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Note

Quantitation of active pharmaceutical ingredients and excipients in powder blends using designed multivariate calibration models by near-infrared spectroscopy

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Abstract

This research note demonstrates the simultaneous quantitation of a pharmaceutical active ingredient and three excipients in a simulated powder blend containing acetaminophen, Prosolv® and Crospovidone. An experimental design approach was used in generating a 5-level (%, w/w) calibration sample set that included 125 samples. The samples were prepared by weighing suitable amount of powders into separate 20-mL scintillation vials and were mixed manually. Partial least squares (PLS) regression was used in calibration model development. The models generated accurate results for quantitation of Crospovidone (at 5%, w/w) and magnesium stearate (at 0.5%, w/w). Further testing of the models demonstrated that the 2-level models were as effective as the 5-level ones, which reduced the calibration sample number to 50. The models had a small bias for quantitation of acetaminophen (at 30%, w/w) and Prosolv® (at 64.5%, w/w) in the blend. The implication of the bias is discussed.

Keywords: Near-infrared spectroscopy; Multivariate calibration; Pharmaceutical blend; Magnesium stearate

Near-infrared spectroscopy (NIR) is a powerful tool in non-invasive qualitative and quantitative analyses for various sources of samples (Hildrum et al., 1992). In the last few years, NIR has received increasingly wider applications in pharmaceutical analysis. For example, NIR has been used for identification of bulk drug substance, excipients, and drug products (Corti et al., 1991; Plugge and Van der Vlies, 1993) for determination of water in various ingredients (Maggard et al., 2002; Mattes et al., 2004) for analysis of tablets for a variety of properties (Yeboah and Wang, 1984; Kirsch and Drennen, 1995) and for manufacturing process control (Callis et al., 1987).

In manufacturing of pharmaceuticals, blending is one of the important unit operations that can benefit from the use of NIR. Currently, the regulatory guide-

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Table 1
Blend composition

Component	% (w/w)	Weight (mg)/dose unit
Acetaminophen	30	210
Prosolv®	64.5	451.5
Crospovidone	5	35
Magnesium stearate	0.5	3.5
Total	100	700

lines only require validation of the blending operation for active pharmaceutical ingredient(s) (APIs). There are no requirements for excipients even though they play important roles in delivery of the API(s). The shortcomings regarding the current industry wide practices in blend uniformity and the future new direction guided by the new PAT guideline have been discussed in the literature (El-Hagrasy et al., 2001; Forcinio, 2003).

In order to successfully apply NIR in blend uniformity studies, one area of research is the development and validation of calibration models for quantitative analysis (Filho et al., 2004). In this research note, we introduce an approach of designing a multi-level calibration sample set for quantitative analysis of API and three excipients in a simulated pharmaceutical blend. The purpose of the study is to demonstrate that it is feasible to quantify multiple components (or all components) simultaneously and accurately in powder blends by NIR. This can be achieved by including multi-level (%, w/w) components into the calibration set, and then build multivariate models for quantitation using partial least squares (PLS) regression. We will demonstrate that the precision and accuracy of the calibration models can be adjusted/optimized by adjusting the number of levels in the calibration sample set.

To prove the concept of this approach, a simulated blend formulation was used in this study, which included acetaminophen as the API and Prosolv® (Registered mark of JRS Pharma, Patterson, NY, USA), Crospovidone, and magnesium stearate as excipients (Table 1). The simulated strength is 300 mg per unit dose and the total weight of the unit dose is 700 mg.

The number of samples for the proposed calibration sample set is calculated according to the following equation:

$$N = L^{c-1}$$

Table 2
Designed calibration sample set (all numbers are in %, w/w)

Acetaminophen	Prosolv®	Crospovidone	Mg stearate
10	84.5	3	0.3, 0.4, 0.5, 0.6, 0.7
		4	0.3, 0.4, 0.5, 0.6, 0.7
		5	0.3, 0.4, 0.5, 0.6, 0.7
		6	0.3, 0.4, 0.5, 0.6, 0.7
		7	0.3, 0.4, 0.5, 0.6, 0.7
20	74.5	Same as above	Same as above
30	64.5	Same as above	Same as above
40	54.5	Same as above	Same as above
50	44.5	Same as above	Same as above

where N is the number of samples, L is the number of levels for each component (assuming the same number of levels for each component) and c is the number of components. In this case ($L = 5$ and $c = 4$), the number of calibration samples is 125 as outlined in Table 2. In addition, nine extra samples were prepared for the validation of the calibration models. The samples were prepared by weighing suitable amount of powders into separate 20 mL scintillation vials using an analytical balance with an accuracy of ±0.01 mg. The total powder weight for each sample is approximately 700 mg. Each sample was then mixed manually using a spatula. The uniformity of the samples was visually inspected and later on confirmed in the validation. The following NIR conditions were used for analysis:

- Instrument: FOSS XDS near-infrared rapid content analyzer.
- Sampling module: rapid module without spot size.
- Detector: reflectance.
- Wavelength range: 1100–2400 nm.
- Data selection: Mahalanobis distance in principal component space.
- Math treatment: second derivative.
- Regression: partial least squares
- Number of factors: up to 25.

