A rapid quantitative assay of intact paracetamol tablets by reflectance near-infrared spectroscopy

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Near-infrared (NIR) reflectance spectroscopy was used to determine rapidly and non-destructively the content of paracetamol in bulk batches of intact Sterwin 500 mg tablets by collecting NIR spectra in the range 1100–2500 nm and using a multiple linear regression calibration method. The developed NIR method gave results comparable to the British Pharmacopoeia 1993 UV assay procedure, the standard errors of calibration and prediction being 0.48% and 0.71% m/m, respectively. The method showed good repeatability, the standard deviation and coefficient of variation for six NIR assays on the same batch on the same day being 0.14 and 0.31 and 0.36 and 0.45% m/m, respectively. Applying the calibration to a parallel test set gave a mean bias of −0.22% and a mean accuracy of 0.45%. The developed method illustrates how the full potential of NIR can be utilised and how the ICH guidelines which recommend the validation of linearity, range, accuracy and precision for pharmaceutical registration purposes can be applied. Duplicate determinations on bulk batches could be performed in under 2 min, allowing the potential use of the method on-line for real time monitoring of a running production process.

Near-infrared (NIR) absorption is mainly due to overtone and combination vibrations arising from fundamental vibrations in the mid-infrared region. As these absorptions are weak, NIR spectroscopy should be ideally suited for the analysis of intact tablets. No sample preparation is required and the method is non-destructive. Surprisingly, the majority of published applications of NIR spectroscopy to tablets have not exploited this advantage, but used powdered tablets or solutions. Zappala and Post measured meprobarbinate in tablets after extraction into chloroform and ranitidine chlorohydrate,2 ranitidine hydrochloride3 and cefuroxime acetil4 have all been measured as powdered tablet samples. An early example of an intact tablet analysis was a qualitative study5 to identify adulterated aspirin tablets. Quantitative assays based on intact tablets have included the assay of amiodarone chlorohydrate,6 metoprolol,7 SB 216469-S8 and aspirin.8 Recently, we described the application of transmittance NIR spectroscopy to the analysis of individual intact tablets9,10 and compared the effect of various pre-treatments on partial least-squares regression models; experiments involved the measurement of 20 intact tablets with an analysis time of 10 min per batch.

A problem with intact tablet assays is that normal production batches do not encompass a sufficiently wide range for setting up a reliable calibration equation. Tablets which are both under- and overdosed with respect to the analyte need to be produced without altering other factors that may affect the assay such as particle size, moisture content and compaction characteristics. Although this complicates the calibration, as compared with powdered tablet assays for which it is easy to prepare standards, the long-term gains of no sample preparation and the potential for the use on-line, allowing for real time monitoring of a running production process, make the effort worthwhile.

This paper describes a rapid quantitative assay for the determination of paracetamol in bulk batches of intact tablets using diffuse reflectance NIR spectroscopy and multiple wavelength linear regression. Where possible we applied the recommendations of the International Conference on Harmonisation (ICH) guidelines12 to the assay. The ICH guidelines recommend the validation of linearity, range, accuracy and precision, both short and long term, for analytical procedures which are to be used for pharmaceutical registration purposes.

Experimental

Materials

Forty-five batches of paracetamol tablets were used for the study; 35 were normal production batches of Sterwin 500 mg paracetamol tablets (nominal content 84.175% m/m paracetamol) with a nominal diameter of 12.7 mm and thickness of 3.80–4.15 mm and 10 were development batches (76–92% m/m paracetamol, i.e., 90–110% of the nominal label content). The development batches were of the same dimensions as the normal production batches but were specially manufactured for this study at a pilot scale on a small scale press, by altering the content of paracetamol and the major excipient (starch). The raw materials used were Paracetamol Fine PhEur, Potassium Sorbate PhEur, Povidone K-25 PhEur, Pregelatinised Starch USNF, Starch Maize PhEur, Stearic Acid BP and Sterilised Talc PhEur, which were of the same specification as those used in the tablets and were obtained from Sanofi Research Division, Alnwick Research Centre, Alnwick, Northumberland, UK.

All reagents were of analytical-reagent grade and were obtained from BDH (Poole, Dorset, UK).

