	264	132
Spectra	828 (46 × 6 × 3)	576 (32 × 6 × 3)	792 (44 × 6 × 3)	396 (22 × 6 × 3)
Concentration range (%)	19.5–73.9	22.4–71.6	28.1–70.9	43.3–65.7
Wavelength range (cm^{-1})	5581.2–6962.0 and 8057.4–8971.6		6248.4–5446.2	
Spectra pretreatment	1st derivative, vector normalization		1st derivative, vector normalization	
Rank	11		6	
R^2 (%)	98.84	99.00	95.13	85.53
RMSECV(P)	1.84	1.45	2.31	2.16

the API for roxithromycin were shown in Fig. 5. Then, a total of 25 batches of samples from different manufacturers were also selected for testing the specificity. They included five batches of roxithromycin tablets, five batches of erythromycin ethylsuccinate tablets, five batches of azithromycin tablets, five batches of erythromycin tablets and five batches of clarithromycin tablets. Azithromycin, erythromycin and clarithromycin tablets were selected as the challenge samples because the chemical structures of APIs of these three tablets are very similar to those of roxithromycin and erythromycin ethylsuccinate tablets. As shown in Table 2, all the identifications were correct. When the model for roxithromycin was applied to the above-mentioned five types of tablets, only corresponding Mahalanobis distances of roxithromycin tablets fell within the threshold of the model, 0.24. All of the corresponding Mahalanobis distances of the four other types of tablets, i.e. azithromycin, erythromycin, clarithromycin and erythromycin ethylsuccinate, exceeded the threshold of the model. The same results were observed in this test for the erythromycin ethylsuccinate model. When the model for erythromycin ethylsuccinate was applied to the five types

