Testing seeds protected with an insecticide by near infrared spectroscopy

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Introduction

It is commonplace in agricultural practice to use seeds treated with plant protection products. The purpose of the present research is to develop a quick method for monitoring batches of seeds, to identify the active ingredient in the coating, as well as to determine its concentration and its uniformity. Being a fast and non-destructive technique, near infrared (NIR) spectroscopy seems to be well-suited to solve these problems. The aim of this work is to establish equations to analyse Tefluthrin, an active ingredient present in Austral Plus, a product used to protect wheat seeds.

Tefluthrin is an insecticide from the Pyrethroids family. It controls a wide range of soil insect pests, particulary Coleoptera, Lepidoptera and Diptera. This active ingredient is solid at ambient temperature. It is soluble in acetone, hexane, toluene, dichloromethane, ethyl acetate and methanol. After extraction in one of these solvents, Tefluthrin is analysed by gas chromatography.[1]

Tefluthrin is one of the three active ingredients of Austral Plus. Austral Plus is made up of 40 g L^{-1} Tefluthrin, 60 g L^{-1} Anthraquinone (a bird repellent, in particular rooks) and Fludioxonil (a fungicide). The amount used is usually 500 mL kg^{-1} of wheat seeds.[1]

Materials and methods

Chemical determinations

Tefluthrin was extracted by acetone during 90 min in an ultrasonic bath. Tefluthrin in solution was analysed by gas chromatography [Hewlett-Packard 6890 Series with an Electron Capture Detector (^{63}Ni) (-ECD-) or with a Flame Ionisation Detector (-FID-)] using the external standard calibration.[2,3]

To determine average concentrations in the batches, Tefluthrin was extracted from 35, 50 and 65 seeds with 50 ml acetone. The concentration on one single seed is determined after an extraction with 5 mL acetone.[2,3]

Acquisition of the spectra, data processing

The seed spectra were acquired in reflection mode on an NIRSystems 6500 (Foss-NIRSystems Inc., Silver Spring, MD, USA) spectrometer. This monochromator is able to collect spectral data from 400 to 2500 nm in steps of 2 nm.

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We have been using two types of sample presentation: the "bulk presentation" to determine average concentrations in the batches of seeds and the "single-seed presentation" to characterise the distribution of Tefluthrin among individual seeds.

In the "bulk presentation", the cell is rectangular and can contain 100 g of seed. During measurement, the cell stops at 32 different places. The result is the average of 32 spectra. In the "single-seed presentation", the seed is measured in a rotating drawer. The cell is equipped with a full aluminium disc with a cavity in its middle where the seed is placed. During the spectral analysis, the cell is turning and the sample is also measured at different places. In both cases, each sample is measured in duplicate.

The spectrum of pure Austral Plus was acquired in transmission mode. The pathlength of the used cell is 0.5 mm. Due to the small available quantity of Tefluthrin, its spectrum was acquired in reflection mode, on an AutoIMAGE Microscope connected to a Perkin–Elmer FT-NIR.

The spectral data were treated with the ISI-NIRS 3 ver. 4.0 software (Foss-Infrasoft International, Port Matilda, PA, USA).[4,5] The calibration was obtained by a modified partial least squares (MPLS) regression technique, as available in the ISI package.[4,5] This technique is the classical PLS[6] algorithm with a standardisation of the *X* residuals at each iteration.[7] This regression technique requires cross-validation to prevent overfitting. Cross-validation estimates calibration performances by partitioning the calibration set into several groups.[7] The ISI software allows calibrations, on the basis of raw spectra, of their first or their second derivatives as well as baseline correction.[4,5] Trial and error is the only way to get the best analytical performances.[5] The latter are characterised by the standard error of calibration (*SEC*), the determination coefficient of calibration (*RSQ*), the standard error of cross-validation (*SECV*) and the determination coefficient of cross-validation (*RSQV*). A ratio SD/*SEC* (SD = standard deviation of the population) of more then 3.0 is required for quantitative determination. The higher this value the more accurate the model is.[8]

Sampling

The seed batches were supplied by the Belgian Ministry of Small Enterprises, Traders and Agriculture. Moreover, 30 batches were treated with accurately known quantities of Austral Plus to get a rectangular distribution.

For the bulk determinations, 98 batches of wheat seeds were investigated to build the calibration to estimate the average concentration. For the single seed determinations, 630 seeds within 42 batches were selected for calibration and for testing the homogeneity within the batches.