UV analysis

UV measurements were made using a Perkin-Elmer (Beaconsfield, Buckinghamshire, UK) Lambda 15 UV/VIS spectrophotometer and 1 cm pathlength matched silica cells. Tablet batches were assayed in duplicate using the following procedure based on the British Pharmacopoeia 199313 assay. The
Pharmacopoeia method was modified because out of specification tablets were being analysed. For this assay, 20 tablets were ground to a fine powder and accurately 0.15 g were measured and transferred into a 200.0 ml calibrated flask. A 50 ml volume of 0.1 m sodium hydroxide solution was added and the sample was shaken for 10 min, then 100 ml of distilled water were added and the sample shaken for a further 10 min. The solution was diluted to volume with distilled water, mixed and filtered and 5.0 ml of the solution were pipetted into a 500.0 ml calibrated flask. 50 ml of 0.1 m sodium hydroxide solution were added and the solution made to volume with distilled water. The absorbance was measured at 257 nm using 0.01 m sodium hydroxide solution as a blank. The content of paracetamol was calculated taking 715 as the value of $A_{1\%_{	ext{cm}}}$ at the maximum at 257 nm.

### NIR analysis

NIR reflectance spectra were measured using a NIRSystems 6500 near-infrared spectrophotometer (Foss NIRSystems, Maidenhead, Berkshire, UK) fitted with a reflectance detector (NR-6503), sample transport module (NR-6511) and coarse sample cell (NR-7080). The instrument was governed by NSAS (Near-Infrared Spectral Analyses Software) version 3.52 (Foss NIRSystems). Each measured spectrum was the average of 32 scans and measured over the wavelength range 1100–2500 nm. Batches were measured by pouring approximately 100 tablets into the coarse sample cell and scanning on the quarter full setting. Measurements were made in duplicate, the coarse sample cell being refilled with the same tablets between scans. Spectra were processed using Vision beta software (Foss NIRSystems).

### Results and discussion

#### Feasibility study

NIR reflectance spectra of all the tablet ingredients were recorded as log (1/R), where R is the reflectance, and their second derivative absorbance spectra were examined to identify any unique spectral features of the active constituent (paracetamol). The second derivative spectrum of paracetamol showed characteristic spectral features at about 1525 and 1625–1675 nm; the minimum at about 1525 nm appeared to be any unique spectral features of the active constituent (paracetamol). The second derivative absorbance NIR spectra of intact paracetamol tablets were being analysed. For this assay, 20 tablets were ground to a fine powder and accurately 0.15 g were measured and transferred into a 200.0 ml calibrated flask. A 50 ml volume of 0.1 m sodium hydroxide solution was added and the sample was shaken for 10 min, then 100 ml of distilled water were added and the sample shaken for a further 10 min. The solution was diluted to volume with distilled water, mixed and filtered and 5.0 ml of the solution were pipetted into a 500.0 ml calibrated flask. 50 ml of 0.1 m sodium hydroxide solution were added and the solution made to volume with distilled water. The absorbance was measured at 257 nm using 0.01 m sodium hydroxide solution as a blank. The content of paracetamol was calculated taking 715 as the value of $A_{1\%_{	ext{cm}}}$ at the maximum at 257 nm.

#### Reference analysis

Reference values (‘true’ values) for the paracetamol content of all batches of tablets were determined in duplicate using the UV assay procedure. The precision of the UV assay procedure was estimated by pooling the results from the duplicate measurements made on the 35 calibration and validation batches and calculating the standard deviation (s) using the equation

$$s = \sqrt{\frac{1}{n-1} \sum_{k=1}^{n} \left( \frac{x_k - \bar{x}}{\bar{x} - \bar{x}} \right)^2}$$

where n is the number of duplicates, $k_1$ and $k_2$ are the individual duplicate results and $\bar{x}_k$ is the mean of the duplicates. The standard deviation was 0.31% m/m, giving a standard error (SE) for a UV determined reference value of 0.22% m/m using the equation

$$SE = \frac{s}{\sqrt{2}}$$

#### Method development and calibration

The NIR spectra were transferred to the Vision software along with the mean UV assay values for the paracetamol content of each batch. Absorbance spectra were treated mathematically by performing a standard normal variate (SNV) transformation to remove multiplicative interferences of scatter and particle size effectively, followed by calculating the second derivative (segment size of 20 and a gap size of zero data points) to maintain the peak locations but enhance the resolution. Fifteen normal production batches were assigned to a calibration set and 10 to a validation set and forward search multiple linear regression (MLR), using the Vision program, was applied to the data. A poor multiple correlation coefficient ($R^2$) between the UV and NIR values of 0.694 was obtained at 1926 nm, which only improved to $R^2 = 0.762$ when a second wavelength was added. This suggested that it was not possible to generate acceptable calibration equations using only normal production batches owing to the limited range of paracetamol concentrations in normal production batches. To improve the calibration equation, five development batches, which covered the extremes of concentration, were added to the calibration set and a further five development batches added to the validation set. Forward search MLR gave $R^2 = 0.971$ at 1426 nm, which improved to 0.974 when a second wavelength, 1528 nm, was added. This second wavelength corresponded to the characteristic spectral feature of paracetamol identified in the feasibility study.

