



# Determinations of ephedrine in mixtures of ephedrine and pseudoephedrine using diffuse reflectance infrared spectroscopy

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## Abstract

There are a number of situations where there is a need to determine the concentrations of components in solid-state mixtures without dissolving the sample. Diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) coupled with partial-least-squares (PLS) data analysis has been used to determine the minor component in a mixture of structurally similar solid-state compounds, in this case mixtures of ephedrine and pseudoephedrine. Factors that limit the precision and accuracy of the determinations are discussed. It is shown that when care is taken to produce homogeneous calibration samples very good results can be obtained, in this case cross-validated standard error of predictions of 0.74 wt% when the minor component spanned the concentration range of 0–50 wt%, and 0.11 wt% when the minor component spanned the concentration range of 0–5 wt%. Results are presented that indicate that the amount of data available to the PLS calibration routine relative to the range over which the calibration is performed can limit the precision and accuracy of the determinations.

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## 1. Introduction

Although new routes for the administration of drugs are continually being investigated, the most common form of administration of drugs is still the solid state. In general, the active ingredient in a solid-state formulation of a drug is a crystalline material. The compounds

considered here are diastereoisomers and have significantly different biological properties (Schmidt et al., 1988). An analysis of samples containing a mixture of the two crystalline materials can be carried out using infrared spectroscopy where the samples are not dissolved, i.e. kept in the solid state. The differences in the infrared spectra of ephedrine and pseudoephedrine are due to the differences in the intermolecular interactions in the crystalline forms of the molecules. This

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situation is analogous to that found for many crystalline pharmaceutical compounds that can exist as different polymorphs. In the case of polymorphs the analysis of a mixture also must be carried out in the solid state. The crystal form of a compound can have significant effects on the bioavailability of a material and/or influence the manufacturing process of a formulation. As a result, polymorphism is a significant problem in the pharmaceutical industry and there is a need for analytical methods for the characterization of solid-state mixtures of polymorphs or diastereoisomers.

There is a rather large spectrum of techniques that can be used to characterize solid-state materials depending on the property, e.g. structure, spectral characteristics, thermodynamic stability, solubility behavior, etc., to be measured. Methods for the characterization of polymorphs have been discussed in a number of reviews of the area (Threlfall, 1995; Caira, 1998). Many of these techniques measure bulk solid-state properties and are not particularly useful for characterization of mixtures, however some are applicable to quantitative analysis of mixtures.

Solid-state  $^{13}\text{C}$  NMR has been shown to be quite useful in the characterization of polymorphs in both bulk drug substances and dosage forms (Aboul-Enein, 1990; Brittain et al., 1991; Saindon et al., 1993; Byrn et al., 1995). Most of the applications of  $^{13}\text{C}$  NMR have been qualitative, but a few quantitative applications have appeared (Suryamarayanan and Wiedmann, 1990; Gao, 1996). For example, the  $^{13}\text{C}$  NMR detection limit for the minor component in a mixture of delavirdine mesylate polymorphs has been reported to be 2–3 wt% (Gao, 1996).

Methods based on vibrational spectroscopy have the potential for characterization of mixtures of polymorphs, i.e. performing quantitative analysis of mixtures. Relatively recent instrumentation improvements as well as the development of multivariate data analysis techniques have made vibrational methods such as near infrared (NIR), infrared (IR), and Raman spectroscopies all much more useful for performing quantitative determinations.

Quantitative analysis of mixtures of polymorphs using NIR (Gimet and Luong, 1987; Blanco and Villar, 2000; Patel et al., 2000), IR (Doff et al., 1986; Deeley et al., 1991; Hartauer et al., 1992; Salari and Young, 1998; Lee et al., 2000; Aboul-Enein et al., 2002) and Raman (Deeley et al., 1991; Tudor et al., 1993;

Jalovszky et al., 1995) have been reported. The studies carried out using NIR and IR have used both attenuated reflectance and diffuse reflectance sampling. Diffuse reflectance sampling, although not without problems, seems to offer some advantages when compared to other solid-state sampling methods for IR spectroscopy. This is the method that has been utilized here.

In this paper, we report on the determination of the minor component in mixtures of two crystalline forms of 1-phenyl-2-aminomethylpropanol. The presence of two asymmetric carbons in the structure of 1-phenyl-2-aminomethylpropanol leads to two diastereometric pairs, known as ephedrine and pseudoephedrine. As will be discussed below, the differences in the IR spectra of ephedrine and pseudoephedrine are due to differences in the intermolecular interactions between the molecules in the solid-state forms, hence they have properties similar to that of polymorphs. The differences in the IR spectra of ephedrine and pseudoephedrine have been previously discussed (Ciurczak, 1987), and as shown here can be used as the basis of quantitative determination of the minor component in mixtures of the two.

## 2. Materials and methods

Ephedrine hydrochloride (Aldrich) and pseudoephedrine hydrochloride (Sigma) both specified to have a purity of 99.8% were used as received. The KBr used to dilute the samples for DRIFTS measurement was FTIR grade (Wilmad) and used after drying in a vacuum at 110 °C. All sample handling was carried out in a purged glove bag.

