NIR for Improved Fertilizer Predictions II
-for Rice

A report for the Rural Industries Research and Development Corporation

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Foreword

This project aimed to continue to research and improve NIR spectroscopy technology relevant to the NIR Tissue Testing Service, operated for rice growers by SunRice. The major new initiatives included:

a) implementing a growth stage calibration, based on findings from the previous project, to check the growth stages indicated by growers when they submit samples.

b) increasing the through-put of samples analysed per hour whilst reducing dust pollution.

c) further automating data handling to reduce the chance of errors and provide more reliable advice to growers.

d) determining the ability of the NIR to report the Mg : K ratios in plant tissue as an indicator of potential grain quality.

In 1986 the researchers first demonstrated that the technique of near infrared reflectance (NIR) spectroscopy could accurately determine the total nitrogen concentration on ground samples of rice shoot tissue. After a successful pilot tissue testing service in the 1987-88 season the SunRice Rice Tissue Testing Service was established to analyse rice samples collected from growers. Since the third season of the service being offered over 40% of the rice growers were sending samples from crops for analysis. On average about 2 crops are sampled from each rice farm.

Surveys of rice growers revealed that the advantages they obtained from the information provided by the Tissue Test included confidence to apply more, or less, fertilizer than they expected was needed by the crop and an overall gain in yield by about 0.6 tonne/ha. Surveys further indicate that producers who have used the service since it became available in 1987/88 continue to use the tissue test recommendation before making the final decision as to how much, if any, fertilizer to apply. This success led to further funding from the Rice Research Committee of the IREC, later the Rice Committee of the RIRDC, to further develop the capabilities of the NIR analyses and to provide the necessary backup to the SunRice laboratory.

This report assesses the current conduct, implementation, grower use, and environmental and economic consequences of the NIR Rice Tissue Test.

This project was funded from industry revenue which is matched by funds provided by the Federal Government.

This report, a new addition to RIRDC’s diverse range of over 1500 research publications, forms part of our Established Industries R&D program, which aims to improve the profitability and sustainability of the Australian rice industry. Most of our publications are available for viewing, downloading or purchasing online through our website:

- downloads at www.rirdc.gov.au/reports/Index.htm
- purchases at www.rirdc.gov.au/eshop

Peter O’Brien
Managing Director
Rural Industries Research and Development Corporation
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Abbreviations

FT  Fourier Transformation
IREC Irrigation Research and Extension Committee
maNage Rice computer software that calculates the response of rice crops to topdressed nitrogen at panicle initiation
NIR Near Infrared Reflectance
PI  panicle initiation
PF  permanent flood
Ricecheck a guide to objective crop management for improving yield, grain quality and profits
RIRDC Rural Industries Research and Development Corporation
Executive Summary

Rice crops accumulate about 166 kg N/ha, of which about 100 kg N/ha is exported from the farm in grain. To ensure high yields farmers apply an average of 124 kg N/ha - the majority just prior to permanent flooding in October and the remainder immediately after panicle initiation (PI) which occurs in January. To improve the understanding of the nitrogen requirements of crops at PI, tissue testing of commercial rice crops is now an integral part of crop management for more than 40% of rice producers.

The SunRice Rice Tissue Testing scheme has been developed with Industry and R & D Corporations support during previous projects: IREC R14 (which commenced 1 July 1986); IREC R21 (1 July 1988), RIRDC DAN 82A (1 July 1992), RIRDC DAN 148A (1 July 1996) and this project RIRDC DAN 148A which commenced 1 July, 1999.

The test operates as follows: Whole shoot samples are collected, weighed in fresh state, and dried in a microwave oven by farmers. These samples are conveyed to the SunRice Appraisals Centre in Leeton where each sample is checked, numbered, ground and analysed using near infrared spectroscopy (NIR). Within 48 hours farmers receive the analysis of each sample and a fertilizer recommendation which may be zero, or as much as 120 kg N/ha.

Project DAN-185A provided significant backup to the SunRice Rice Tissue Testing Service. Over the period of the project the following advances in efficiency and accuracy were achieved:

NIR calibrations for total nitrogen, starch, moisture, potassium, phosphorus and zinc in whole shoot samples were improved, and

Calibrations for magnesium and for the Mg/K ratio were developed. Mg and K in rice grain influence the potential value of rice grain for the quality-conscious market in Japan and Korea. Studies in RIRDC Project DAN175 and CRC projects in Program 2.1 indicate the relations between minerals in the soil, plant and grain. The ability to measure the Mg/K ratio in shoots by NIR at PI has proved to be feasible and this has allowed us to broadly predict the Mg/K ratio in grain. The ultimate aim is to identify crops with quality suited to specific markets to be segregated well before harvest.

In collaboration with staff at SunRice and NSW Agriculture annual revisions have been made to:-
the contents of the Ricecheck manual,
the instructions sent to growers re the operation of the Tissue Test,
the Crop Data Forms sent by growers to the SunRice Tissue Testing Laboratory with each sample and
the Tissue Test Report Forms used to advise the growers and their District Agronomists of the results on each sample analysed.

A database (the Rice Tissue Test Database) is used to record all information on every sample sent for testing, and also to calculate the nitrogen uptake and fertilizer recommendations for each crop, and is revised in line with changes to the forms and recommendations mentioned above. This database has facilitated useful summaries of the information gathered from rice growers such as the influence of seasonal conditions on crop nitrogen and starch ratios, patterns of use of fertilizer, etc. These summaries have been invaluable to gaining a better understanding of the limits to rice production and for informing growers of best fertilizer management. The database also forms the basis of the extended database for the Ricecheck programme.
1. For the 2001/2002 Tissue Test the computer consulting firm, CodeCreations of Albury, developed more efficient NIR to computer and computer to fax software. After some initial problems were resolved this has reduced the time needed to produce reports and recommendations, reduced the chance of errors, and provided more rapid and reliable advice to growers.

2. The SunRice Rice Tissue Test, especially if used in conjunction with the maNage Rice software package for Amaroo, Doongara, Jarrah, Langi, Millin, Namaga, and Opus crops, provides rice producers with relevant, accurate and economic information which aids in deciding the fertilizer program needed to achieve high rice grain yields through nitrogen fertilizer management. Additional benefits include analysis of the nitrogen:starch ratio which indicates if a stress factor, other than nitrogen, is limiting the growth of the crop and the chance of obtaining the expected response to fertilizer nitrogen.

3. This project has also aided the objective to reduce pressure on the environment beyond the rice farm by reducing some of the wastage of fertilizers, eg in situations where inappropriate forms or amounts of fertilizer were applied.

4. Guidelines for the N management of under-fertilized crops prior to PI have been established.

**Value of the Rice NIR Tissue Test to the Industry**

Surveys have indicated that growers who make use of the SunRice NIR Tissue Testing Service achieve, on average, 0.6 tonnes/ha more paddy grain than the average for all rice producers. While there are confounding factors in this comparison it indicates substantial real benefits, perhaps as high as $13 m/year directly to producers, can be attributed to the NIR tissue testing technology.

