OPUS

Spectroscopy Software

Version 6

User Manual

OVP PhEur 2.2.40

BRUKER

Table of Contents

1 Introduction

This manual describes the OPUS Validation Program (OVP) for spectrometer qualification according to the guidelines set forth by the European Pharmacopoeia 5 (PhEur). OVP for PhEur 2.2.40 is an integral part of the OPUS spectroscopic software.

The tests and test criteria intend to check the Bruker line of spectrometers and modules according to the PhEur regulations. They are designed to follow the guidelines outlined in section 2.2.40 *Near-Infrared Spectrophotometry* by the European Pharmacopoeia 5.

The OQ (Operational Qualification) test protocol checks the instrument performance and compares this performance to the instrument specifications. OQ tests should normally run after installation, maintenance or major repair, exchange of optical components which may influence the instrument performance, on a regular yearly or semi-yearly basis. The PhEur 2.2.40 test protocol is intended to be run as part of the OQ test.

1.1 PhEur 2.2.40 Tests

- Photometric Linearity
- Photometric Noise (high light flux and low light flux)
- Wavelength Uncertainty
- Wavelength Reproducibility

For additional information on the different testing methods, refer to European Pharmacopoeia 5, section 2.2.40.

For further general information on OVP refer to the OPUS Reference Manual.

2 Test Setup

OVP PhEur 2.2.40 supports reflectance channels as well as transmittance channels. Depending on the specified measurement channel OVP PhEur 2.2.40 detects the proper configuration automatically. If you select a reflectance channel, only the tests for reflectance measurements will be activated. If you select a transmission channel, only the test for the transmission measurement will be activated. In case of fiber optic ports you can select between reflectance and transmission mode, depending on the type of probe connected to the fiber port. Once selected, the mode cannot be changed later.

Depending on the spectrometer type and test mode additional external reference standards may be required. The following table shows instrument types, test modes and standards which are used for the different tests. Before setting up the tests make sure that the required standards are available.

2.1 Reference Standards

Photometric Linearity Test:

Instrument Type	Test Mode	Internal Standard	External Standard
MPA MATRIX VECTOR 22/N	Reflection	None	Gold Standard (for stray-light correction spectra only) Labsphere Standard Set with 99% (Reference), 80%, 40%, 20%, 10% optional: 5%
MPA with BRM 2065 and Glass filters NG4, NG9 and NG11	Transmission	Filter NG4 Filter NG9 Filter NG11	None
MPA, MATRIX, VECTOR 22/N with Polystyrene and Glass filter NG4 and NG9	Transmission	Filter NG4 Filter NG9	Filter NG11

High Flux and Low Flux Noise Test:

Instrument Type	Test Mode	Internal Standard	External Standard
MPA MATRIX VECTOR 22/N	Reflection	None	Labsphere Standards 99% and 10%
MPA with BRM 2065 and Glass filters NG4, NG9 and NG11	Transmission	Filter NG9	None
MPA, MATRIX, VECTOR 22/N with Polystyrene and Glass filter NG4 and NG9	Transmission	Filter NG9	None

Wavelength Uncertainty and Wavelength Reproducibility Test:

Note: This test requires only a single standard - if standards are listed in both the internal and external standard column, you can either use the internal or external standard. Alternatively, you can use water vapor instead of reference standards for both reflection and transmission mode. Details are given in attachment 1.

Instrument Type	Test Mode	Internal Standard	External Standard
MPA MATRIX VECTOR 22/N	Reflection	None	Gold Standard or Labsphere 99% for reference measurement SRM 1920a or KTA 1920x or SRM 2036 or CH_2Cl_2 (Suspension in TiO_2)
MPA with BRM 2065 and Glass filters NG4, NG9 and NG11	Transmission	BRM 2065	SRM 2065 or SRM 2035 or BRM 2065 or CH_2Cl_2
MPA, MATRIX, VECTOR 22/N with Polystyrene and Glass filter NG4 and NG9	Transmission	None	SRM 2065 or SRM 2035 or BRM 2065 or CH_2Cl_2

2.2 Setting up OVP for PhEur 2.2.40

If you have already set up OVP for OQ and/or PQ (Performance Qualification)[1], you can skip this chapter.