Two sets of calibration mixtures were prepared. The first set of calibration samples consisted of mixtures of ephedrine and pseudoephedrine of about 3 g total mass with ephedrine as the minor component. Eleven calibration samples were prepared spanning a range of ephedrine concentration of 0 to ~50 wt%, with the design points every 5 wt%. A second set of calibration samples was prepared with the minor component, the ephedrine, concentration ranging from 0 to about 5 wt%. There were 20 calibration samples in this set, with 8 of the 20 samples having concentrations between 0 and 1 wt%, and the remaining twelve samples having concentrations between about 1.3 and 5 wt%. A

set of five validation samples was also prepared with ephedrine concentrations of 0.904, 1.688, 2.001, 2.665, and 3.068 wt%.

The masses of the two components to be mixed were determined to 0.01 mg using a Mettler Toledo balance (Model AX 205). In order to assure that homogeneous mixtures were produced, the following procedure was followed when mixing the two sample components. The contents of the weighing boat containing the minor component were transferred to a mortar. An amount of the major component about equal in mass to that of the minor component was transferred to the mortar and the two components mixed thoroughly. A second quantity of the major component about equal in mass to the mixture in the mortar was then transferred to the mortar and thoroughly mixed in. This process was continued until the entire amount of the major component had been mixed in.

The IR spectral data were collected using a Nicolet Nexus Model 870 FTIR equipped with a diffuse reflectance accessory (Nicolet model 0031-999) mounted in the sample compartment. A deuterated triglycine sulfate detector was used and all spectral measurements consisted of 128 coadded scans at  $4\text{ cm}^{-1}$  resolution. All samples were diluted by thoroughly mixing with KBr using 10 parts KBr to 1 part sample, each carefully weighed, prior to diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) measurement. The mixing of the sample with KBr was carried out using the same mixing protocol as that described above. In the cases where the samples were ground prior to the DRIFTS measurement, the grinding was performed using a mortar and pestle. The sample was ground until it reached a “flour” like consistency. The KBr/sample mixtures were poured into the sample cup of the DRIFTS apparatus and the top surface of the sample smoothed by carefully pulling a straight edge across the sample cup. A gold mirror was used for the background for all spectral data used in the calibration modeling, and pure KBr was used for the background of the pure component spectra. The samples of the pure components were ground to uniform particle size prior to mixing with KBr for DRIFTS analysis.

All data processing, including the partial-least-squares (PLS) modeling, was carried out using the PLS2000 (Sandia National Laboratories) package that runs in the GRAMS (Thermo-Galactic Corp.) environment. The linear baseline correction used forces the end

points of the spectral region being corrected to zero. The scattering correction used is a multiplicative scatter correction (MSC) (Isaksson and Næs, 1988). The derivative processing is based on the Savitsky–Golay algorithm. All PLS calibrations were carried out using cross-validation with all spectra from a specific calibration sample simultaneously removed. The results of the calibrations using various frequency regions and different preprocessing are reported as the cross-validated standard error of prediction (CVSEP).

### 3. Results

The IR spectra of the (1*R*,2*S*)-(+)-ephedrine hydrochloride (ephedrine) and (1*S*,2*S*)-(+)-ephedrine hydrochloride (pseudoephedrine) are shown in Fig. 1. The structures of these isomers of ephedrine have been determined and although the arrangement of molecules in the two structures are similar, there are significant differences in the intermolecular hydrogen bonding in the two structures (Bergin, 1971; Mathew and Palenik, 1977). There is, for example, a difference in the frequency of the O–H stretching vibration in the two forms, as can be seen in Fig. 1. The O–H bands would be expected to be spectral features significantly affected by changes in hydrogen bonding. The OH–Cl bond distances are 2.38 and 2.16 Å in ephedrine and pseudoephedrine respectively. These distances correlate with the O–H stretching frequencies of  $3330\text{ cm}^{-1}$  in ephedrine and  $3273\text{ cm}^{-1}$  in pseudoephedrine.

The first set of calibration samples was prepared by mixing ephedrine and pseudoephedrine, with 11 mixture samples spanning the range of 0–50% ephedrine. Over such a concentration range the changes in the IR spectral data collected from these samples can be readily seen. Fig. 2 contains spectra of three of the mixture samples, 0, 25, and 50% ephedrine. The changes in the spectral features where there are significant differences in the pure component spectra, such as the –OH stretch in the region between  $3260$  and  $3300\text{ cm}^{-1}$ , are apparent. Fig. 3 contains the same three spectra plotted over the frequency region from  $950$  to  $1540\text{ cm}^{-1}$ . Bands due to the minor component (ephedrine), e.g. at  $993$ ,  $1242$ , and  $1355\text{ cm}^{-1}$ , can be seen to increase in intensity with concentration. Not so apparent, is the baseline shift in the spectra that also correlates (negatively) with the ephedrine concentration. The baseline