The NIR Rice Tissue Test was devised to assist growers to decide on the amount of nitrogen to apply to the growing crop. The test has resulted in an overall gain in yield of about 0.6 tonne/ha. A recent economic evaluation indicated that the NIR Tissue test, coupled with the managed Rice program, has returned to rice growers some 8.8 times the money spent on them over the last 16 years.
1. Introduction

In 1986 the researchers first demonstrated that the technique of near infrared reflectance (NIR) spectroscopy could accurately determine the total nitrogen concentration on ground samples of rice shoot tissue. After a successful pilot tissue testing service in the 1987-88 season the SunRice Rice Tissue Testing Service was established to analyse rice samples collected from growers. In the third season of the service being offered almost 40% of the rice growers were sending samples from crops for analysis. Since then approximately 2500 farms have made use of the service, although in any one year participation is still only about 40 to 44% of farms growing rice.

Figure 1: Use of the Rice Tissue Test from 1988 to 2003
On average 2 crops are sampled from each rice farm, and most farms have been sampled in more than one year. For example over 400 farms have been tested twice and 16 have been tested every year for 16 years (Figure 2).

Figure 2: “Return” users of the Rice Tissue Test

Surveys of rice growers revealed that the advantages they obtained from the information provided by the Tissue Test included confidence to apply more or less fertilizer than they expected was needed by the crop and an overall gain in yield by about 0.6 tonne/ha. Surveys further indicate that producers who have used the service since it became available in 1987/88 refer to the tissue test recommendation before making the final decision as to how much, if any, fertilizer to apply.

This success led to further funding from the Rice Research Committee of the IREC, later the Rice Committee of the RIRDC, to further develop the capabilities of the NIR analyses and to provide the necessary backup to the SunRice laboratory. These goals were continued during this project RIRDC DAN 185A.
2. Objectives
This project continued to research and improve NIR spectroscopy technology relevant to the NIR Tissue Testing Service, operated for rice growers by SunRice. The major new initiatives for this quadrennium included:
1. implementation of a growth stage calibration, based on findings from the previous project, to check the growth stages indicated by growers when they submit samples.
2. an increase in the through-put of samples analysed per hour whilst reducing dust pollution.
3. further automation of data handling to reduce the chance of errors and provide more reliable advice to growers.
4. determination of the ability of the NIR to report the Mg : K ratios in plant tissue as an indicator of potential grain quality.

3. Methodology

3.1 Techniques – NIR and chemical analysis
The development of new and improved calibrations for the NIR instrument was based on best practice techniques. These are clearly and comprehensively summarised in documents ASTM E275 (1993) and ASTM E1655 (1994)

For this program samples were readily available from those collected at PI, microwave dried by rice growers and ground by SunRice when tested. While it has not been difficult to select appropriate samples for the nitrogen and starch calibrations, it has been time consuming and expensive to select and analyse samples with a suitable range in concentrations of minerals such as sulphur, phosphorus, potassium, magnesium and zinc. For these elements acid digestion of ground samples, followed by analysis using ICP spectroscopy, was necessary. To aid in the selection of suitable ranges of the various elements use was made of the new software, WINISI, which has been developed by Foss NIRSystems for the NIRSystems 6500 spectrophotometer. The software is able to select those samples with uniquely different spectra for subsequent laboratory analysis, thus reducing costs by eliminating the analysis of redundant samples.

3.2 Development of NIR calibrations

3.2.1 Growth Stage Calibration
Many farmers have difficulty estimating the growth stage as accurately as required for the NIR Tissue Testing laboratory to make a reliable fertilizer recommendation because in most developing crops plants with up to five different growth stages are present. Calibrations were developed for both days after sowing and growth stage. As the current WINISI software can analyse many constituents of any one sample simultaneously, both the calibrations for days after sowing and growth stage were included in the 2001/2002 Rice Tissue Test. The calibrations had been developed from trial samples, collected over two years, in which the different growth stages had been carefully segregated.

High-power scans of plant sections at pre –PI, at PI and post PI stages were incorporated into a poster and distributed to the District Agronomists for use in grower education.

3.2.2 Calibration maintenance
The scanning NIR unit (NIRSystems 6500, Foss – NIRSystems Silver Spring MD) is now being used at the SunRice Appraisal Centre to determine nitrogen, starch, sulphur, phosphorus, potassium and zinc in rice shoots at the panicle initiation stage. The calibrations for these constituents have been checked and updated using WINISI software and the more powerful Partial Least Squares data
manipulation method. The management and updating of calibrations is an exacting and time-consuming task requiring a thorough knowledge of both NIR technology and the software. Calibrations need to be updated with each new season, instrument repair and service, particularly if a new lamp has been added. This involves not only manipulation of data but also re-scanning of the samples used in the calibration. Calibration transfer will be feasible in the new generation of instruments being developed, but with the NIRSystems 6500 calibration maintenance is a continuing process.

During the course of this project the instrument has required three major repairs, all requiring re-scanning of all calibration samples.

3.3 Increase in the through-put of samples analysed.
With the incorporation of the improved and updated data transfer and handling it is apparent that the “bottleneck” in the operation of the tissue Testing service is the actual presentation of each sample to the instrument, the time taken for the analysis to occur and then the re-packing of the sample in its plastic bag. This also causes dust pollution, leading to instrument and human contamination. In order to address these problems and that of the advancing age (12 years) of the current instrument two possible alternative instruments have been trialled.

3.4 Further automation of data handling.
There was a need to speed up the entry of the data needed to generate the grower reports and to ensure less manual entry of data. In the second last year of this project a consultant was engaged to enable the transfer of the analysis data from the NIR spectrophotometer to a computer. The generation of reports was updated to a Windows format and the reports faxed directly to the growers. After many initial problems, this proved to be an efficient and accurate method of information transfer.

3.5 Magnesium: Potassium ratio
Mg and K in rice grain influence the potential value of rice grain for the quality-conscious market in Japan and Korea. Studies in RIRDC Project DAN175 and CRC projects in Program 2.1 indicate the relations between minerals in the soil, plant and grain. The ability to measure the Mg/K ratio in shoots by NIR at PI has proved to be feasible and a calibration for the Mg/K ratio was developed. This has allowed us to broadly predict the Mg/K ratio in grain. The ultimate aim is to identify crops with quality suited to specific markets to be segregated well before harvest.
4. Detailed Results

4.1. Implementation of a growth stage calibration

Based on findings from the previous project, steps were taken to check the growth stages indicated by growers when they submit samples. The calibrations developed, using accurately growth-stage assessed samples from separate trials, are shown in the two lower shaded sections of the table of calibrations used for the 2001/2003 rice season. Both the “Days after Sowing” and the “Zadok No.” calibrations developed have RSQ s of greater than 0.98 and standard errors of calibration (SEC) of 3.3 and 2.3 days respectively. However, when used on grower samples the calibrations proved unsatisfactory, indicating both the growth variability existing within and between plants in a sample and the inaccuracy in identifying panicle initiation.