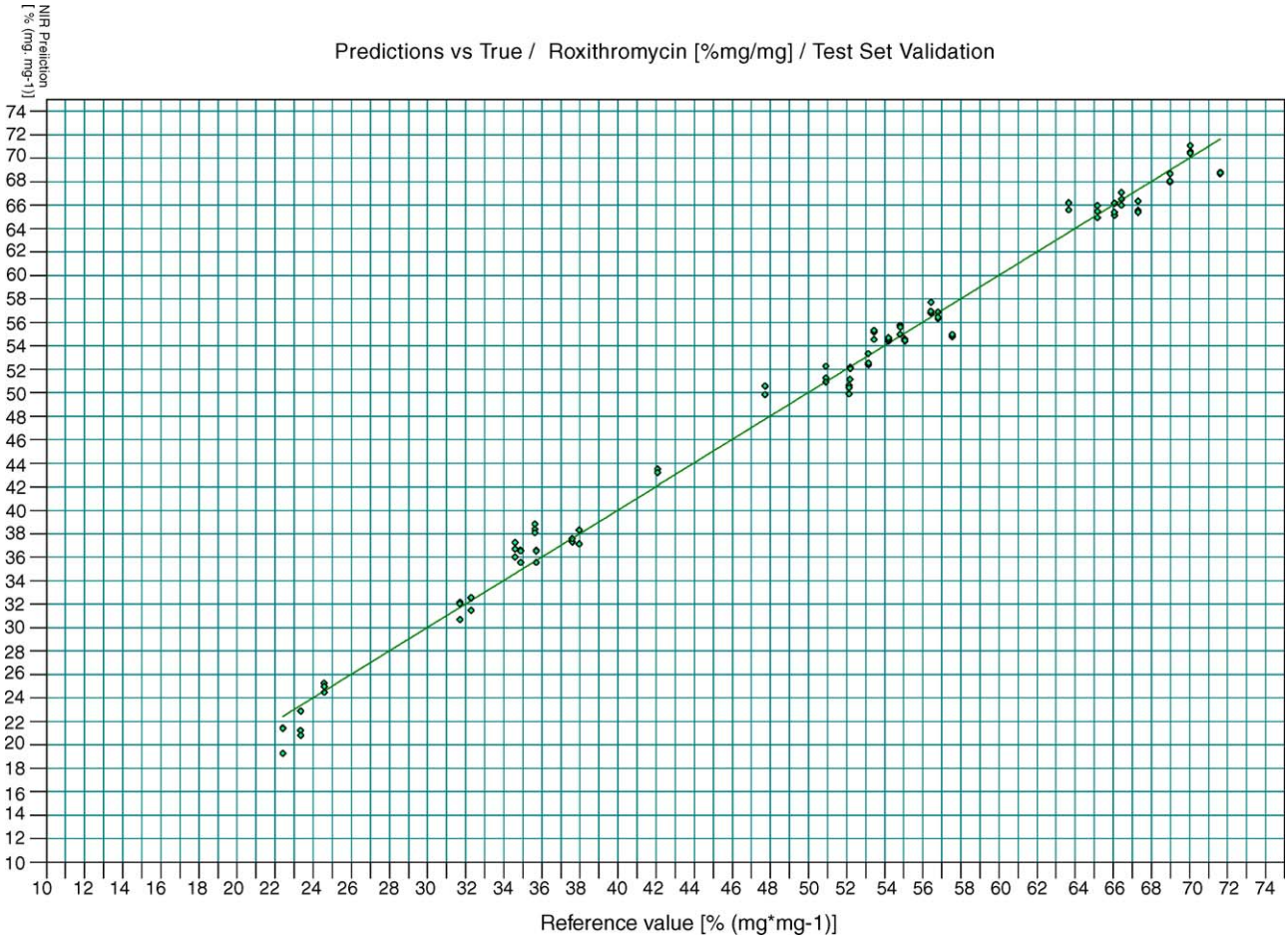

Fig. 3. The external validation results for roxithromycin tablets RMSEP = 1.45, R^2 = 99.00%, eleven PLS factors.