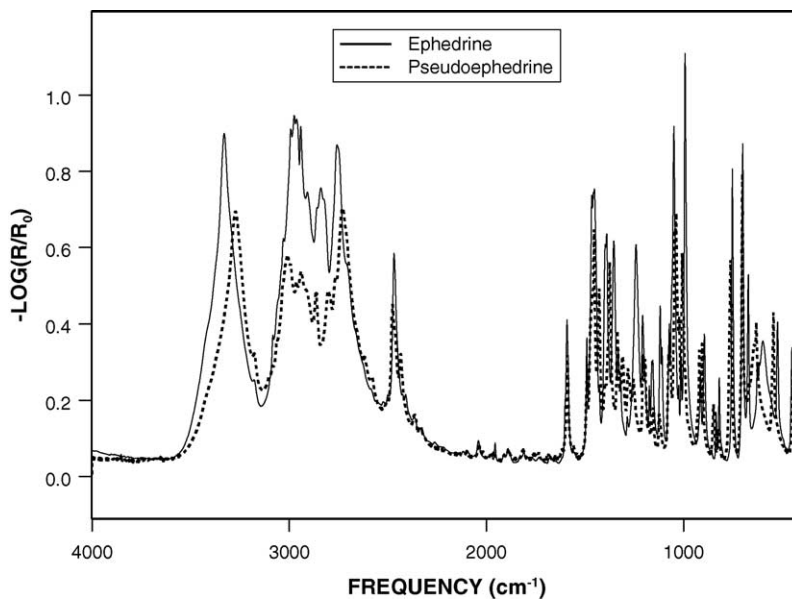


Fig. 1. DRIFTS spectra of ephedrine and pseudoephedrine.

shift can be best seen in the high-frequency region of Fig. 2. The baseline shift is due to scattering (Geladi et al., 1985; Isaksson and Næs, 1988), and is due to the fact that the two pure component samples have different crystal sizes. The calibration samples were made

up using the two pure components as received, and aliquots of the calibration samples were weighed and mixed with KBr diluent without any attempt to alter the crystal size when preparing the samples for DRIFTS analysis.

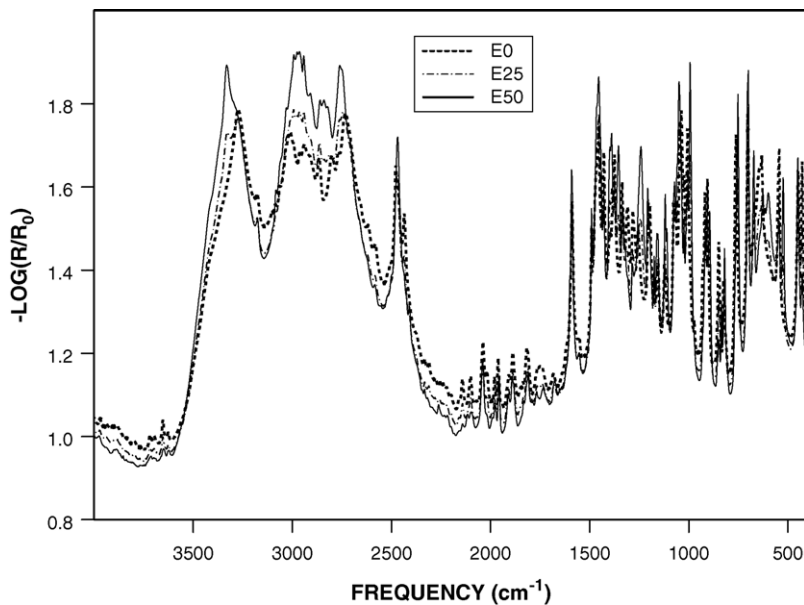


Fig. 2. DRIFTS spectra of the calibration samples containing 0, 25, and 50 wt% ephedrine.

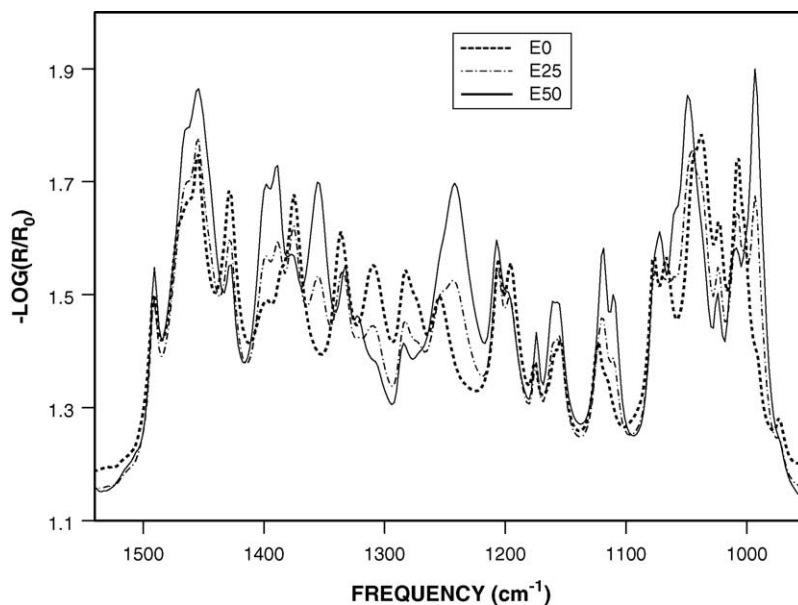


Fig. 3. DRIFTS spectra of the 0, 25, and 50 wt% ephedrine sample across the 1540–950  $\text{cm}^{-1}$  frequency region.