Panicle initiation is a difficult growth stage to accurately identify and as an aid for growers a poster was developed from scans of plants before, at, and after PI. These posters were distributed to District Agronomists for use at grower meetings (see Figure 3, below).
### Table 1: Summary of NIR calibrations developed for the 2002/2003 Rice Tissue Test using WINISI

<table>
<thead>
<tr>
<th></th>
<th>Calibration Statistics</th>
<th>Validation Statistics</th>
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<td><strong>Range</strong></td>
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<td>N</td>
<td>190</td>
<td>0.67-3.68%</td>
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<tr>
<td>St</td>
<td>137</td>
<td>1.23-32.8%</td>
</tr>
<tr>
<td>P</td>
<td>647</td>
<td>0.11-0.48%</td>
</tr>
<tr>
<td>S</td>
<td>285</td>
<td>0.10-0.24%</td>
</tr>
<tr>
<td>K</td>
<td>635</td>
<td>1.27-3.70%</td>
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<tr>
<td>Zn</td>
<td>183</td>
<td>11-36 ppm</td>
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<tr>
<td>Mg</td>
<td>284</td>
<td>0.12-0.29%</td>
</tr>
<tr>
<td>H2O</td>
<td>31</td>
<td>4.04-12.0</td>
</tr>
<tr>
<td>DAS</td>
<td>91</td>
<td>27-126 ppm</td>
</tr>
<tr>
<td>Mg/K</td>
<td>557</td>
<td>0.04-0.16 ppm</td>
</tr>
</tbody>
</table>
Figure 3 Poster identifying Panicle Initiation

Identifying Panicle Initiation (PI) in Rice

**Rice grown in deep water**
(note the large air spaces)

**Rice grown in shallow water**

Panicle appears as a 2 mm "furry tip" on top of a white bottle-shaped section of elongated stem.
4.2. Increase in the through-put of samples analysed per hour whilst reducing dust pollution.

With the incorporation of the improved and updated data transfer and handling it is apparent that the “bottleneck” in the operation of the tissue Testing service is the actual presentation of each sample to the instrument, the time taken for the analysis to occur and then the re-packing of the sample in its plastic bag. This also causes dust pollution, leading to instrument and human contamination. In order to address these problems and that of the advancing age (12 years) of the current instrument two possible alternative instruments have been trialled.

The first of these was a diode array instrument manufactured by the Perten company (see Figures 4 to 6). This instrument is now in production again (July 2003) after a period of unavailability due to patenting problems. When trialled it proved to be excellent but concerns exist concerning the longevity of the diodes. Samples did not need to be emptied from the plastic bags, mixed and loaded into a sample cup, thus enabling quicker, cleaner analysis. The current Perten diode array instrument is configured differently to the earlier model illustrated here in that the sample is now illuminated from above, rather than below. This is to eliminate the possibility of triggering an epileptic response to the flashing light.
Figure 4: Perten Diode Array – 1

Figure 5: Perten Diode Array – 2

Perten DA7000
with mask to read tissue samples in plastic bags.

Black non reflective paper covered wood block to press plastic bags against platen and provide a uniform background.
The second instrument is a Fourier Transfer (FT) instrument from Bruker (Germany). In addition to being able to scan the sample whilst it is still in its plastic bag, it also offers the option of automated sampling from a 30-piece carousel (Figures 7 to 10). This instrument had only just been released when it was trialled in July 2003.

The spectra produced by a FT-NIR are derived by a different algorism to that of a grating instrument like the NIRS 6500 and have increased data resolution and increased baseline sensitivity but decreased variation (which decreases the need for re-calibration). Figure 11 (typical NIRS 6500 spectrum) and Figure 12 (typical FT-NIR spectrum) illustrate the difference in the spectra.

The 60 samples analysed were presented to the instrument both in plastic bags and in glass vials on the automatic carousel. Both methods produced nitrogen calibrations comparable to that produced by the current NIRSystems 6500 scanning instrument (see Figures 13 to 15). This instrument would be a suitable replacement for the current instrument, allowing a dust-free environment for both the operator and the optics, rapid through-time and, in the case of the carousel, automated analysis of 30 samples at a time.
Figure 7: The Bruker MPA FT-NIR Interferometer

Figure 8: Bruker FT-NIR showing the circular window through which samples are analysed
Figure 9: Bruker FT-NIR - sample presentation in plastic bag

Figure 10: Bruker FT-NIR 30 sample carousel
Figure 11: A Spectrum of rice tissue from Burker FT-NIR interferometer

Figure 12: A spectrum of rice tissue from a NIRSystems 6500 scanning spectrometer
Figure 13: N calibration for rice tissue scanned on NIRS 6500 (current instrument) $R^2 = 0.99$

Figure 14: N calibration for rice tissue in bags, scanned on FT-NIR. $R^2 = 0.99$
Figure 15: N calibration for rice tissue in vials on carousel, scanned on FT-NIR. $R^2 = 0.99$
4.3 Further automation of data handling to reduce the chance of errors and provide more reliable advice to growers.

A new data input and farmer report generating database was developed by Code Creations of Albury for the 2001/2002 Rice Tissue Test. The idea envisaged by the researchers who conceived, developed and who have been involved with the Test since its inception was that the change from the DOS based system to a Windows based one would involve all programming being performed by SunRice personnel. This would have enabled changes and alterations to be carried out as needed. By outsourcing the programming many problems developed, not the least of which was the delay in getting problems fixed. This meant many interruptions to the normal running of the Test which, if the researcher overseeing the Test had been allowed access to the program, could have been averted. The programmer at Code Creations refused to incorporate calculated fields in the resulting database. This was expensive in terms of the time then devoted to the task in re-calculating the data and also in terms of the delay in the relevant information becoming available to the District Agronomists for use with the growers. These problems were addressed before the 2002-2003 season and control of the database returned to the project by SunRice personnel taking over.

Many aspects of the more automated system were particularly beneficial to the efficiency to the Test, namely the downloading of the analyses results from the NIR spectrophotometer to the computer, the report generation and the direct faxing of the reports to the growers.

Previously the term “database” has referred to both the software used for data input and that used for data storage functions (that is, the database so generated and used to develop further extension programs and to track trends).

With the advent of the new software developed for data input and forwarding reports to growers it is now useful to separate these functions. In the future the data input software will be known by this name and the data storage software will be referred to as “the rice tissue test database”.

4.4. Determination of the ability of the NIR to report the Mg : K ratios in plant tissue as an indicator of potential grain quality

Mg and k in rice grain may influence the potential value of rice grain for the quality-conscious market in Japan and Korea. The ability to measure the Mg/K ratio in shoots at PI to predict the Mg/K ratio in grain would be very valuable. Prior knowledge of crops with “good” Mg/K ratios would allow segregation to be planned well before harvest.

Over 600 samples were acid-digested and analysed by ICP to provide the chemical data for the determination of the mineral calibrations. All samples which had poor duplicates for a particular element were removed from the set as each calibration was performed. This ensured that the set of samples was well constructed and contained the reliable chemical information possible.

The calibrations for both magnesium and potassium were robust and provided a sound basis for the calibration developed from the magnesium:potassium ratio.