When the first wavelength was selected manually from the characteristic spectral region, 1530 nm gave the best correlation with $R^2 = 0.860$. When a second wavelength of 1426 nm, selected by the Vision software, was added, $R^2$ improved to 0.974, the same as the initial two wavelength MLR selected by the Vision software. On addition of a third wavelength, $R^2$ only improved to 0.976, a minor improvement considering the subsequent risk of overfitting the data.

The two wavelength calibration was therefore chosen and the equation obtained was:

$$Y = 87.22 - 35.11A_{1530} + 169.65A_{1426}$$

where $Y$ is the predicted paracetamol content (% m/m) and $A_{1530}$ and $A_{1426}$ are the ordinate values of the transformed spectra (SNV plus the second derivative) at 1530 and 1426 nm, respectively. The fit had a residual sum of squares (RSS) of 3.89 [eqn. (4)], giving a standard error of calibration (SEC) of 0.48% m/m [eqn. (5)].

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**Fig. 1** Second derivative absorbance NIR spectra of intact paracetamol tablets: (a) 76, (b) 84 and (c) 93% m/m paracetamol content.
where \( y \) is the ‘true’ paracetamol content (i.e., mean UV assay value), \( n \) the number of batches and \( p \) the number of coefficients in eqn. (3). Fig. 2 shows a plot of predicted values versus reference values for the calibration set. Ideally, the intercept \( (a) \) and slope \( (b) \) should be 0 and 1, respectively, if there is no fixed systematic error or relative systematic error in the calibration equation. Linear regression was applied and the 95% confidence intervals for the intercept [eqn. (6)] and slope [eqn. (7)] were calculated.

\[
a \pm t \times \text{RSD} \times \sqrt{\frac{1}{n} \frac{\bar{x}^2}{S_{xx}}} \\
b \pm t \times \frac{\text{RSD}}{\sqrt{S_{xx}}} \\
\text{RSD} = \sqrt{\frac{\text{RSS}}{n - 2}} \\
S_{xx} = \frac{(\Sigma x)^2}{n}
\]

The confidence interval for the intercept (−3.56 to 8.08) included 0, and there was therefore no evidence to suggest a non-zero intercept. Similarly, the confidence interval for the slope (0.90–1.04) included 1, suggesting that there was no evidence for a relative systematic error in the calibration equation.

**Validation of calibration equation**

The calibration equation was validated by using it to calculate the paracetamol content of each of the batches in the validation sample set. The standard error for prediction (SEP) was 0.71% m/m (equation as for SEC with \( p = 0 \)). A plot of predicted versus UV assay values is shown in Fig. 3. Again, the 95% confidence intervals for the intercept (−11.52 to 11.92) and slope (0.93–1.07) suggest that there is no evidence for relative or fixed systematic errors.

**Parallel test set**

Further validation was performed on batches which were independent of both the calibration and validation sets. The validation set used above was not totally independent of the calibration set as they were scanned at the same time. Validation batches are often used to tune a calibration procedure by, for example, choosing optimum wavelengths for a multiple regression equation so the use of a parallel test set avoids overfitting. Also the performance of the calibration on the parallel test set is a more reliable indicator of its future performance on normal production batches. A month after the calibration and validation sets had been measured, 10 totally independent production batches were used to evaluate the performance of the calibration. The UV and NIR results (Table 1) were then compared using the paired sampled Student’s \( t \)-test [eqn. (10)] to show if the results were statistically equivalent.

\[
t_{\text{calc}} = \frac{\bar{d}}{s_d / \sqrt{n}}
\]

where \( \bar{d} \) is the mean residual (NIR − UV) and \( s_d \) is the standard deviation of the residuals. A value of 1.069 for \( t_{\text{calc}} \) was obtained. The two-sided critical value of Student’s \( t \) at the 5% significance level for \( n - 2 \) degrees of freedom was 2.262, suggesting that there was no evidence for a difference between the NIR and UV assay results.