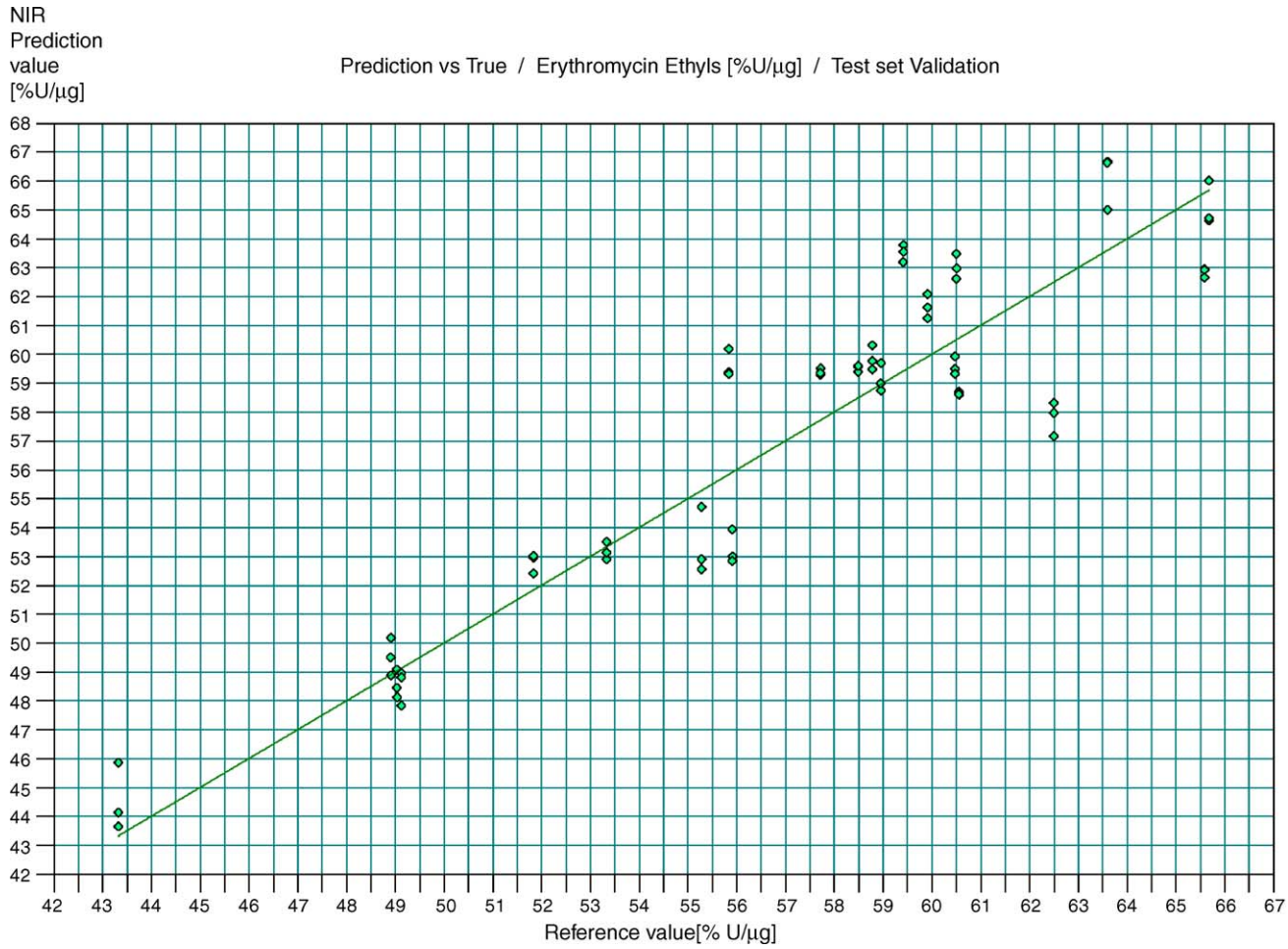

Fig. 4. The external validation results for erythromycin ethylsuccinate tablets RMSEP = 2.16, R^2 = 85.53%, six PLS factors.

of tablets, only corresponding Mahalanobis distances of erythromycin ethylsuccinate tablets fell within the threshold of the model, 0.14. All of the corresponding Mahalanobis distances of the four other types of tablets, i.e. azithromycin, erythromycin, clarithromycin and roxithromycin, exceeded the threshold of the model. It can be concluded that the universal models developed for roxithromycin and erythromycin ethylsuccinate showed the required specificity.

3.3.2. Linearity

Unlike a univariate calibration, the analytical signal can be directly plotted as a function of the analyte content; a multivari-

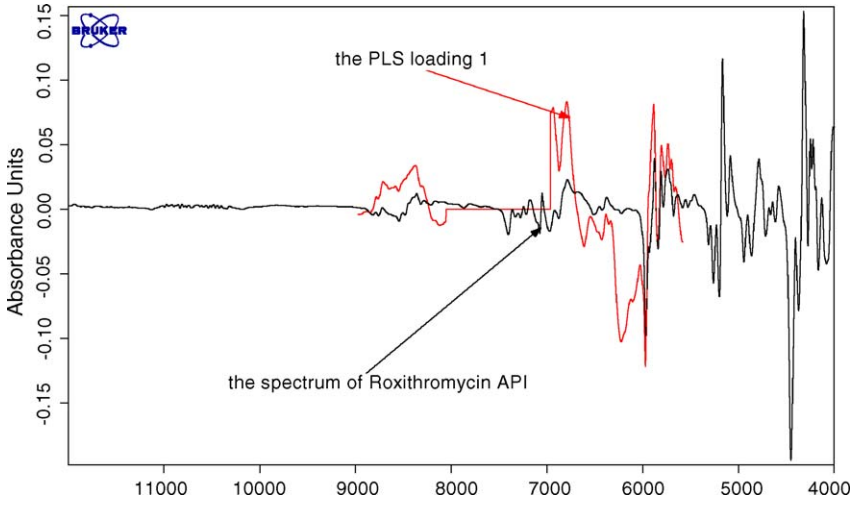

Fig. 5. The comparison between the PLS loading 1 with the spectrum of the API for roxithromycin (first derivative followed by vector normalization).