4.5 Changes to sample collection

On the basis of the fresh weight data and the maps provided by farmers on the crop data forms a review of current sampling practice was initiated and new guidelines for appropriate sampling procedures were developed.

A new sampling tube, developed by the rice physiology research group in conjunction with the district agronomists, was evaluated over the last two years. It was agreed at a meeting that included the district agronomists, representatives of the Rice Research Committee and the rice physiology
research group to begin the use of this tube in the 2002/2003 season. Growers would have the opportunity to continue to take 9 samples, each of 0.2 sqm, instead of the 0.1 sqm, and so increase precision, or to sample approximately the same area as before without loss of precision by taking 6 samples each of 0.2 sqm. However, due to the restrictions placed on the industry due to the drought, the introduction of the new tube has been delayed indefinitely.

In the 2002-2003 rice season some growers participated in a remote sensing trial aimed to help them determine which areas in their crops to sample. This exercise was enthusiastically adopted by the participating growers and will be expanded, in conjunction with commercial agronomists, during the next season.

4.6 Compilation of Manuals
Two manuals were compiled to facilitate the operation of the NIR Tissue Test and are included as Appendices 1 and 2
A detailed manual describing the standard operating procedure for near infrared analysis using a NIRSystems 6500 spectrophotometer and WINISI software (Appendix 1).
A “help” manual for the operation of the data input software developed in conjunction with the IT consultant from Code Creations, Albury (Appendix 2).

A third manual which details the principles of calibration of a NIR spectrophotometer is included as a supporting document (Appendix 3). This manual was prepared by Dr B Osborne, Dr Ian Wesley and Dr R Anderssen as part of GRDC Project No: BRI 82
5. Discussion of Results

The NIR Rice Tissue Test has been in operation since 1988. It is a valuable service for rice growers because it is a rapid and objective measurement of the nutrient status of the developing rice crop. Knowledge of the nitrogen uptake by the crop allows informed decisions to be made as to how much, if any, fertiliser is needed for the crop yield to be economically maximised. This is particularly important in a year of unusual climatic conditions, as in the 2002-2003 season, when the average N-uptake was 151 kg N / ha, compared to only 114 kg N / ha the previous season and a long term average of 126 kg N / ha.

The NIR Rice Tissue Test was devised to assist growers to decide on the amount of nitrogen to apply to the growing crop. The test has resulted in an overall gain in yield of about 0.6 tonne / ha. A recent economic evaluation indicated that the NIR Tissue test, coupled with the maNage Rice program, has returned to rice growers some 8.8 times the money spent on them over the last 16 years.

Since the commencement of the NIR testing service in 1988, when 125 farms were tested, the service is now used by over 40% of rice farmers; it provides more information, with a shorter turn around time and requires fewer staff.

Significant gains have been made to improve the efficiency of operation of the SunRice Rice Tissue test during the course of this project. In particular, the transfer of data from the spectrometer to computer and the generation and automated faxing of reports to growers have streamlined the process and ensured accuracy in the calculation of results. The problem of grower sampling has been evident over the years and has been addressed during this project by the inclusion of grower maps on the crop data forms and the participation by some growers in a remote sensing trial.

New software (WINISI) has been utilised and allows the simultaneous determination of an unlimited number of constituents. This is powerful software and has been successfully interfaced with the Rice Tissue Test database.

As the current instrument (NIRSystems 6500) is aging and beginning to require expensive annual repairs it is time to seriously consider steps towards the purchase of a successor. Two instruments have been trialled and both found to be satisfactory and cost effective. In particular, the Bruker interferometer offers the possibility of real-time savings in the actual analysis time as it is able to automatically analyse 30 samples loaded on a carousel. Both instruments offer the option of much “cleaner” analysis as the samples do not need to be tipped into a container, stirred, loaded into a sample analysis cup, and then unloaded into a plastic bag after analysis. For some time this has been a concern, both for the health of the operators and the instrument, as large amounts of green dust are generated.

Other research projects continue to use and rely on the calibrations developed in this project in the rapid analysis of research samples.
6. Implications

The provision of the Tissue Test is an aid to production efficiency within the industry and guards against unnecessary fertilizer use with its associated environmental contamination. It has led to reduced costs, an increase in the information available to growers, and an increase in yield.

7. Recommendations

Continue to develop NIR technology to better serve the rice industry and to keep abreast of the technological advances in both instrumentation and software.

As the current NIR spectrometer is now twelve years old, it is timely to begin to prepare for the future purchase of a new instrument. It is suggested that, if growers were to be charged a fee to cover the expenses of conducting the service, then a proportion of this could be retained by SunRice as the basis of an instrument replacement fund.

8. Intellectual Property

While there have not been any commercial outcomes, SunRice has been the beneficiary of all information generated during this project. Future directions re commercialisation will be the subject of discussions between SunRice and interested parties prior to the 2003-2004 Rice Tissue Testing Season.

9. Communications Strategy

All findings and developments have been published in papers or incorporated into the SunRice Tissue Testing Service directly.
Appendix 1.

Standard Operating Procedure for
Near Infrared Analysis using
NIRSystems 6500 spectrophotometer
and
WinISI software

Method No :  NIR ANALYSIS
Date        :  October 30, 2002
Prepared by :  S. Ciavarella
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<td>C RISK ASSESSMENT</td>
<td>20</td>
</tr>
</tbody>
</table>
A Amendment Record

On receipt of this amendment record and the accompanying new or revised entries for your copy of the current authorised method, you must:

(a) Insert each new or revised entry in its proper sequence in the document.

(b) Return superseded entries to document controller for disposal.

(c) Attach this amendment record to your copy of the authorised method.

<table>
<thead>
<tr>
<th>Amendment No.</th>
<th>Date</th>
<th>DISCARD</th>
<th>INSERT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Section</td>
<td>Sheet</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Section</td>
<td>Sheet</td>
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<td></td>
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<td>Section</td>
<td>Sheet</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Section</td>
<td>Sheet</td>
</tr>
</tbody>
</table>
B NIR Analysis

1. INTRODUCTION

Near Infrared spectroscopy is the measurement of the intensity of radiation in the region of the electromagnetic spectrum between visible light and the mid infrared. When near infrared radiation falls on a sample, some of the energy is absorbed by chemical bonds between atoms (e.g., C-H, N-H or O-H). The rest of the energy is either reflected by or transmitted through the sample. An NIR spectrometer measures the amount of infrared light reflected by (or transmitted through) the sample.