Results and discussion

Raw spectra

Before calibration, it is interesting to study the raw spectra. Figure 1 shows specific absorbance bands of Tefluthrin, which are clearly recognisable in the spectrum of Austral Plus and two treated wheat seed batches. As expected, higher peaks in the spectra of treated seed batches correspond to larger quantities of Austral Plus. Some wavelengths (1654–1666, 1944–1954, 2142–2152, 2254–2260, 2308–2312, 2360– 2368, 2442–2446 nm) are common to the spectra of Tefluthrin, Austral Plus and treated batches.

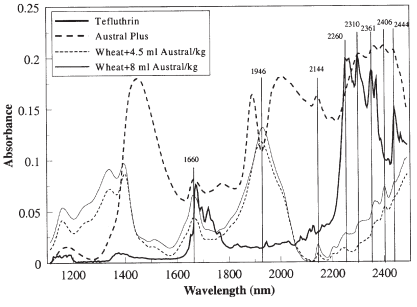

Figure 1. Spectra of Tefluthrin, Austral Plus and two batches of wheat seeds treated at two levels with Austral Plus.

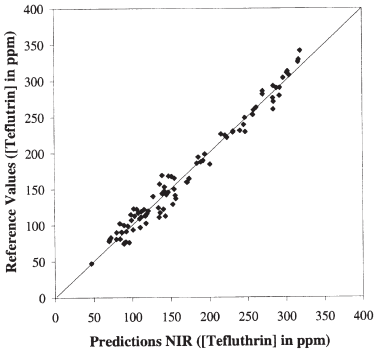

Figure 2. Scatter plots of the regression to predict average concentrations of treatment in batches of seeds.

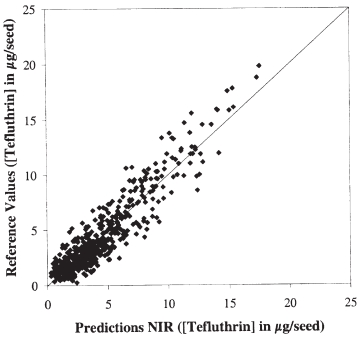

Figure 3. Scatter plots of the regression to predict distribution of treatment in batches of seeds.

Calibrations

Figures 2 and 3 show the two scatter plots of the regression to predict (1) average concentrations and (2) distribution of treatment in batches of seeds. The wavelengths from 400 nm to 700 nm (visible range) were not used in developing the equations. The two databases were searched for outliers using the Mahalanobis distances (*H* statistic). Three samples in the first database (for bulk determinations) and five in the second database (for single-seed determinations) with *H* values higher than three were discarded to avoid singular samples. In the second database, 30 samples with very low concentrations of Tefluthrin were discarded.

In both cases, the best treatment of the spectral data is 2, 5, 5 (2 for the second derivative, 5 for the subtraction gap and the smoothing segment expressed in data points, respectively) without any scatter correction. The number of cross-validation groups is six in the first database and four in the second database. During the calibration, five samples of the first database (5.1% of the population) and 26 of the second database (4,4% of the population) were discarded, owing to too high residual values. The two calibrations (Table 1) are acceptable but the equation to determine the average concentrations of

Table 1. Performance of equations to predict (1) average concentrations and (2) the distribution of the single seed treatment.

	N	Range	Mean	SD	*SEC*	*SD/SEC*	*RSQ*	*SECV*	*RSQV*	PLST
Tefluthrin (ppm)	90	47.3 – 341.6	170.8	76.94	12.85	5.99	0.97	18.80	0.94	7
Tefluthrin (µg/seed)	569	0.31 – 19.80	4.29	3.445	1.32	2.61	0.85	1.56	0.80	12

N: number of samples
SEC: standard error or calibration
SECV: standard error of cross-validation
PLST: number of PLS terms
SD: standard deviation
RSQ: determination coefficient of calibration
RSQV: determination coefficient of cross-validation

Tefluthrin is better than the other one (SD/*SEC* = 5.99 v. 2.61; *RSQ* = 0.97 v. 0.85). The bulk absorbances are stronger than single seed ones as the instrument design has not been modified to focus the light beam on the single seed.

Conclusions

The NIR technique may be used to predict the active ingredient Tefluthrin. The bulk, as well as the single seed measurement, yield satisfactory results. The average concentration of Tefluthrin in batches of wheat seeds can be obtained with good accuracy on the basis of bulk measurements. The single seed measurements allow a good estimate of the distribution of Tefluthrin within seed batches. In future, it would be interesting to build models for other active ingredients and thus other important products used for coating other seeds. A similar approach is currently being developed to predict Imidacloprid, which is an active ingredient in Gaucho, a product used to protect barley seeds.

Acknowledgements

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