Table 2
Specificity

Preparation	Sample no.	Mahalanobis distance based on the model for roxithromycin threshold = 0.24	Outlier	Mahalanobis distance based on the model for erythromycin ethylsuccinate tablets threshold = 0.14	Outlier
Erythromycin tablets	111	6.9	Yes	11	Yes
	120	5.6	Yes	10	Yes
	126	5.9	Yes	11	Yes
	127	5.9	Yes	11	Yes
	129	4.1	Yes	15	Yes
Azithromycin tablets	10	1.4	Yes	7.4	Yes
	100	1.8	Yes	1.6	Yes
	102	1.9	Yes	6.7	Yes
	103	1.9	Yes	9.4	Yes
	104	1.9	Yes	1.8	Yes
Clarithromycin tablets	2	2.5	Yes	15	Yes
	3	1.4	Yes	14	Yes
	4	1.3	Yes	15	Yes
	5	1.6	Yes	13	Yes
	6	1.4	Yes	14	Yes
Roxithromycin tablets	11	0.023	No	2.1	Yes
	16	0.079	No	3.9	Yes
	18	0.041	No	1.5	Yes
	28	0.11	No	3.2	Yes
	44	0.11	No	3.2	Yes
Erythromycin ethylsuccinate tablets	42	6	Yes	0.0088	No
	55	6	Yes	0.013	No
	246	5.7	Yes	0.019	No
	292	5.9	Yes	0.036	No
	148	5.6	Yes	0.0097	No

ate calibration (e.g. PLSR) does not allow one to determine the linearity of the method in the same way. One way to establish the linearity of a NIR model is to exam the slope and intercept in a plot of NIR predicted values vs. reference values. This may be accomplished during the calibration and validation stage of the NIR method. Ideally the intercept, a, and slope, b, should be zero and one, respectively, if there was no relative systematic error or fixed systematic error in the reference method. The 95% confidence interval for the intercept and slope were calculated. The results are shown in Table 3 . As can be seen, all the confidence intervals included zero or one; therefore there was no evidence to suggest a non-zero intercept and a relative systematic error.

3.3.3. Accuracy

The accuracy of the proposed methods was assessed by comparing the NIR predicted results with those provided by the reference method for several validation batches. A validation set consisting of 32 batches from 12 different manufacturers with an API content range from 22.4% to 71.6% was used to test the model for roxithromycin while a validation set consisting of 22 batches from 18 manufacturers with an API content range from 43.3% to 65.7% was used to test the model for erythromycin ethylsuccinate. The NIR predictions of the two models were shown in Tables 4 and 5. A paired *t*-test was also performed to check whether the NIR values and the reference value were significantly different. The results of *t*-test were shown in Table 3. As shown in the table, the *p* values for both model were bigger than 0.05, so the NIR predicted values and reference results were not significantly different using these two models.

3.3.4. Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. In this study, we considered the precision at only two levels: repeatability and intermediate precision.

Repeatability was determined by making six measurements of the same sample by the same operator on the same day. A single batch of roxithromycin tablets and erythromycin ethylsuccinate tablets was measured and quantified. As shown in Table 3, the reference values were within the confidence intervals for both determinations. Intermediate precision expresses within laboratory variations. In this study a single batch of each variety was analyzed from measurements made by two different analysts on 3 days. The variances due to operator and day were determined jointly by analysis of variance (ANOVA). Based on the results of Table 3, the factors of day and operator had no significant influence on either model.

3.3.5. Robustness

The robustness of an analytical method is a measure of its capacity to remain unaffected by small, but deliberate varia-

Table 3
Validation of the calibration models for the determination of roxithromycin and erythromycin ethylsuccinate content