The Vision software provided by the instrument vendor was used in development of the calibration models. The sample selection step using Mahalanobis distance in principal component space showed no outliers in the calibration sample set. Therefore, all 125 samples were used in calibration model development. Fig. 1 shows the plot of the logarithm of the predicted residual error sum of squares (PRESS) versus number of factors for magnesium stearate. The plot indicates that

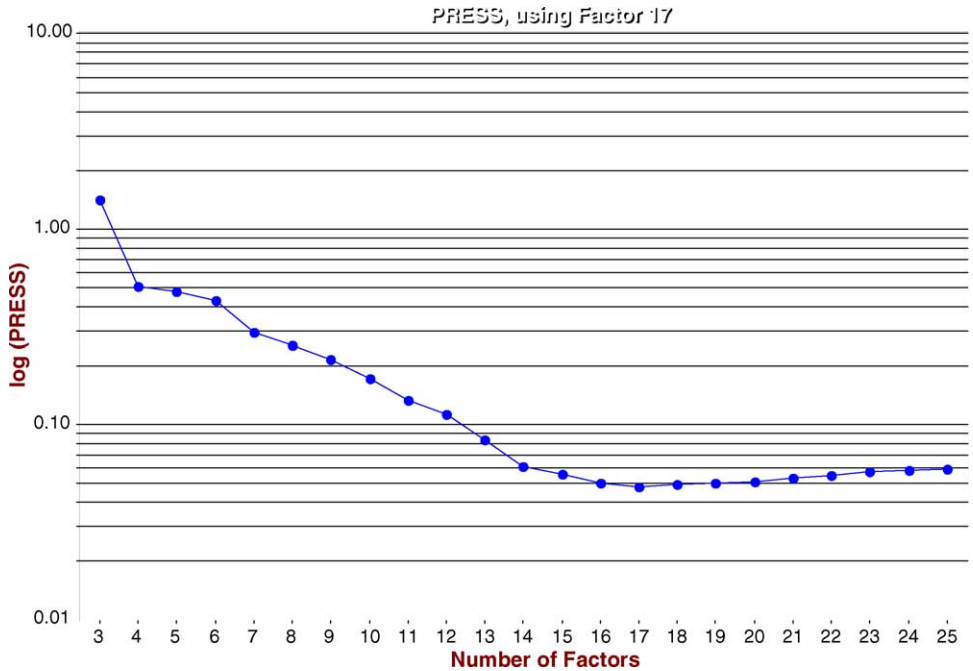

Fig. 1. PRESS vs. factor plot for the 5-level calibration model for magnesium stearate.

Table 3
NIR predicted results for magnesium stearate using the 17-factor/5-level model

Sample ID	% (w/w)	NIR predicted (%)[a]	%R.S.D.[b]	Residual
1	0.21	0.21	3.15	0.00
2	0.67	0.66	3.79	−0.01
3	0.96	0.96	4.92	0.00
4	0.21	0.22	1.31	0.01
5	0.55	0.55	2.81	0.00
6	0.98	0.96	2.31	−0.02
7	0.52	0.50	2.27	−0.02
8	0.79	0.80	1.58	0.01
9	0.20	0.20	6.02	0.00
Mean	0.566	0.562		
S.D.	0.312	0.308		
95% CI for mean difference	(−0.00526, 0.0119)			
t	0.89			
p	0.397			

[a] Mean of three measurements.
[b] Percent relative standard deviation of the three NIR measurements.