Spectral data were collected from three repeat sample preparations of each of the 0–50% mixture samples, for a total of 33 spectra. These spectral data were converted to  $-\log(R/R_0)$  and Kubelka–Munk units, assembled into data files, and subjected to PLS analysis. The calibration results obtained with the Kubelka–Munk transformed data were not as good as, i.e. always higher CVSEP, than the results obtained using the  $-\log(R/R_0)$  transformed data. Although the Kubelka–Munk transformation is often employed with diffuse reflectance data, a number of papers have shown that the theory does not accurately describe the situation where scattering samples have strong absorption bands (Brimmer and Griffiths, 1988; Loyalka and Riggs, 1995; Vargas and Niklasson, 1997). Other authors developing quantitative applications of diffuse reflectance data have also found that better results were obtained with data in “absorbance” units, rather than Kubelka–Munk units (Reeves and Reeves, 2002). Thus, all results presented here are based on  $-\log(R/R_0)$  transformed data. Calibrations were performed using a large number of different frequency regions, and a variety of data preprocessing options including baseline correction, scatter correction, and derivative processing were tested. The scattering correction made a significant

improvement in the CVSEP of the calibration when the high-frequency data were included, but did not seem to impact the calibration significantly when the calibration was limited to a portion of the low-frequency region. The best calibration based on these data applied only baseline correction to the frequency region from 950 to 1540  $\text{cm}^{-1}$ . The CVSEP produced was 2.1 wt%.

The calibration samples were rerun, but after the calibration samples were ground to uniform particle size prior to mixing with KBr powder. The grinding step minimized the baseline shifts, especially in the high-frequency region, that were correlated to the ephedrine concentration. Again, various forms of data pretreatment were tested in an attempt to determine the best conditions for a quantitative analysis based on these data. Again, the data pretreatment methods included frequency selection, baseline correction, scattering correction, and taking derivatives. A summary of these results is included in Table 1. A number of other frequency regions were tested with varying results, some producing CVSEPs close to the best shown in Table 1. However, none of the regions produced calibrations that were better, i.e. lower CVSEP, than the best shown in Table 1.

In most calibrations carried out some spectral files were identified as outliers, i.e. one to three spectra

Table 1  
Summary of calibration results obtained across the 0–50 wt% range

| Frequency region (cm <sup>-1</sup> ) | Pretreatment     | CVSEP (wt%) | No. of PLS factors | No. of outliers |
|--------------------------------------|------------------|-------------|--------------------|-----------------|
| 400–4000                             | Baseline         | 0.75        | 5                  | 3               |
| 400–4000                             | MSC              | 2.27        | 3                  | 1               |
| 400–4000                             | First derivative | 1.46        | 3                  | 0               |
| 950–1540                             | Baseline         | 0.74        | 3                  | 3               |
| 950–1540                             | MSC              | 2.55        | 5                  | 1               |
| 950–1540                             | First derivative | 1.08        | 3                  | 2               |

were found to be outliers during the cross-validation process. The outlier metrics used included concentration *F*-ratio, spectral *F*-ratio, and Mahalanobis distance. As can be seen in Table 1 good calibrations result from the data using only baseline correction as the preprocessing step. This was the case for all frequency regions tested. A calibration plot based on the data over the 950–1540 cm<sup>-1</sup> frequency region with only baseline correction is shown in Fig. 4. Wavelength selection made only small differences in the final CVSEP, although models based on data limited to the fingerprint region of the spectrum tend to have fewer factors than models that include data from the high-frequency region. Use of a scattering correction did not produce good results for any of the frequency regions tested for these data. Since the samples had

been ground to uniform particle size, this result might not be unexpected. First derivative preprocessing accounts for baseline shifts, but is not as effective as a simple linear baseline correction for these data.

One of the advantages of using multivariate calibration is that the signal averaging inherent to the calibration process allows for the measurement of small signals relative to the noise. In our experience, calibration models often get better, i.e. the CVSEP gets smaller, as the amount of data available to the modeling process increases relative to the range over which the calibration is carried out. The increased amount of data allows for more effective signal averaging, and in some cases the increased amount of data allows for better modeling of any nonlinearities in the data. The calibration covering the concentration range of 0–50 wt% with

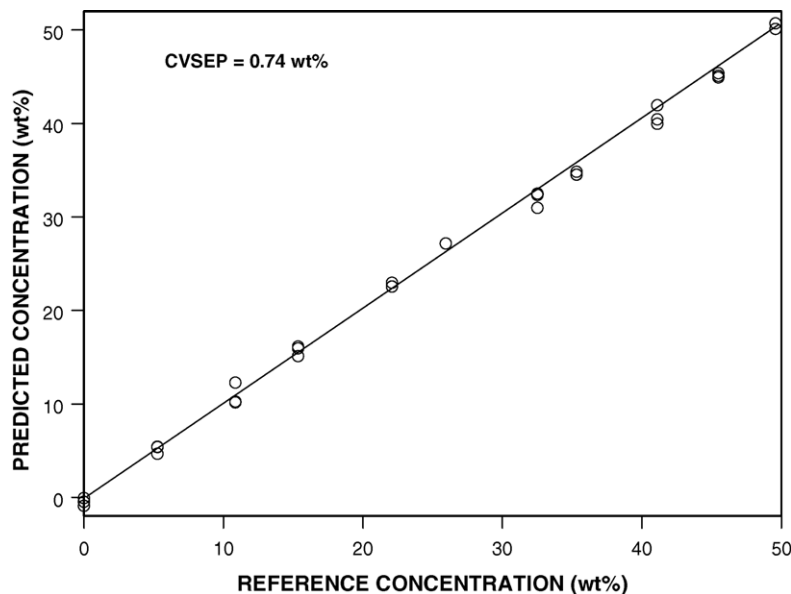


Fig. 4. Cross-validated calibration plot for the 0–50 wt% mixtures obtained using the spectral data collected from the 1540–950 cm<sup>-1</sup> frequency region after baseline correction.