An NIR spectrometer must be calibrated against a standard laboratory method. Calibration involves taking spectra, using an NIR instrument, from many samples covering the measurement range and also determining the required constituents by standard reference methods. The spectral data is then correlated with the measured constituents, enabling unknown samples to be compared and the constituent concentration determined.
2. FLOW DIAGRAM FOR CREATING A CALIBRATION IN WinISI

**Diagnostics**
- 1. Instrument Performance
- 2. Adjustments

**Scan Samples (Routine Analysis)**
- Collect and Store Spectra

**Population Structuring #1**
*(Make & Use Scores)*
- 1. Eliminate Spectral Outliers
- 2. Calculate PCs & scores

**Add Lab Reference Data**
*(View & Modify Files)*

**Population Structuring #2**
*(Make & Use Scores)*
- 1. ID samples adding variance
- 2. Eliminate redundant samples
- 3. Calculate PCs & scores

**Generate Equation/Model**
*(Regression)*
- 1. Select scatter correction & math treatment
- 2. Select regression method
- 3. Validate equation/model

**Validate Model**
*(Monitor)*
- Use & separate set of samples

**Update Model**
*(View & Modify Files/Merge)*
- Add scans & lab data for some samples not represented in the equation/calibration database

**Implement Model**
*(Routine Analysis)*
- 1. Scan & predict unknowns
- 2. ID samples to add to calibration
3. ABBREVIATIONS AND DEFINITIONS

<table>
<thead>
<tr>
<th>ABBREVIATION</th>
<th>DEFINITION</th>
</tr>
</thead>
<tbody>
<tr>
<td>File extension</td>
<td></td>
</tr>
<tr>
<td>*NIR</td>
<td>Spectra only file</td>
</tr>
<tr>
<td>*CAL</td>
<td>Spectra and lab data</td>
</tr>
<tr>
<td>*ANL</td>
<td>NIR predicted values only</td>
</tr>
<tr>
<td>*EQA</td>
<td>Equation (formula for predicting)</td>
</tr>
<tr>
<td>*PCA (PL1, PL2)</td>
<td>For global H values (centre)</td>
</tr>
<tr>
<td>*LIB (LB1, LB2)</td>
<td>For neighbourhood values (select)</td>
</tr>
<tr>
<td>STD</td>
<td>Standardisation file</td>
</tr>
<tr>
<td>SEC</td>
<td>Standard error calibration</td>
</tr>
<tr>
<td>SEP</td>
<td>Standard error prediction</td>
</tr>
<tr>
<td>SECV</td>
<td>Standard error cross validation</td>
</tr>
<tr>
<td>RSQ</td>
<td>Coefficient of determination. i.e. the amount of variance in the calibration set explained by the calibration. An RSQ of 1 means that 100% of the constituent variance in the calibration samples is explained by the equation l-variance ratio. An RSQ type term from the cross validation set that explains how much of the constituent variance is explained by the calibration equation. A 1-VR of 1 means that 100% of the constituent variance in the calibration samples is explained by the calibration equation during the cross validation process. RSQ is to SEC as 1-VR is to SECV</td>
</tr>
<tr>
<td>1-VR</td>
<td></td>
</tr>
</tbody>
</table>
4. MATH TREATMENTS

a) **SNV**
   Standard normal variance, scales each spectrum to have a standard deviation of 1.0 from the baseline. It helps to reduce particle size effects.

b) **Detrend**
   Removes linear and quadratic curvature from each spectrum.

c) **MSC**
   Multiplicative scatter correction – each spectrum in the set is regressed onto the average spectrum of the set. The result is sample set dependent.

d) **Derivatives**
   These are used to enhance spectra through minimising baseline variations, eliminating slope, and reducing particle size effects. 1\text{st} and 2\text{nd} derivatives are most commonly used. It is not recommended to apply 3\text{rd} and 4\text{th} derivatives as they can cause overfitting of the data.

e) **Common math treatments**

<table>
<thead>
<tr>
<th>1\text{st} digit (derivative)</th>
<th>2\text{nd} digit (gap)</th>
<th>3\text{rd} digit (1\text{st} smooth)</th>
<th>4\text{th} digit (2\text{nd} smooth-rarely used)</th>
<th>Example</th>
<th>Typical Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>4</td>
<td>1</td>
<td>1,4,4,1</td>
<td>Uniform, fine ground, homogenous samples</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>4</td>
<td>1</td>
<td>2,6,4,1</td>
<td>Coarse, wholegrain, non-homogeneous samples</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>10</td>
<td>1</td>
<td>2,10,10,1</td>
<td>Totally unground samples</td>
</tr>
</tbody>
</table>

5. SECURITY

Entry levels limit access to programs, operating parameters and files. The default passwords are:

Entry Level Two – “two” in lower case
Entry Level three – “three” in lower case
6. DIAGNOSTICS

a) Diagnostic Tests: WinISI provides a full diagnostic program to test and evaluate the performance of an instrument.

- **Instrument Response.** This is a measure of the absolute reflectance from the ceramic and tests the performance of the detectors. It reports the “gain factor”. “Auto gain” (not available on the Yanco instrument) sets the detector gain for maximum resolution on each sample for best precision – turns gain “up” on dark samples and “down” on bright samples.

- **Wavelength Accuracy.** (Also called wavelength linearisation). Measure of the wavelength alignment of the instrument using didymium for the visible region and polystyrene for the near infrared region. Sets the wavelength position of the instrument. Automatically downloads the new settings to the instrument.

- **NIR Repeatability.** This is a measure of the deviations in optical data at each wavelength, sometimes called “noise”. The ceramic is scanned first as reference, then as a sample and again as a reference. The sequence is repeated and the two scans are subtracted. The difference between the scans is the internal instrument “noise”.

b) Auto Diagnostics

When selected, all three tests will be run in sequence. The operator is provided with a pass/fail report. This is not available on the Yanco instrument.

c) General Diagnostic Limits

<table>
<thead>
<tr>
<th>Instrument Response</th>
<th>Minimum, maximum numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visible maximum</td>
<td>30,000 – 62,000</td>
</tr>
<tr>
<td>NIR maximum</td>
<td>30,000 – 62,000</td>
</tr>
<tr>
<td>Dark average of 3 values</td>
<td>500 – 5,000</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Wavelength Accuracy</th>
<th>Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visible error</td>
<td>Change K and Phi if the ratio of suggested/current is &lt;0.5000</td>
</tr>
<tr>
<td>NIR error</td>
<td>Change K and Phi if the ratio of suggested/current is &lt;0.5000</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Photometric Repeatability</th>
<th>Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visible repeatability</td>
<td>0.00 – 30.00</td>
</tr>
<tr>
<td>Visible bias</td>
<td>-60 – +60</td>
</tr>
<tr>
<td>NIR repeatability</td>
<td>0.00 – 30.00</td>
</tr>
<tr>
<td>NIR bias</td>
<td>-60 – +60</td>
</tr>
</tbody>
</table>
Running the Diagnostics

- Make sure the instrument is warmed up for at least 15 minutes before making these tests.
- Click on the Diagnostics icon or select Instruments/Diagnostics/Use Dialogue to Select Tests
- An instrument message “Instrument 15 Firmware version 32” appears. This indicates that the lamp is being turned on. Select OK.
- The three diagnostic test, Repeatability, Accuracy and Response are listed.
- Select “Display Schedule” on the right hand side of the screen. For each of the tests set the schedule period to weekly. With this setting, after a week has passed since the last testing of these parameters, a red warning will appear over the Project Manager screen to alert that it is time to perform the instrument diagnostics. You can either run the diagnostics at that time or cancel the screen to perform the tests later.
- Each of the performance tests has to be set up individually under the relevant heading:

**Instrument Response**
- **Output Options:** The ceramic scans can be stored in an NIR file and/or the statistical results in an ASCII file if required.
- **Scanning options:** Number of Repetitions: 5 (default)
- Click on Test to check the instrument response. After the test is complete Cancel to return to the Instrument Diagnostics screen.