Before you can use OVP for PhEur 2.2.40 you have to set up the corresponding PhEur 2.2.40 parameters and define the OVP test channels and OVP tests. Select the *OVP Setup* command from the *Validation* menu to open the following dialog:
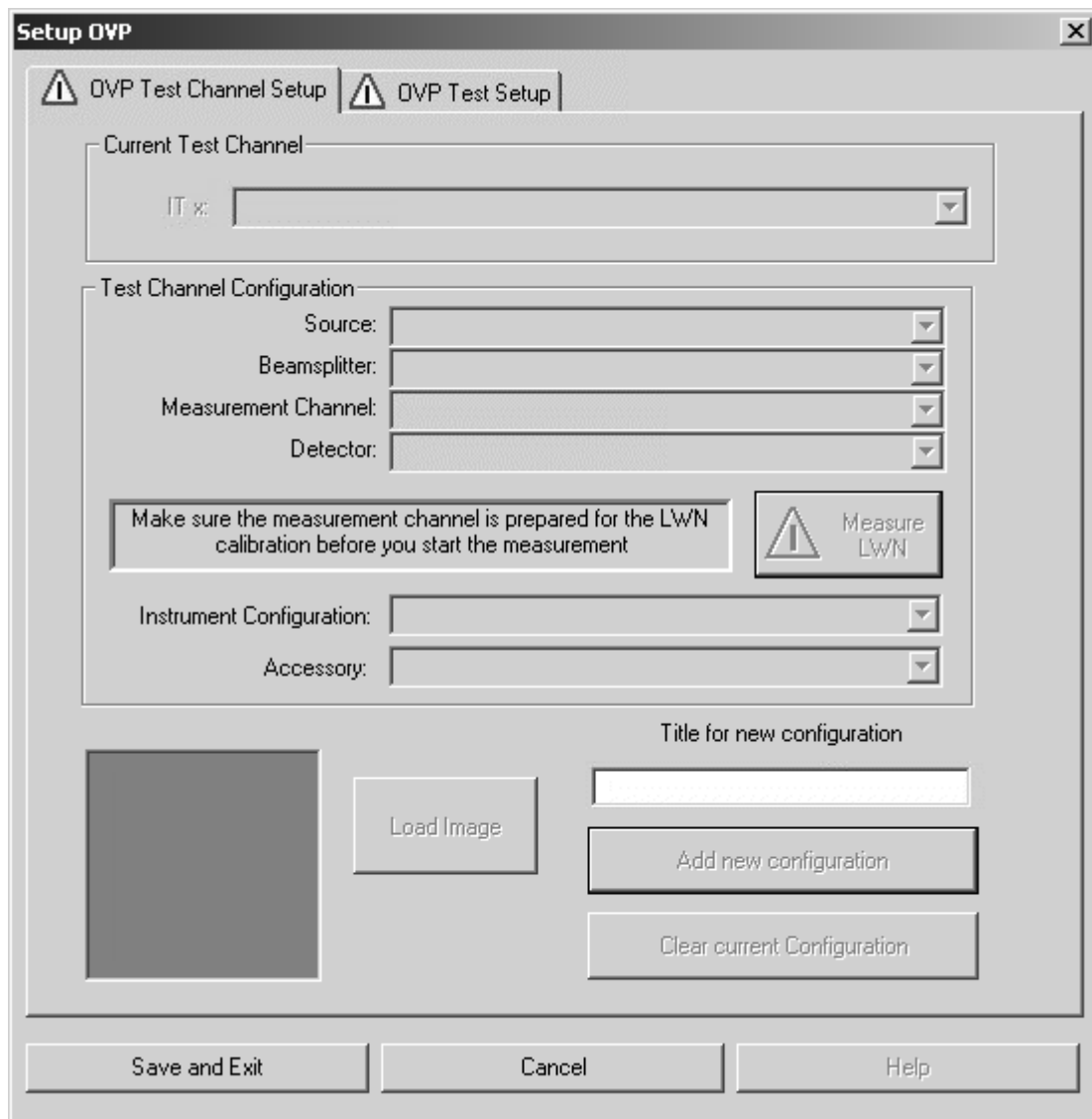

Figure 1: Setup OVP

Enter a title for the current configuration into the *Title for new configuration* entry field, to specify the current setup by a descriptive text.

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1. Short-term test which evaluates the spectrometer performance and proper spectrometer functioning.