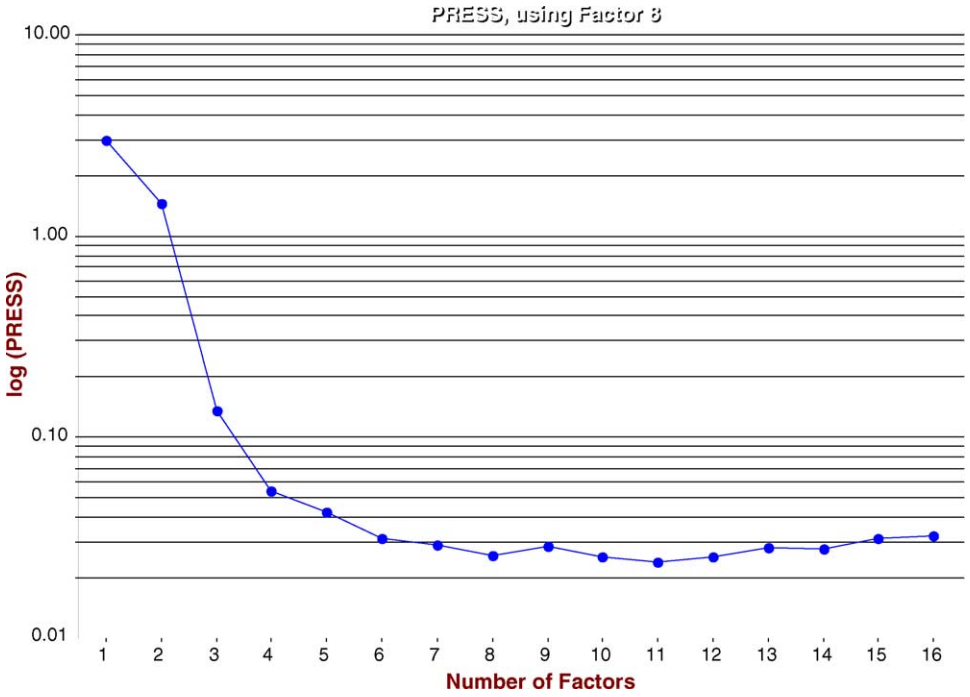

Fig. 2. PRESS vs. factor plot for the 2-level calibration model for magnesium stearate.

the optimum number of variables (factors) is 17 for the calibration model for magnesium stearate. The model was validated by the leave-one-out cross-validation approach. Further validation was performed using nine additional validation samples. Each validation sample was analyzed three times to obtain a %R.S.D. for the measurements. The samples were re-mixed manually after each measurement. The results in Table 3 verify

Table 4
NIR predicted results for magnesium stearate using the 8-factor/2-level model

Sample ID	% (w/w)	NIR predicted (%)[a]	%R.S.D.[a]	Residual
1	0.21	0.18	11.60	−0.03
2	0.67	0.66	6.41	−0.01
3	0.96	0.98	1.51	0.02
4	0.21	0.17	6.17	−0.04
5	0.55	0.56	0.71	0.01
6	0.98	1.00	2.10	0.02
7	0.52	0.49	1.17	−0.03
8	0.79	0.75	1.44	−0.04
9	0.20	0.20	9.61	0.00
Mean	0.566	0.554		
S.D.	0.312	0.325		
95% CI for mean difference	(−0.00789, 0.0301)			
t	1.35			
p	0.214			

[a] See Table 3.

Table 5
NIR predicted results for acetaminophen using the 5- and 2-level models

Sample ID	% (w/w)	NIR[a] 2-level	%R.S.D.[a] 2-level	NIR[a] 5-level	%R.S.D.[a] 5-level	Residual 2-level	Residual 5-level
1	22.2	23.0	1.43	22.2	3.45	0.7	0.0
2	18.7	20.2	2.42	19.6	1.96	1.5	0.9
3	20.4	21.5	1.78	21.4	1.83	1.1	1.2
4	29.2	30.3	0.58	30.6	0.27	1.1	1.4
5	29.2	29.8	1.49	30.4	1.04	0.6	1.2
6	26.4	26.8	1.78	27.5	2.13	0.4	1.1
7	39.7	40.4	0.50	39.9	1.04	0.7	0.2
8	41.9	43.5	1.74	43.1	0.84	1.6	1.2
9	41.1	43.0	1.25	42.8	0.54	1.9	1.7
Mean	29.9	30.9					
S.D.	9.04	9.21					
95% CI for mean difference	(−1.46651, −0.69004)						
t	−6.41						
p	0.000						

[a] See Table 3.

the accuracy and precision of the calibration model. A paired *t*-test at the 95% confidence level did not indicate any differences in the magnesium stearate % (w/w) results by weighing and by NIR.