**Accuracy**

For the purposes of this study, the accuracy was taken as how close the NIR values were to the UV assay values. The standard errors of calibration (0.48% m/m) and prediction (0.71% m/m) gave an indication of the accuracy of the NIR determination and a further estimate of accuracy was given by the standard deviation of the residuals obtained in the parallel test set (0.53% m/m). As expected, these values were greater than the standard error (0.22% m/m) for the UV measurement itself, although acceptable and therefore suitable for batch release purposes.
The mean bias [eqn. (11)] and the mean accuracy [eqn. (12)] for the parallel test set (n = 10) were determined to be −0.22 and 0.45% with standard deviations of 0.63 and 0.48%, respectively.

\[
\text{Mean bias} = \frac{\sum_{i=1}^{n} (\text{NIR}_{\text{value}} - \text{UV}_{\text{value}})}{\text{UV}_{\text{value}}} \times 100 \quad (11)
\]

\[
\text{Mean accuracy} = \frac{\sum_{i=1}^{n} (\text{NIR}_{\text{value}} - \text{UV}_{\text{value}})}{\text{UV}_{\text{value}}} \times 100 \quad (12)
\]

Repeatability

The short term precision (within-day) was determined by measuring the paracetamol content of a single batch six times within one day by both UV and NIR methods. The standard deviation and coefficient of variation (CV) for the NIR assay procedure were 0.14% m/m and 0.16%, respectively, and for the UV assay procedure they were 0.41% m/m and 0.48%, respectively. The mean (±95% confidence limit) UV and NIR assay values were 84.53 ± 0.43% and 84.38 ± 0.14% m/m, respectively. The confidence intervals overlap, further suggesting that there was no evidence for a difference in values obtained by the two procedures (cf., parallel test set).

Intermediate precision

The between-day precision was measured by assaying a single batch on six consecutive days by both UV and NIR methods. The standard deviation and CV for the NIR assay procedure were 0.31% m/m and 0.36%, respectively, and for the UV assay procedure they were 0.52% m/m and 0.61%, respectively. As in the repeatability study, the mean (±95% confidence limit) UV and NIR results, 84.52 ± 0.54% m/m and 84.15 ± 0.32% m/m, respectively, gave no evidence for a difference in the methods.

The between analyst precision with the NIR procedure was determined by six different analysts testing the same batch six times on the same day (Table 2). Reference scans were taken between the different analysts’ scans and the coarse sample cell was refilled between each scan. The hypothesis that all the analysts’ results were equally precise was tested using Cochrans’s test. This test compares the largest variance (s²) with the other variances by dividing the largest variance by the sum of all the variances. This was determined to be 0.413, which was less than the critical value of 0.445 (5% significance level), so there was no evidence that the analysts were not equally precise.

### Table 2: Analyst intermediate precision data

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Conclusions

The developed one step NIR method for the quantitative assay of paracetamol in intact Sterwin 500 mg tablets was rapid and statistical analysis of the data indicated that the NIR method was comparable to the British Pharmacopoeia 1993 reference method.

The NIR procedure had the advantages over the reference technique of requiring no sample preparation or the use of potential environmentally harmful reagents. Although the ICH guidelines were developed mainly for the validation of analytical procedures primarily based on the analyte being in solution, it was found possible to apply them successfully to the validation of a reflectance NIR intact bulk tablet assay. This paper illustrates how the full potential of NIR can be utilised and the ICH guidelines applied to the quantification of active ingredients in bulk samples of intact tablets and it is hoped that this will lead to others developing rapid tablet assays. Although the calibration process requires the preparation of intact tablets covering the calibration range and analyses by a reference method, once the calibration has been established duplicate quantitative determinations on bulk batches can be performed in under 2 min.

The mean bias (−0.22%) and mean accuracy (0.45%) were comparable to those for the assay on individual intact tablets by transmission NIR spectroscopy, which had values of −0.08% and 0.59%, respectively, but was five times quicker and therefore ideally suited to the analysis of bulk tablets on-line, allowing real time monitoring of a running production process.

A method similar to the above using a sample transport module, coarse sample cell and a reflectance detector has been
accepted by the Medicines Control Agency for the assay of paracetamol in intact Sterwin tablets.

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