Click on the *Add new configuration* button. Now, all the other selection fields will no longer be hidden and can be filled in.
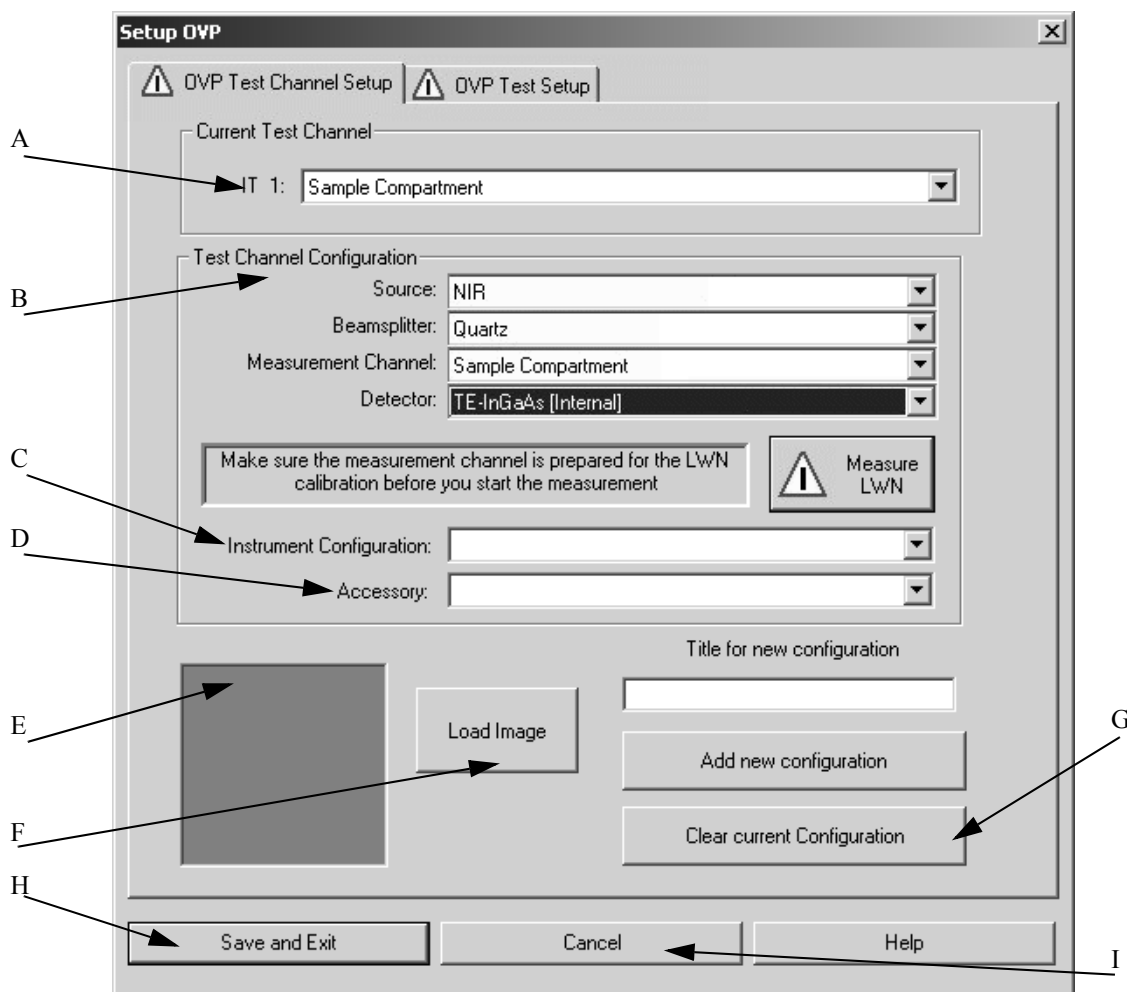

Figure 2: Setup OVP - Activated drop-down lists

A) The drop-down list shows the test channel used by OVP.

B) For each spectrometer test channel you have to assign a measurement configuration to be used during actual validation measurement. This is a valid combination of source, beam splitter, measurement channel and detector. As soon as you have selected all components the appropriate instrument configuration (C) and the bitmap (E) specified for the measurement channel will automatically be displayed.

C) In general, the instrument configuration is automatically displayed as soon as you have selected all components.

D) Depending on the test channel, the drop-down list contains appropriate accessories.

E) Depending on the measurement channel selected a configuration-specific bitmap will be displayed.

F) Use the *Load Image* button to load further instrument images available in OPUS. The bitmaps are stored in the OPUS\VALIDATION\Bitmaps directory. Select the *Thumbnail* option from the pop-up menu when clicking on the ▦▾ button to be able to see at once which bitmap belongs to which file name. For details on the bitmaps refer to the OPUS Reference Manual, chapter 13. You can also make your own photo with a size of 90 x 90 and store it into the bitmap directory using the *ITnn.bmp* file name (*nn* is a consecutive number).