**Wavelength Accuracy**
- **Output options:** As for Instrument Response
- **Instrument options:** The two spectral regions (visible and NIR) must be tested separately.
- **Scanning options:** scan numbers 32, 32, 32 with 2 repetitions
- Click on Test to measure wavelength accuracy.
- If the test passes click OK. If the instrument does not pass the test, ie, the Suggested Error is less than 0.5 times the Current Error then change the instrument settings by clicking on the Accept Settings button. If the settings are changed re-run the test to confirm its accuracy.
- Click OK and then Cancel to return to the Instrument diagnostics screen.

**NIR Repeatability**
- **Output options:** As for Instrument Response
- **Edit Wavelength Range:** 800 – 1098 and 1100 – 2498 (separate VIS and NIR regions)
- **Scanning options:** 32, 32, 32 with 10 repetitions
- Save the settings, then click on Test to start repeatability measurements.
- Click OK, then Cancel to return to the Instrument Diagnostics screen.
e) **Note**: Instrument Diagnostics should be performed, in their entirety, a minimum of once a month. The fan filter should be cleaned/changed at that time or as needed. The diagnostic tests should be performed when the instrument is installed or altered in any way (lamp change, repair, etc) and should also be run when the Check cell fails in order to identify the area of concern.

7. **CHECK CELLS**

The check cells are designed for instrument standardisation and to be a convenient mode of monitoring the instrument diagnostics. The analyses are stored over a period of time and indicate trends in the hardware. The check cells should be analysed every day or every shift.

Follow the instructions for Routine Analysis below.

8. **ACQUIRING SAMPLE SPECTRA**

Click on the *Routine Analysis* icon or select *Instrument/Routine Analysis*. Click on the *Files* option on the right hand side of the screen. The *Setup for Scanning Samples* dialogue box will appear.

At least one of the storage options must be selected and a name entered or none of the information generated will be saved. To save spectra select *Spectra Filename* and *One File*. Click on *Preferences* on the right hand side of the screen and tick the first two options (*Save Output Options and Save Output Filenames*).

Click *OK* to return to the previous screen. Click *Advanced*, enter the password (“two”) and the *Edit Product File Information* dialogue screen will appear.

Highlight *check cells* in the *Product Code Description* box

**Options:**

- **Equation File**: Yes, select equation (or set up new INI file) if predicting or *No* if not predicting
- **Product Code**: eg 1
- **Database file**: None (for use with local Calibrations)
- **Report Form**: 0
- **Number of copies**: 1
- **Number of subsample scans**: 1
- **Check cell**: Not checked
- **As rec Moisture**: None
- **Std Moisture Level**: 100
- **DM or Moisture**: DM
- **Test**: DM
- **Global H***: 3.00
- **Global H****: 4.00
- **Neighbourhood H***: 1.0
- **Neighbourhood H****: 1.2
- **T***: 2.5
- **T****: 3.0
<table>
<thead>
<tr>
<th>Instrument Setup</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Instrument Type</td>
<td>6500</td>
</tr>
<tr>
<td>Com port</td>
<td>1</td>
</tr>
<tr>
<td>Baud Rate</td>
<td>38400</td>
</tr>
<tr>
<td>Attachment</td>
<td>Transport</td>
</tr>
<tr>
<td>Cup Type</td>
<td>Small ring cup</td>
</tr>
<tr>
<td>Reflectance/Transmission</td>
<td>Reflectance</td>
</tr>
<tr>
<td>Cup Fullness</td>
<td>Full</td>
</tr>
<tr>
<td>Drawer Switches</td>
<td>Check</td>
</tr>
<tr>
<td>Reference Offset</td>
<td>0.09691</td>
</tr>
<tr>
<td>Fourier Smooth</td>
<td>4.0</td>
</tr>
<tr>
<td>No. Ref Scans to Av Before sample</td>
<td>16</td>
</tr>
<tr>
<td>No. Ref Scans to Av After sample</td>
<td>16</td>
</tr>
<tr>
<td>No. Sample Scans to Average</td>
<td>32</td>
</tr>
<tr>
<td>No. Complete Scans to Average</td>
<td>1</td>
</tr>
<tr>
<td>RMS limit of subsamples</td>
<td>1000</td>
</tr>
<tr>
<td>Derivative for RMS</td>
<td>No check</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Data Collection</th>
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</thead>
<tbody>
<tr>
<td>Spectra Source</td>
<td>Instrument</td>
</tr>
<tr>
<td>Product/Sample # Filename</td>
<td>None (used for autosamplers)</td>
</tr>
<tr>
<td>Standardisation Filename</td>
<td>Yes (Select name of file from standardsiation disk)</td>
</tr>
<tr>
<td>Plot Spectra</td>
<td>Check</td>
</tr>
<tr>
<td>View Subsample Results</td>
<td>No check</td>
</tr>
<tr>
<td>Auto Scan Samples</td>
<td>No check</td>
</tr>
<tr>
<td>Number of Auto Scans</td>
<td>0</td>
</tr>
<tr>
<td>Seconds Between Scans</td>
<td>0</td>
</tr>
<tr>
<td>Spectra Storage Prompt</td>
<td>Yes</td>
</tr>
<tr>
<td>Use Time for Sample Number</td>
<td>No check</td>
</tr>
<tr>
<td>Current Sample Number</td>
<td>1</td>
</tr>
<tr>
<td>Auto Increment Value</td>
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</table>

<table>
<thead>
<tr>
<th>General Analysis</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Analysis Storage Prompt</td>
<td>Yes</td>
</tr>
<tr>
<td>Product Auto Detect</td>
<td>No</td>
</tr>
<tr>
<td>Display H Values</td>
<td>Check</td>
</tr>
<tr>
<td>Display T Values</td>
<td>Check</td>
</tr>
</tbody>
</table>

Select OK to return to the *Setup for Scanning Samples* dialogue box. Enter the desired output filenames.

Click on the *Done* button.

Click on the *Scan* button. The *Sample Confirmation box* appears with the sample numbering appearing (or enter the sample number)

Select *Analyze* and scanning will begin. When scanning is completed the spectrum will be displayed (if that option was selected).

Win ISI will prompt “Store spectra in NIR file?” Click OK. A file ID is now requested. This is not a sample ID so enter information about the product. Re-enter filename.

Enter – results will display.

Enter, input the next sample number and click *Analyze*. Repeat to analyse all samples.


9. **VIEW AND MODIFY FILES**

This program is used to view and modify sample numbers, add or edit laboratory edit laboratory reference values, and sort spectra into different files for evaluation or testing. In addition this program can also be used too make adjustments to equations and to store customer names and addresses.