The calibration model for magnesium stearate was further tested by reducing the number of calibration samples from five levels/125 samples to two levels/50 samples. The samples with 20 and 40% (w/w) acetaminophen were used. Fig. 2 shows the log(PRESS) versus number of factors for the new model. The plot indicates that the optimum number of factors is reduced to 8. Validation results using the same nine samples and the same procedure show that the model remains effective with some acceptable increase in %R.S.D. and residual for the predicted results (Table 4). Again, a paired *t*-test at the 95% confidence level did not indicate any differences in results obtained by the two methods. It should be pointed out that the 5-level model might be necessary when lower limit of quantitation (e.g. 0.2%, w/w) is needed.

The similar calibration models were also developed for the other components. The log(PRESS) versus factor plots for the 5- and 2-level model for acetaminophen indicated an optimum factor number of 10 and 7, respectively. The additional validation results using the same nine samples are presented in Table 5. It should be pointed out that the % (w/w) acetaminophen amounts in the validation samples are within the calibrated range. Again the results show that the 2-level model is as good as the 5-level model with regard to precision and accuracy. However, the paired *t*-test indicates that the acetaminophen results obtained by the NIR methods (both the 5- and 2-level models) are statistically different from those obtained by weighing. The residuals in Table 5 indicate a positive bias. Interestingly, the NIR (5-level model) predicted results for Prosolv® are also statistically different from the weighing results (Table 6). The residuals for Prosolv® show a negative bias. The magnitude of the bias may not necessarily prevent the use of these models. On the other hand, the observation of the bias may have important implications. This demonstrates that the variation of one major component may affect the accuracy of the NIR calibration model for the other. The current calibration models reveal the presence of the bias but cannot completely correct for them. Therefore, further improvement is needed for these models.

On the other hand, the calibration models are accurate for the minor components. Both the Crospovidone and magnesium stearate models show no bias (Tables 3, 4 and 6). This may be because the Crospovidone and magnesium stearate models can easily detect the variations of acetaminophen and Prosolv®, both present in large quantities. On the contrary, the acetaminophen and Prosolv® model may not be able to detect the variation of magnesium stearate at 0.5%. This

Table 6
NIR predicted results for Prosolv® and Crospovidone using the 5-level models

Sample ID	Prosolv® % (w/w)	Prosolv® NIR	Residual	CrosPVD % (w/w)	CrosPVD NIR	Residual
1	73.9	73.1	−0.8	3.72	3.76	0.04
2	75.1	73.7	−1.4	5.51	5.30	−0.21
3	72.1	71.5	−0.6	6.53	6.41	−0.12
4	66.1	65.9	−0.2	4.49	4.38	−0.11
5	65.5	65.0	−0.5	4.76	4.88	0.12
6	66.6	66.4	−0.2	5.97	6.02	0.05
7	55.6	55.2	−0.4	4.15	4.20	0.05
8	53.3	52.7	−0.6	4.02	3.91	−0.11
9	52.5	51.5	−1.0	6.23	6.10	−0.13
Mean	64.5	63.9		5.04	5.00	
S.D.	8.77	8.69		0.347	0.335	
95% CI for mean difference						
	(0.336, 0.931)			(−0.0397, 0.1330)		
t	4.91			1.25		
p	0.001			0.248		

Table 7
Coefficient of determination and standard error of calibration for NIR models

Constituent	Model	Optimum factor number	R^2	SEC
Acetaminophen	Five levels/125 samples	10	0.9991	0.42
Acetaminophen	Two levels/50 samples	7	0.9982	0.46
Prosolv®	Five levels/125 samples	13	0.9994	0.37
Crospovidone	Five levels/125 samples	14	0.9963	0.092
Mg stearate	Five levels/125 samples	17	0.9983	0.011
Mg stearate	Two levels/50 samples	8	0.9966	0.016

is supported by the fact that the 5-level acetaminophen model only has 10 usable variables whereas the 5-level magnesium stearate model has 17. In addition, the regression coefficient (R^2) and standard error of calibration (SEC) data for all the calibration models discussed in this article are presented in Table 7.

In conclusion, NIR is suitable for quantitative analysis of API(s) and excipients in pharmaceutical powder blends. Calibration samples can be prepared by weighing and mixing the appropriate powders in small vials in the laboratory. Suitably designed calibration sample sets may allow the calibration model to account for variability caused by the varied amounts of each of the components in the blend. This will, to a certain extent increase the precision and accuracy of the calibration models. The calibration sample designs presented in this research note appear to be very effective for the determination of two minor components at the 5% (Crospovidone) and 0.5% (magnesium stearate) levels. The quantitation models for the major components show a small bias and may need to be further optimized.

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