Table 2  
Summary of calibration results obtained across the 0–5 wt% range

| Frequency region (cm <sup>-1</sup> ) | Pretreatment      | CVSEP (wt%) | No. of PLS factors | No. of outliers |
|--------------------------------------|-------------------|-------------|--------------------|-----------------|
| 400–4000                             | Baseline          | 0.11        | 4                  | 9               |
| 400–4000                             | MSC               | 0.11        | 5                  | 6               |
| 400–4000                             | First derivative  | 0.14        | 3                  | 4               |
| 400–4000                             | Second derivative | 0.13        | 4                  | 4               |
| 950–1540                             | Baseline          | 0.12        | 4                  | 4               |
| 950–1540                             | MSC               | 0.16        | 2                  | 1               |
| 950–1540                             | First derivative  | 0.12        | 3                  | 2               |
| 950–1540                             | Second derivative | 0.12        | 3                  | 3               |

33 spectra might be limited by the information available to the PLS model relative to the relatively large concentration range to which the calibration model applies.

A second calibration sample set was produced to test whether or not a better calibration could be produced by limiting the calibration to a narrower concentration range. In this set of 20 calibration samples the concentration of ephedrine in the mixtures of ephedrine and pseudoephedrine spanned the range of 0 to ~5 wt%. Three repeat DRIFTS preparations were carried out for each mixture, spectra collected from each, and the spectral data assembled into a matrix for processing using the PLS algorithm. As with the 0–50 wt% set, calibrations were carried out using frequency selection

and a variety of data preprocessing steps. Table 2 contains some of the most significant results obtained.

As can be seen in Table 2 the CVSEP when working with this larger data set is a factor of five or six better, i.e. lower, than that achieved with the 0–50 wt% data. Other frequency regions than those included in Table 2 produced CVSEPs that are equivalent to, but not any better than, those shown in Table 2. A calibration plot using the first derivative preprocessed spectral data over the 950–1540 cm<sup>-1</sup> frequency range is shown in Fig. 5. It should be noted that when preparing the samples for DRIFTS measurement, the aliquots of the calibration samples were mixed with the KBr diluent without any grinding. Thus, there will be scattering differences

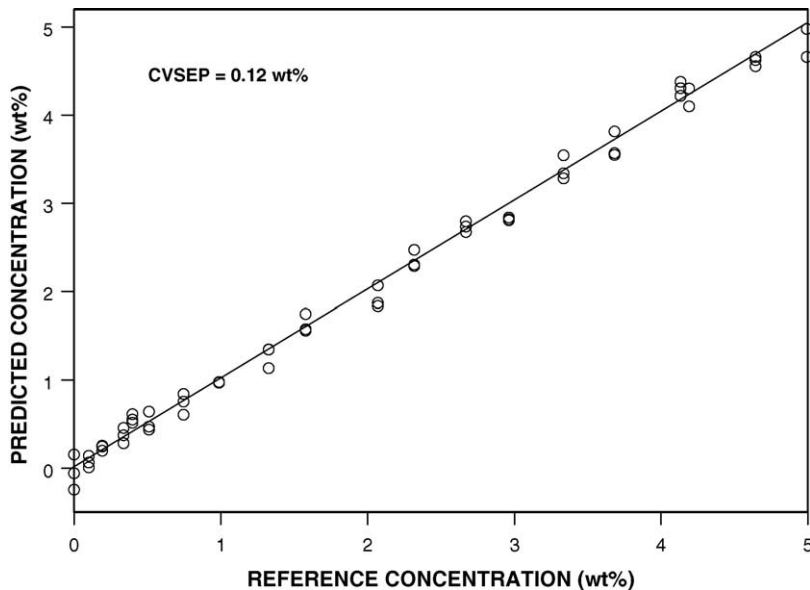


Fig. 5. Cross-validated calibration plot for the 0–5 wt% mixtures obtained using the spectral data collected from the 1540–950 cm<sup>-1</sup> frequency region after first derivative preprocessing.

across the spectral data to the degree the mixtures vary in particle size distribution across the concentration range of 0–5 wt% ephedrine. As can be seen in Table 2, at least across the 950–1540  $\text{cm}^{-1}$  frequency region the MSC and derivative data preprocessing steps seem to account for the degree of scattering encountered.

As with the 0–50% data discussed above, a number of concentration outliers were identified during the cross-validation process. The number of outlier spectral files identified during each calibration is shown in column five in Table 2. When the entire spectral region was included the number of outliers during the cross-validation process was relatively high. For example, nine samples were flagged as outliers using baseline correction and six were flagged using the scatter correction. While the CVSEPs produced are as good as those produced when limiting the spectral data to portions of the low-frequency region, they are the result of removing a significant number of spectral files from the calibration. In most of the low-frequency models employing an MSC correction, or derivative preprocessing, one to three spectral files are identified as outliers. One spectral file, one of the repeats of the 4.99 wt% sample, had a high spectral  $F$ -ratio and was identified as a significant concentration outlier regardless of the spectral region or preprocessing option used when developing the PLS calibration model. It is possible some error was made when collecting this spectrum.

The calibration model was validated by determining the concentration of ephedrine in a set of five independent validation samples. Three repeat preparations of each of the validation samples were prepared, an IR spectrum collected from each, and the concentration predicted using the calibration model (the first derivative preprocessed data across the 950–1540  $\text{cm}^{-1}$  range). The resulting SEP was 0.13 wt%, i.e. very good agreement with the calibration model CVSEP of 0.12 wt%.