**Adding Constituents and laboratory Reference Data**
Select the *File View* icon or *Files/View & Modify/Spectra Files*.
Select the spectra file (*.nir) to work with.
Click on *Reference values / Reorder, Add, Delete Constituents*.
Click of the *Add* button and then enter the constituent name.
Answer *Yes* to the question “All lab values will be dry matter based?”
To add values for the constituents click on *Reference Values / Enter / Edit Values*.
Hold Ctrl key down and press the right arrow key to move across the spreadsheet to the column for the constituent.
Save values when prompted

Your *.nir file has now become a *.cal file with the addition of lab data.

**Create a new file**
Highlight a *.cal file and enter *Files / View & Modify Files / Spectra files*.
Click on *Functions / Select / Merge / Create new file*.
Enter filename and file ID when prompted.
Answer *No* to “File the remaining samples?” The original file has not been altered at all.
The samples were only copied to a new file.

**Deleting / Purging Spectra**
From the same screen as above, click on *Functions / Select By Position*. Enter the position numbers.
Samples may also be selected using the *Select* icon on the toolbar.
Click on *Function / Delete / Delete Selected Samples*.
Confirm the deletion. These samples will disappear from view and will not be used in any calculations but can be undeleted until they are purged.
To purge the samples click on *Functions / Delete / Purge deleted Samples*. 
10. PLOT SPECTRA & SCORES

After spectra have been acquired it is advisable to view them to identify any obvious bad scans.

Select the *.nir file you wish to view.
Click on the Plot Spectra and Scores icon and select the spectrum option. Different scatter corrections and math treatments can be viewed.

11. MAKE AND USE SCORES

The next step in creating an equation is to perform population structuring. This program is used to define the spectral boundaries of the population of spectra.

Part 1
Population structuring begins with the identification of sample spectra which are statistically, significantly different from the rest of the samples in the file.
Click the Make and Use Scores icon and then Create Score File from Spectra File.
Select an input filename. It can be either a *.nir or a *.cal file.
Use Loading Type: PCA (if using *.cal file could also have PL1 or PL2)
This procedure will explain the spectral variation in the file.
Select Purge all outlier variation in the file and recalculate.
Change the Loading and Score filenames and enter an outlier and a selected filename.

Options: Measure Distance from Mean
          Cutoff by H and R 3.0
          Select H-statistic

Choose appropriate Wavelengths and Math Treatments.
Click OK
Click the Calculate button to start the calculations.

When the Fraction of Explained variance in Spectra dialogue box appears, the Enter components used to measure H is estimated by the software. This is the calculated number of principal components needed to explain the statistically significant amount of variation in the file. This will appear a second time after the outliers have been removed.
Click Enter.
The Spectral Distances from the Mean dialogue box will appear. By scrolling to the end of the file the outliers can be viewed. You can view the distribution of the samples by clicking on Graphs.

Part 2
The second part of population structuring involves the identification of unique sample spectra within the data set and the subsequent elimination of redundant spectra.
Click Make and Use Scores icon and then click Select Samples from a Spectra File.
Input the filename created under Selected Filename previously in Part 1.
Select Purge non-selected Samples and update loading –scores files and rename the output filename. As this is done the loading and score filenames will also be changed.

Options:  
          Cutoff by H and R: 0.8
          H or R measurement H-statistic
Set the Wavelengths and Math Treatments
Click Calculate to start the calculations.
When the *Fraction of explainable variance in spectra* dialogue box appears, the *Enter components used to measure H* is estimated by the software. The *input file neighbours* dialogue box will appear. Click on *Tables/Selected Samples* to see the number of samples selected. These are the samples identified as adding spectral variation to the file. They are the ones that should be sent out for laboratory reference values and used for the equation development.

12. **EQUATION DEVELOPMENT**

There are several techniques available in the WinISI software for developing an equation. The most commonly used approach for “natural” products is Modified Partial Least Squares (MPLS).

Click the *Regression Equations* icon and select *Global Equations*. Select *Develop equation with the full spectrum* and then click the *Calibration file* button. Choose the *.cal* file generated during Make & Use Scores Part 2. Enter filenames for at least the Equation and Loadings files. Under *Choose a Regression Method* select *Modified PLS*.

**Options:**

Click *Constituents* button and then *Clear*. Highlight the constituents to use and then *OK* in the *Pick Constituents* dialogue box.

- Selected Samples to Delete: 0
- Max Number Terms: 9 (calculated by the software)
- Cross Validation Groups: 5 (calculated by the software)
- Number Outlier Elimination Passes: 2
- Missing Data Value: 0
- Critical T Outlier Value: 2.5
- Critical H Outlier Value: 10.0
- Critical X Outlier Value: 10.0
- Pause Data: Until uses presses continue
  
  For a period of seconds = 5
- Scatter Correction: None

Then click on *Calculate* and *Extend the file*. Also try a 2nd derivative with and without scatter correction.

13. **SELECTING THE BEST EQUATION**

When more than one math treatment and/or scatter correction has been used to develop an equation under one file name, each of the equations is stored separately. The equations resulting from the various math treatments applied need to be examined to determine which is the best for the particular samples.

- For each constituent, the standard error of calibration (ESC) and coefficient of determination (R2) for each calibration equation are important criteria with respect to equation selection. As wavelengths are added to the equation, the SEC and R2 will decrease in stepwise regression.
- The standard error of performance (SEP) is an indication of the performance of the equation on a set of independent samples. For each math treatment the equation SEP will reach a minimum value as terms are added to the equation.
- As a guide, the values of SEC and SEP should be within 20% of each other.
• The math treatment should have the lowest SEC value and the fewest Number of terms (wavelengths) to prevent overfitting. A PLS equation should be chosen on the basis of the lowest SECV not SEC.
• The slope of the regression line should be close to 1.

Click on View & Modify Files/Calibration Equation and Support Files. The statistics for each of the math treatments are displayed in a spreadsheet format. Visually identify which of the equations gave the lowest SECV and highest 1-VR value.

Select Functions/Display Equation and click of the equation selected above. Review the screen to identify the scatter correction and math treatment used to get these “best” statistics. Exit the screen and move the cursor to Files/Calibration equation files. Highlight the chosen equation and click OK to return to the listing of the equations that are not being kept. Then Functions/Delete Selected Equations to remove the unwanted equations. The only remaining constituent equation under the equation name is the one that provided the “best” statistics.

14. MONITOR RESULTS
After an equation is developed it should be validated using a set of samples NOT used to develop the equation. WinISI performs a statistical comparison between the laboratory reference values and the NIR predicted values for a data set to evaluate the equation performance.

Click the Monitor Results icon and then Compare Predicted and Reference Values. Highlight the *.cal file and then the equation filename.

Prediction Basis Dry matter
Missing Value 0.00

Click on Select Pairs button and highlight a constituent pair and choose Add. Repeat for remaining constituents you wish to monitor. Then Calculate. The summary statistics for the 1st constituent will be displayed, as well as the individual Lab vs Predicted results, the residual, and the Global and Neighbourhood H values.