Aspects	Procedure	Roxithromycin determination	Erythromycin ethylsuccinate determination
Validation results			
Linearity	NIR value = $a + b \times$ reference value ($a = 0$; $b = 1$)	Forty-six calibration samples: Conc. range 19.5–73.9%; $a = 0.3045 \pm 0.3353$; $b = 0.9924 \pm 0.01830$; $R^2 = 0.9884$ Thirty-two validation samples: conc. range 22.4–71.6%; $a = 0.8701 \pm 1.05228$; $b = 0.9853 \pm 0.02049$; $R^2 = 0.99$	Forty-four calibration samples: conc. range 28.1–70.9%; $a = 2.0732 \pm 2.1026$; $b = 0.9627 \pm 0.03830$; $R^2 = 0.9515$ Twenty-two validation samples: conc. range 43.3–65.7%; $a = 4.1105 \pm 5.4410$; $b = 0.9334 \pm 0.09494$; $R^2 = 0.8659$
Accuracy	Paired t-test of NIR values and reference values of prediction samples	Thirty-two prediction samples: avg. diff. = 0.05; S.D. = 1.41; $t = 0.3672$	Twenty-two prediction samples: avg. diff. = −0.33; S.D. = 2.12; $t = 1.2539$
		$p = 0.7143$ ($\alpha = 0.05$)	$p = 0.2144$ ($\alpha = 0.05$)
Repeatability	Only the one sample analyzed six times by one operator confidence interval avg. $\pm t \times S/\sqrt{n}$	Reference content 56.0%, mg/mg	Reference content 50.2%, U/μg
		Avg. = 55.9; S.D.=0.07; conf. int. = 55.9 ± 0.1	Avg. = 50.2; S.D. = 0.11; conf. int. = 50.2 ± 0.1
Intermediate precision	Only the one sample analyzed in 3 days by two different operators confidence interval and ANOVA	Reference content 56.0%, mg/mg	Reference content 50.2%, U/μg
		Avg. = 55.0; S.D. = 0.95; conf. int. = 55.0 ± 1.0; no significant effect of day and operator	Avg. = 50.1; S.D. = 0.38; conf. int. = 50.1 ± 0.4; no significant effect of day and operator
Robustness	Paired t-test of NIR values and reference values of validation samples that were not from the manufacturers in the calibration sets	Seven validation samples; avg. diff. = −0.695; S.D. = 1.50; $t = -2.1302$; $p = 0.04580$ ($\alpha = 0.05$); significant effect between prediction and true	Twelve validation samples; avg. diff. = 0.092; S.D. = 1.68; $t = 0.3275$; $p = 0.7452$ ($\alpha = 0.05$); no significant effect between prediction and true

Table 4
Accuracy of roxithromycin model: comparing the NIR predictions with the reference values of the validation set

Sample no.	True (HPLC %, mg/mg)	Prediction (NIR %, mg/mg)		
		MATRIX-F (2I011104)	MPA	EQUINOX55
67	34.9	36.6	36.6	35.6
68	35.7	36.6	36.5	35.6
69	34.6	36.7	37.3	36.0
70	52.2	52.2	51.2	52.1
71	52.1	49.9	50.6	50.4
72	50.9	52.3	51.3	50.9
73	23.3	21.2	22.9	20.8
100	67.3	65.6	65.4	66.3
102	32.3	32.5	32.6	31.5
103	31.7	32.2	32.0	30.7
109	42.1	43.5	43.5	43.2
110	65.2	64.9	66.0	65.5
111	66.4	66.0	67.1	66.5
113	47.7	50.6	50.6	49.8
11	57.5	54.9	55.0	54.8
16	35.6	38.9	38.1	38.4
1	70.1	71.1	70.4	70.6
22	56.8	56.4	56.4	56.9
24	54.2	54.5	54.7	54.4
32	37.6	37.3	37.6	37.3
33	38.0	37.2	38.3	38.3
34	24.6	25.0	24.5	25.3
39	53.1	53.3	52.5	52.4
50	56.4	56.8	56.9	57.7
76	22.4	19.3	21.5	21.4

Table 4 (*Continued*)

Sample no.	True (HPLC %, mg/mg)	Prediction (NIR %, mg/mg)		
		MATRIX-F (2I011104)	MPA	EQUINOX55
82	54.8	55.0	55.8	55.6
85	55.1	54.4	54.6	54.5
89	69.0	68.0	68.7	68.1
8	66.1	65.4	66.2	65.1
90	71.6	68.7	68.8	68.8
92	53.4	55.2	55.3	54.5
98	63.7	66.2	66.2	65.6
RMSEP		1.61	1.40	1.31
Mean accuracy (%)			2.6	

Table 5
Accuracy of erythromycin ethylsuccinate model: comparing the NIR predictions with the reference values of the validation set