It is clear that the CVSEP is much improved by using more data, i.e. more calibration samples relative to the concentration range being modeled. Ultimately the CVSEP will be limited by a noise source or nonlinearity in the data that the PLS algorithm cannot adequately model. In the experiments discussed here we believe that the limiting noise source is likely the variability of the sample surface when the sample is introduced to the spectrometer, as will be discussed below. Thus, the relatively large number of samples and small concentration range provide adequate information so that the limit is the noise source, not a less than adequate amount of information necessary for the model. A measure of the impact of the number of samples relative to the information needed by the PLS algorithm to model a particular data set can be determined by removing data from the matrix of calibration data and repeating the calibration process. Results of such a study are

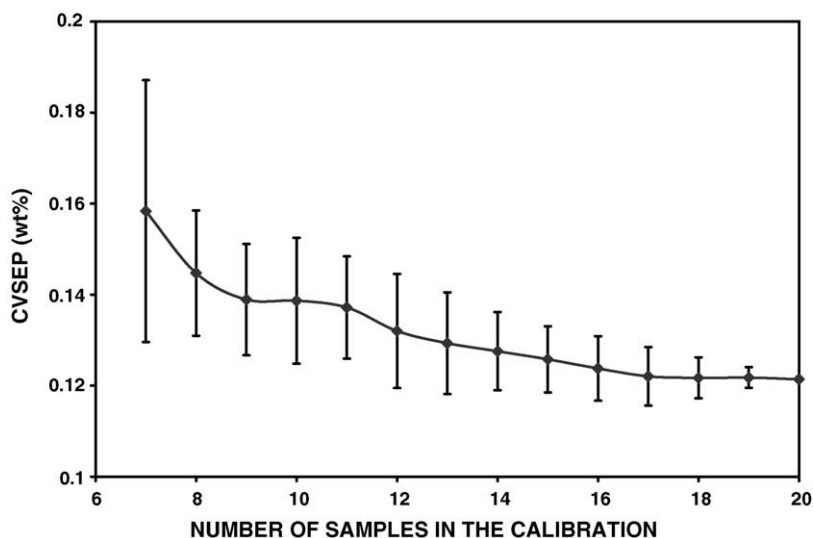


Fig. 6. The effect of removing samples from the calibration data set on the CVSEP.



shown in Fig. 6. The “starting point” of Fig. 6 is the CVSEP of 0.12 wt% achieved modeling the data collected from the 0–5 wt% calibration samples across the 950–1540  $\text{cm}^{-1}$  frequency region using first derivative preprocessing. The three repeat spectra collected from a randomly selected sample were removed from the calibration data and a PLS model was produced from the remaining data. This process (randomly selecting a sample and removing all the spectral data collected from that sample) was repeated 10 times, and the average CVSEP and standard deviation of the 10 trials plotted relative to 19 samples. Analogous calibrations were performed using six spectra collected from two randomly selected samples, nine spectra collected from three randomly selected samples, etc. As can be seen in Fig. 6 there is virtually no change in the average until four samples have been removed. The average CVSEP increases slowly as more samples are removed until about 13 samples remain. From that point both the average CVSEP and the standard deviation of the CVSEP increase more rapidly as additional samples are removed. For this specific experiment, three repeat spectra of 14 or 15 samples would yield a good CVSEP. A small gain, i.e. lower CVSEP, as the number of samples increases to eighteen is realized. The addition of further samples does not impact the CVSEP. This suggests that the PLS model has extracted the maximum amount of information that it can, and that some noise source is limiting the CVSEP.

The magnitude of various noise sources can be determined by carrying out repeat measurements. In this experiment, 10 repeat spectral measurements of the 2.67 wt% ephedrine sample were carried out using different procedures. A PLS calibration model was built using data collected from the other nineteen samples, i.e. there were no data included in the calibration model from the 2.67 wt% sample, and this model used to predict the ephedrine concentration of the spectra collected from the 2.67 wt% sample using the different procedures. Table 3 contains the results of these experiments. The first row of Table 3 contains the standard deviation obtained when 10 spectra were collected from the sample without any movement of the sample, and the concentration of the ephedrine predicted from each of the 10 repeat spectra. This should represent the spectrometer noise, which is expected to be relatively low. The second row contains the results obtained by moving the same sample in and out of the DRIFTS apparatus

Table 3

The standard deviation of 10 repeat determinations of the sample containing 2.67 wt% ephedrine as a function of procedure

| Experiment           | Standard deviation (wt%) |
|----------------------|--------------------------|
| No movement          | 0.02                     |
| Sample in/out        | 0.08                     |
| Sample in/out—smooth | 0.12                     |
| Sample cup repacked  | 0.11                     |

between repeat data collects. The sample holder was moved carefully, i.e. an attempt was made to reposition the sample holder in the DRIFTS apparatus precisely and to avoid any perturbation of the smoothed sample surface. The third row in Table 3 contains the results obtained when removing the sample from the spectrometer and resmoothing the sample surface between data collects. The results in the fourth row were obtained using 10 different aliquots of the sample, i.e. a complete new sample preparation including weighing out the sample and KBr, mixing the sample into the KBr, introducing the mixture to the sample cup, and smoothing the surface were carried out for each data collect step. The results in row two are not significantly different than those in rows three and four (*F* test at the 95% confidence level). When the sample is moved in and out of the spectrometer the sample surface is likely disturbed. The largest standard deviations in Table 3, rows three and four, both have a sample surface smoothing step. The lack of ability to generate an exactly reproducible sample surface seems to be a significant source of noise in this experiment.