15. ROUTINE ANALYSIS
Once an NIR equation has been developed to predict constituents in a product, the next step is to implement this procedure into the production environment.

a) Loading and unloading the cell:
• Thoroughly clean the glass of the cell with a 50:50 mixture of ethanol and water. Dry with a lint-free tissue
• Pour the sample into a stainless steel mixing cup and stir the sample 10 times with a spoon.
• Place a heaped teaspoon into the cell, spread evenly, and cover with the tape-backed foam pad. Screw the lid on securely
• Position the cell in the instrument with the mark on the cover pointing upwards. This ensures that the effect of any imperfections in the glass is minimised.
• After analysis, remove the cell, unload the sample and repeat the process.

b) The Analysis
Click on the Routine Analysis icon, then Advanced.
Options
Inside the Edit Product File Information dialogue box click the Add button. Key in a product number and description for the new product.
Under Associated Field select the equation name and click OK.
Select Equation Setup and check new equation is selected. All other parameters are as previously set up.
Check Instrument Setup is correct.
Under Data Collection click the Standardisation Filename/Yes and make sure the correct file is selected.
Exit to the Setup for Scanning Samples dialogue box. Enter a Spectra Filename and an Analysis Filename so the predicted results can be saved.
Click Scan and highlight the correct equation and OK.
Enter the sample number and follow prompts.
Click Analyse and when the spectrum is displayed click Exit to view the predicted results for the sample.
Select Scan Accept Sample and then repeat these steps for the remaining samples.

16. BIAS ADJUSTING AN EQUATION
Click View Modify Files/Calibration Equation and Support Files. Select your equation and then highlight Bias and Slope Adjustment. Under the equation select the constituent.
Enter Slope Adjustment: 1 (never change this)
Then follow the prompts and select Adjust Slope and Bias. Check the old results against the new and then click Bias Equation. Repeat for other constituents if necessary.

A bias adjustment can be removed. This is done by returning to View & Modify Files/Calibration Equation and Support Files/Functions/Bias and Slope the Equation then click on the Unbias Equation button.

17. EXPANDING AN EQUATION
Because of the continuing changes in natural products, an equation is never completely finished. An equation should be periodically updated with new spectra and reference data in order to address ongoing product variation.
Click on Regression/Global Equations/Develop Expandable Equations and then New File for Expansion. Select the *.cal file that will be used to expand the equation.
Under Product Library Score File highlight the *.LIB file of the equation to be expanded and key in a unique name for the new equation.

Preferences
Save options on changes
Prompt before overwriting/extending
Automatically save output files when paused and output file(s) entered

Options
Pause Data for a period of seconds 5
Max number term for display use preset number from equation file
Check Create loading and score files box.
Click on Calculate. The software will proceed to generate the new expnded equation.

18. ADDING SAMPLES TO A FILE
During Routine Analysis some samples may have high neighbourhood H values (ie they are not represented in the file). These samples should be added to your database and included in equation development/updating. If samples are received from a new location or are of a variety not represented in the database they should be added to the file and the equation redeveloped.
Select *.cal file to be expanded in the Project Manager and then click View & Modify Files/Spectra Files/Functions/Merge/Merge file(s) to this file/Add to end of current file. Answer “Yes” to Save the Original File and follow prompts. Enter a new name for the expanded *.cal file and then select the *.cal file that contains the data to expand the original file.

Confirm the constituents of the file and follow prompts. Move the cursor to File on the tool bar and select Spectra Files. Highlight the expanded *.cal file. The statistics for this file will appear at the bottom of the screen.

19. EQUATIONS FOR MORE THAN ONE CONSTITUENT

- To use >1 equation in an analysis create what is really a product equation file that comprises several constituent equations.
- Select one of the equations, copy it in the File Manager and re-name the copy (eg 2003Rice).
- Go to Modify Files/Function and add the other equations using Import Files.
- When setting up the Product.INI file for the product you use the product equation and not the individual constituent equation files.
Appendix 2.

Risk assessment for the operation of a NIRSystems 6500 spectrophotometer

WORK PROCESS RISK ASSESSMENT

<table>
<thead>
<tr>
<th>Location</th>
<th>YAI</th>
<th>Sub-Location</th>
<th>NIR Laboratory</th>
</tr>
</thead>
<tbody>
<tr>
<td>Work Process</td>
<td>NIR ANALYSIS</td>
<td>Assessment date</td>
<td>15/08/02</td>
</tr>
<tr>
<td>Assessment performed by:</td>
<td>S Ciavarella</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**HAZPAK RISK ASSESSMENT**

<table>
<thead>
<tr>
<th>Steps in process</th>
<th>Hazards identified</th>
<th>How severe?</th>
<th>How likely?</th>
<th>What priority?</th>
<th>Control measure (Use hierarchy of controls)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loading NIR cell</td>
<td>Dust inhalation</td>
<td>!!</td>
<td>+</td>
<td>4</td>
<td>Turn on extraction fan&lt;br/Use dust mask</td>
</tr>
<tr>
<td>Sitting at fume hood and computer</td>
<td>Back strains</td>
<td>!!</td>
<td>+</td>
<td>4</td>
<td>Make sure laboratory chair is adjusted to correct height and back support is used.&lt;br/&gt;Make sure computer monitor is at the correct height and angle for viewing</td>
</tr>
<tr>
<td></td>
<td>Neck strains</td>
<td>!!</td>
<td>+</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Typing on key board</td>
<td>Repetitive Strain Injury</td>
<td>!!</td>
<td>+</td>
<td>3</td>
<td>Ensure keyboard is at the correct height for operator and arm rest area below keyboard is adequate. Take adequate breaks during long periods of data input</td>
</tr>
<tr>
<td>Standing</td>
<td>Back strain</td>
<td>!!</td>
<td>+</td>
<td>4</td>
<td>Either slide the chair from the fume hood to the instrument &amp; computer or stand on rubber mat.</td>
</tr>
</tbody>
</table>

Approved by:  

Date
<table>
<thead>
<tr>
<th>SUMMARY OF CONTROL MEASURES</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Personal Qualifications and Experience:</strong></td>
</tr>
<tr>
<td><strong>Personnel, Duties and Responsibilities:</strong> Do pause gymnastics each hour or as required</td>
</tr>
<tr>
<td><strong>Training Required to Complete Work:</strong></td>
</tr>
<tr>
<td><strong>Engineering Details/Certificates/WorkCover Approvals:</strong></td>
</tr>
<tr>
<td><strong>Codes of Practice, Legislation:</strong></td>
</tr>
<tr>
<td>OHS Induction</td>
</tr>
<tr>
<td><strong>Plant/Equipment:</strong> Computer, chair and table</td>
</tr>
<tr>
<td><strong>Maintenance Checks:</strong></td>
</tr>
<tr>
<td>Approved by:</td>
</tr>
<tr>
<td>Signed by:</td>
</tr>
</tbody>
</table>
Appendix 3.

NIR Calibration Guidelines - Project No: BRI 82

NIR CALIBRATION GUIDELINES

Project No: BRI 82

September 2002

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