Sample no.	True (biological assay %, U/μg)	Prediction (NIR %, U/μg)		
		MATRIX-F (2I011104)	MATRIX-F (2I008003)	EQUINOX55
133	59.4	64.7	64.3	64.8
148	58.5	58.9	59.0	59.5
149	59.0	59.4	59.4	60.0
156	60.5	63.8	63.7	64.4
187	65.6	64.8	64.5	65.1
192	43.3	45.8	43.9	45.1
196	58.8	59.7	59.3	59.9
246	53.3	53.5	53.6	53.2
251	55.8	58.9	58.4	58.7
252	51.8	51.8	51.3	51.8
291	60.5	59.7	59.1	59.7
292	62.5	58.9	59.5	59.6
29	49.0	50.4	50.1	49.2
308	65.7	64.0	63.1	63.2
42	59.9	61.5	60.9	61.5
44	63.6	63.5	61.9	63.7
45	48.9	51.2	50.8	51.0
55	57.7	59.6	59.6	60.2
67	60.6	60.1	60.1	60.3
76	55.9	53.3	52.5	51.8
87	55.3	53.8	52.6	52.7
99	49.1	48.5	49.1	47.8
RMSEP		2.09	2.05	2.27
Mean accuracy (%)			2.9	

Table 6
Robustness of roxithromycin model

Sample no.	True (HPLC %, mg/mg)	Prediction (NIR %, mg/mg)		
		EQUINOX55	MATRIX-F (2I011104)	MPA
113	47.7	49.8	50.6	50.6
67	34.9	35.6	36.6	36.6
68	35.7	35.6	36.6	36.5
69	34.6	36.0	36.7	37.3
70	52.2	52.1	52.2	51.2
71	52.1	50.4	49.9	50.6
72	50.9	50.9	52.3	51.3
RMSEP		1.18	1.82	1.80
Mean accuracy (%)			3.2	

Table 7
Robustness of erythromycin ethylsuccinate model

Sample no.	True (biological assay %, U/μg)	Prediction (NIR %, U/μg)		
		EQUINOX55	MATRIX-F (2I011104)	MATRIX-F (2I008003)
148	58.5	59.4	58.9	59.0
246	53.3	53.2	53.5	53.6
251	55.8	58.7	58.9	58.4
252	51.8	51.8	51.8	51.3
291	60.5	59.7	59.7	59.1
292	62.5	59.6	58.9	59.5
308	65.7	63.2	64.0	63.1
42	59.9	61.4	61.5	60.9
44	63.6	63.7	63.5	61.9
55	57.7	60.2	59.6	59.6
67	60.6	60.3	60.1	60.1
99	49.1	47.8	48.5	59.0
RMSEP		1.70	1.66	1.65
Mean accuracy (%)			2.1	

tions in method parameters and provides an indication of its reliability during normal usage. In this study, variations in NIR spectra of the same product mainly come from the difference of the manufacturers. Therefore the predictive ability of the model for a sample that was not from the manufacturers included in the calibration set must be assessed. Seven batches from three new manufacturers were studied for roxithromycin while 12 batches from eight new manufacturers were examined for erythromycin ethylsuccinate. No outlier based on Mahalanobis distance thresholds was found in these samples. Comparison between the NIR predictions and the corresponding reference values for both models were shown in Tables 6 and 7. A paired *t*-test was performed to check whether the NIR value and the corresponding reference value were significantly different. As shown in Table 3, the *p* value of erythromycin ethylsuccinate was 0.7452 and bigger than 0.05, so the NIR and reference results were not significantly different. The *p* value of roxithromycin was 0.046 and slightly smaller than 0.05, based on the statistics, it can be concluded that the NIR and reference results were significantly different. The purpose to develop these quantitative models is, however, not used for accurate analysis but for quick prescreening of sub-standard drugs at the scene. As shown in Table 6, the RMSEP for roxithromycin was lower than that from cross validation, and the mean accuracy is only 3.2%, which was not significantly different from that value of the external validation set. As a result, this value was acceptable for prescreening of drugs at the scene.

3.4. Transferability

Transferability is important because many developed methods need to be implemented in multiple instruments at different