#### 4. Discussion

In our experience the CVSEP of the determination of the minor component in a solid-state mixture is dependent on a number of experimental variables. However, the homogeneity of the calibration samples is of paramount importance. The components used to prepare the solid-state calibration mixtures have to be accurately weighed and very carefully mixed to ensure homogeneity. The method presented above, in Section 2, involving sequential addition/mixing of approximately equal volumes consistently produces homogeneous samples.

The use of a multivariate calibration routine, such as the PLS routine used here, generally produce better

results than do conventional univariate analysis methods. One significant factor in the use of multivariate calibration is the opportunity to employ outlier detection. There are a number of commercially available multivariate calibration software systems and virtually all of them have the capability of performing cross-validation. The use of cross-validation allows for outlier detection to be applied to the calibration samples. Elimination of outlier samples in the calibration sample set can improve the quality of calibration, as well as make the calibration model more sensitive to outlier samples in the future. A major source of variance in the spectral data collected from solid-state samples such as discussed here is scattering. As shown above, the number of outlier samples identified during the cross-validation process is dependent upon frequency selection and choice of data preprocessing options. It is important that these options be explored when developing a multivariate calibration model if optimum performance is to be achieved.

The amount of information available to the calibration model relative to the complexity in the data that the calibration model must account for is an important factor in the determination of how well the calibration model performs. DRIFTS data are potentially quite nonlinear; hence a calibration model applied to DRIFTS data might be limited by the calibration model's ability to account for the nonlinearity. In all experiments discussed above the amounts of sample and KBr that were mixed to prepare a sample for DRIFTS measurement were carefully weighed out. Thus, the total amount of sample introduced to the spectrometer was nearly a constant, hence limiting the range of variation in the measured signals.

It is well known that highly scattering samples result in band shape differences and nonlinear intensities in DRIFTS measurements. It is not surprising that the results of the calibration over the 0–50 wt% range improved after grinding the samples. Over the 0–5 wt% range the difference in particle size distribution across the range of samples is small, hence the changes due to scattering across the calibration sample set are not great. For example, the baseline shift in the high-frequency region is not highly correlated with the ephedrine concentration as it was in the 0–50 wt% samples. However, the effects of scattering are present in the 0–5 wt% spectra as indicated by the data in [Table 2](#). The calibration models that employ prepro-

cessing steps that minimize scattering effects, e.g. MSC or derivative preprocessing, have fewer factors than the model produced using baseline correction only. The fact that the CVSEP from the baseline correction model is equivalent to those of the other models suggests the PLS algorithm attempts to account for the scattering effects by adding factors to the model, but even then must reject more samples as outliers to achieve CVSEPs equivalent to those yielded by models where MSC or derivative preprocessing was employed. These observations agree with those of other researchers who explored scattering effects on near infrared data sets ([Blanco et al., 1997](#)). The ability to account for scattering effects, whether by preprocessing or adding additional PLS factors, is probably related to the amount of data available relative to the range of variation in properties the calibration model must account for.

The fact that the repeat experiments demonstrated standard deviations equivalent to the CVSEP of the calibration suggests that a noise source limited the calibration. In this case the noise source appears to be the variability of the sample surface when the sample is inserted into the DRIFTS apparatus. Special care should be taken to achieve reproducible sample surfaces when preparing materials for quantitative measurements using DRIFTS.

In conclusion, the results presented above show DRIFTS measurements can be used to produce sensitive determinations for the minor component in a solid-state mixture. The quality of results depends on careful experimental procedure; critical factors include preparation of homogeneous calibration mixtures and controlling the surface of the sample when it is inserted into the DRIFTS apparatus. The magnitude of the CVSEP of the calibration will be limited by the amount of data available relative to the range properties over which the calibration is carried out. If the target of the determination is very small quantities of the analyte, better results will be obtained for a limited number of calibration samples if the range of concentration over which the calibration is carried out is kept small.

## References

- Aboul-Enein, H.Y., 1990. Applications of solid-state nuclear magnetic resonance spectroscopy to pharmaceutical research. *Spectroscopy* 5, 32–40.