Table 8
Transferability of roxithromycin model in eight spectrometers

Sample no.	Prediction (NIR %, mg/mg)							
	Recorded spectra from instruments used for model construction			Recorded spectra from instruments not used for model construction				
	MPA(1)	EQUINOX55	MATRIX-F 2I011104	MATRIX-F 2I008003	MPA(2)	MATRIX-F 2I002403	MATRIX-F 2I007903	MATRIX-F 2I015904
1	55.0	54.8	54.9	54.3	54.4	54.0	54.4	54.5
2	38.1	38.4	38.9	36.5	37.3	37.3	37.8	38.0
3	64.5	64.7	64.5	63.7	64.4	64.0	64.3	64.2
4	70.4	70.6	71.1	70.3	70.2	69.0	70.5	68.9
5	56.4	56.9	56.3	55.9	56.0	55.8	55.9	56.2
6	54.7	54.4	54.5	53.8	54.1	54.1	54.4	53.5
7	33.9	33.5	33.2	31.8	31.0	31.5	31.5	33.7
8	34.3	33.6	34.4	30.5	31.5	31.2	32.6	36.0
9	37.6	37.3	37.3	36.1	37.1	36.7	37.7	37.7
10	24.5	25.3	25.0	23.3	24.0	23.8	24.3	27.4
11	33.1	33.6	33.7	31.8	31.7	31.6	32.3	34.4
12	37.7	37.5	37.1	36.1	37.2	36.5	36.6	37.1
13	37.8	37.6	37.7	36.0	36.9	36.4	35.5	37.8
RMSEP	1.62	1.59	1.67	1.38	1.36	1.45	1.34	2.05

Table 9
Transferability of erythromycin ethylsuccinate model in seven spectrometers

Sample no.	Prediction (NIR %, U/μg)						
	Recorded spectra from instruments used for model construction			Recorded spectra from instruments not used for model construction			
	MATRIX-F 2I008003	EQUINOX55	MATRIX-F 2I011104	MPA(1)	MATRIX-F 2I002403	MATRIX-F 2I007903	MATRIX-F 2I015904
1	59.0	59.4	58.9	59.4	59.1	58.5	59.5
2	53.6	53.2	53.5	54.3	53.3	53.3	53.9
3	51.3	51.8	51.8	52.4	51.7	51.5	52.5
4	59.1	59.7	59.7	59.9	59.3	59.8	59.8
5	59.5	59.6	58.9	59.3	59.4	59.1	59.8
6	63.1	63.2	64.0	63.5	63.4	63.1	63.9
7	60.9	61.4	61.5	61.7	60.9	61.8	61.8
8	61.9	63.7	63.5	63.0	63.3	62.3	62.7
9	59.6	60.2	59.6	60.1	60.0	60.0	60.1
10	60.1	60.3	60.1	60.5	60.1	59.9	60.2
11	52.5	51.8	53.3	53.9	52.7	52.0	53.2
12	52.6	52.7	53.8	55.1	54.1	52.9	53.6
13	49.1	47.8	48.5	49.5	47.9	48.5	49.4
RMSEP	1.86	1.95	1.57	1.53	1.67	1.99	1.60

locations. Almost all of the previous studies investigated only spectrometers (and software) of the same brand and the same instrument model. In this study, in order to construct a robust calibration model, the spectra of the calibration sets and the validation sets were recorded from three different spectrometers of the same brand but different instrument model. Thirteen batches of roxithromycin not used in the calibration set were measured using eight spectrometers, of which three instruments were used in the model building and five instruments were not used in the model building. These samples were measured by one analyst during several days. The experiment for testing 13 batches of erythromycin ethylsuccinate was the same as roxithromycin. The NIR prediction results and the corresponding RMSEP values for these samples were shown in Tables 8 and 9. The prediction variances between spectrometers were determined by analysis of variance (ANOVA). The prediction from all spectrometers had no significant difference, and the values of RMSEP of all spectrometers were similar, too.

From the results of the transferability, it is found that the transfer of calibration models between different FT-NIR spectrometers with the same band can be done easily and accurately without any mathematical treatments. This experiment implied that a model is possible to be transferred between tens even up to several hundreds of instruments if it is required. Therefore, it is feasible to construct a universal model for prescreening drugs from different manufacturers and to be used by hundreds of NIR systems over the whole country.

4. Conclusion

The samples used for developing models came from almost all the manufacturers of these two products in China, so these models are based on good representative sample sets for the real application in China. From the results mentioned above, we can conclude that these models also have good specificity, linearity, accuracy, precision and robustness. Furthermore, they can be directly used in different FT-NIR spectrometers with the same brand without any special mathematical treatments. The drugs used in this research are only two of the examples. It is possible to build more universal quantitative models for quick screening analysis of substandard pharmaceutical products produced by different manufacturers as well as for other different purposes.

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