- Aboul-Enein, H.Y., Bunaciu, A.A., Fleschin, S., 2002. Analysis of mebendazole polymorphs by Fourier transform IR spectrometry using chemometric methods. *Biopolymers (Biospectroscopy)* 67, 56–60.
- Bergin, R., 1971. Refinement of the structure of (–)-ephedrine hydrochloride. *Acta Cryst.* B27, 381–386.
- Blanco, M., Coello, J., Iturriaga, H., MasPOCH, S., de la Pezuela, C., 1997. Effect of data preprocessing methods in near-infrared diffuse reflectance spectroscopy for the determination of the active compound in a pharmaceutical preparation. *Appl. Spectrosc.* 51, 240–246.
- Blanco, M., Villar, A., 2000. Polymorphic analysis of a pharmaceutical preparation by NIR spectroscopy. *Analyst* 125, 2311–2314.
- Brimmer, P.J., Griffiths, P.R., 1988. Angular dependence of diffuse reflectance infrared spectra. Part III: linearity of Kubelka–Munk plots. *Appl. Spectrosc.* 42, 242–247.
- Brittain, H.G., Bogdanowich, S.J., Bugay, D.E., DeVincentis, J., Lewen, G., Newman, A.W., 1991. Physical characterizations of pharmaceutical solids. *Pharm. Res.* 8, 963–973.
- Byrn, S.R., Pfeiffer, R., Ganey, M., Hoiberg, C., Poochikian, C., 1995. Pharmaceutical solids: a strategic approach to regulatory considerations. *Pharm. Res.* 12, 945–954.
- Caira, M.R., 1998. Crystalline polymorphism of organic compounds. *Top. Cur. Chem.* 198, 163–208.
- Ciurczak, E.W., 1987. Uses of near-infrared spectroscopy in pharmaceutical analysis. *Appl. Spectrosc. Rev.* 23, 147–163.
- Deeley, C.M., Spragg, R.A., Threlfall, T.L., 1991. A comparison of Fourier transform infrared and near-infrared Fourier transform Raman spectroscopy for quantitative measurements: an application in polymorphism. *Spectrochim. Acta* 47A, 1217–1223.
- Doff, D.H., Brownen, F.L., Corrigan, O.I., 1986. Determination of  $\alpha$ -impurities in the  $\beta$ -polymorph of inosine using infrared spectroscopy and X-ray powder diffraction. *Analyst* 111, 179–182.
- Gao, P., 1996. Determination of the composition of delavirdine mesylate polymorph and pseudopolymorph mixtures using  $^{13}\text{C}$  CP/MAS NMR. *Pharm. Res.* 13, 1095–1104.
- Geladi, P., MacDougall, D., Martens, H., 1985. Linearization and scatter-correction for near-infrared reflectance spectra of meat. *Appl. Spectrosc.* 39, 491–500.
- Gimet, R., Luong, A.T., 1987. Quantitative determination of polymorphic forms in a formulation matrix using the near infrared reflectance analysis technique. *J. Pharm. Biomed. Anal.* 5, 205–211.
- Hartauer, K.J., Miller, E.S., Guillory, J. Keith, 1992. Diffuse reflectance infrared Fourier transform spectroscopy for the quantitative analysis of mixtures of polymorphs. *Int. J. Pharm.* 85, 163–174.
- Isaksson, T., Næs, T., 1988. The effect of multiplicative scatter correction (MSC) and linearity improvement in NIR spectroscopy. *Appl. Spectrosc.* 42, 1273–1284.
- Jalšovszky, G., Egyed, O., Holly, S., Hegedus, B., 1995. Investigation of the morphological composition of cimetidine by FT-Raman spectroscopy. *Appl. Spectrosc.* 49, 1142–1145.
- Lee, K.R., Zuber, G., Katrincic, L., 2000. Chemometrics approach to the determination of polymorphism of a drug compound by infrared spectroscopy. *Drug Dev. Ind. Pharm.* 26, 135–147.
- Loyalka, S.K., Riggs, C.A., 1995. Inverse problem in diffuse reflectance spectroscopy: accuracy of the Kubelka–Munk equations. *Appl. Spectrosc.* 49, 1107–1110.
- Mathew, M., Palenik, G.J., 1977. The crystal and molecular structures of (+)-pseudoephedrine and (+)-pseudoephedrine hydrochloride. *Acta Cryst.* B33, 1016–1022.
- Patel, A.D., Luner, P.E., Kemper, M.S., 2000. Quantitative analysis of polymorphs in binary and multi-component powder mixtures by near-infrared reflectance spectroscopy. *Int. J. Pharm.* 206, 63–74.
- Reeves, J.B., Reeves, V.B., 2002. Effects of apodization function, zero filling, background spectra, and absorbance transformation on mid-infrared calibrations for feed composition. *Spectrosc. Lett.* 35, 663–680.
- Saïndon, P.J., Cauchon, N.S., Sutton, P.A., Chang, C.-J., Peck, G.E., Byrn, S.R., 1993. Solid-state nuclear magnetic resonance (NMR) spectra of pharmaceutical dosage forms. *Pharm. Res.* 10, 197–203.
- Salari, A., Young, R.E., 1998. Application of attenuated total reflectance FTIR spectroscopy to the analysis of mixtures of pharmaceutical polymorphs. *Int. J. Pharm.* 163, 157–166.
- Schmidt, W.F., Porter, W., Carstensen, J.T., 1988. Thermodynamics in the prediction of the solubilities of binary mixtures of the diastereoisomers and enantiomers of ephedrine. *Pharm. Res.* 5, 391.
- Suryamarayanan, R., Wiedmann, T.S., 1990. Quantitation of the relative amounts of anhydrous carbamazepine ( $\text{C}_{15}\text{H}_{12}\text{N}_2\text{O}$ ) and carbamazepine dihydrate ( $\text{C}_{15}\text{H}_{12}\text{N}_2\text{O}\cdot 2\text{H}_2\text{O}$ ) in a mixture by solid-state nuclear magnetic resonance (NMR). *Pharm. Res.* 7, 184–187.
- Threlfall, T.L., 1995. Analysis of organic polymorphs: a review. *Analyst* 120, 2435–2460.
- Tudor, A.M., Church, S.J., Hendra, P.J., Davies, M.C., Melia, C.D., 1993. The qualitative and quantitative analysis of chlorpropamide polymorphic mixtures by near-infrared Fourier transform Raman spectroscopy. *Pharm. Res.* 10, 1772–1776.
- Vargas, W.E., Niklasson, G.A., 1997. Applicability conditions of the Kubelka–Munk theory. *Appl. Opt.* 36, 5580–5586.