G) To delete the current configuration click on this button. Once deleted the settings cannot be reloaded. If you have accidentally deleted a channel, you have to repeat the complete setup again. OVP configurations are stored in the *OPUS\VALIDATION\Data-base\<Instrument>* directory.

H) To save the configuration click on the *Save and Exit* button.

I) Click on this button to interrupt the setup without saving.
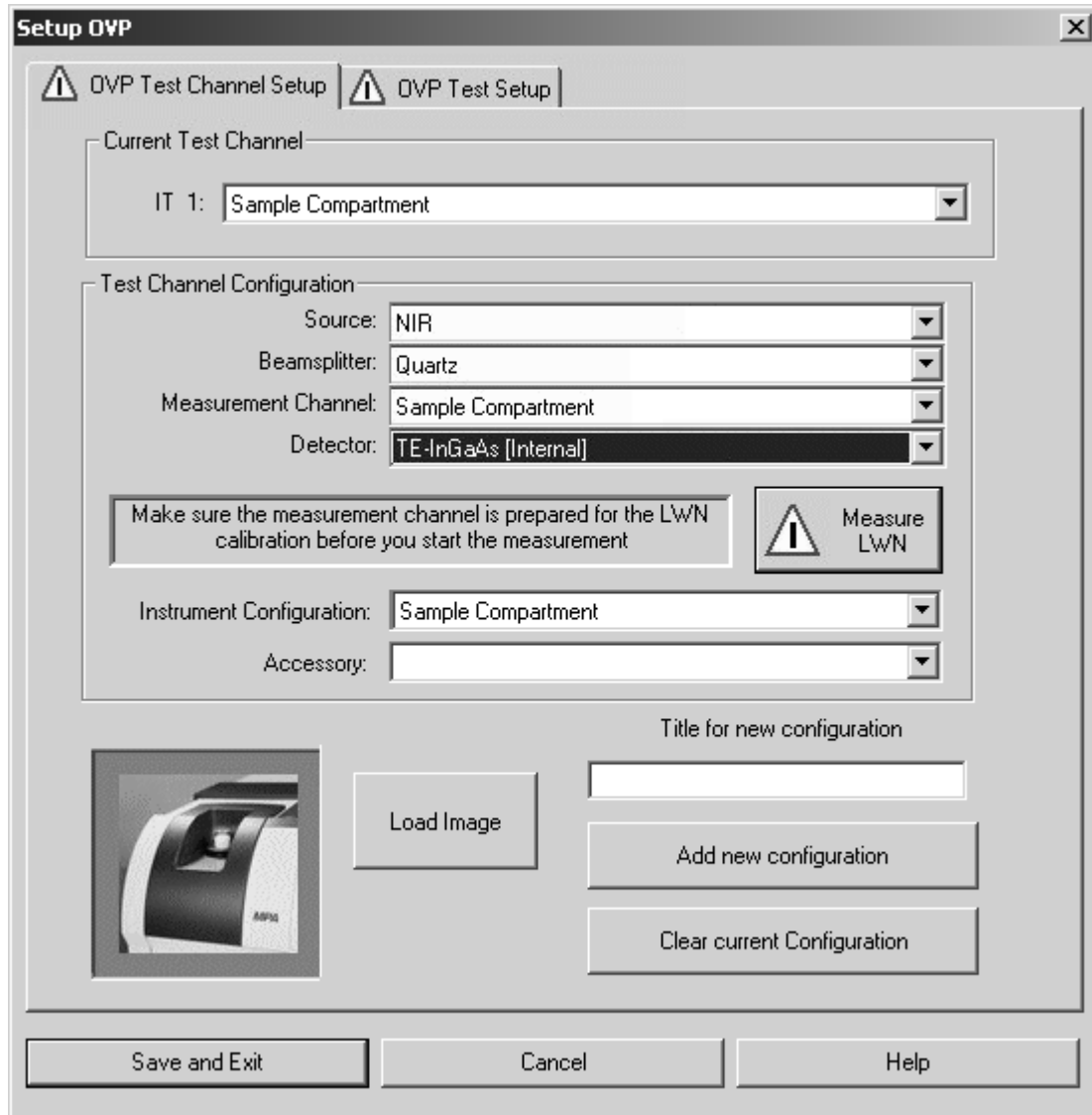
Figure 3: Setup OVP - Bitmap of instrument type

If you click on the *OVP Test Setup* tab, the following dialog opens:
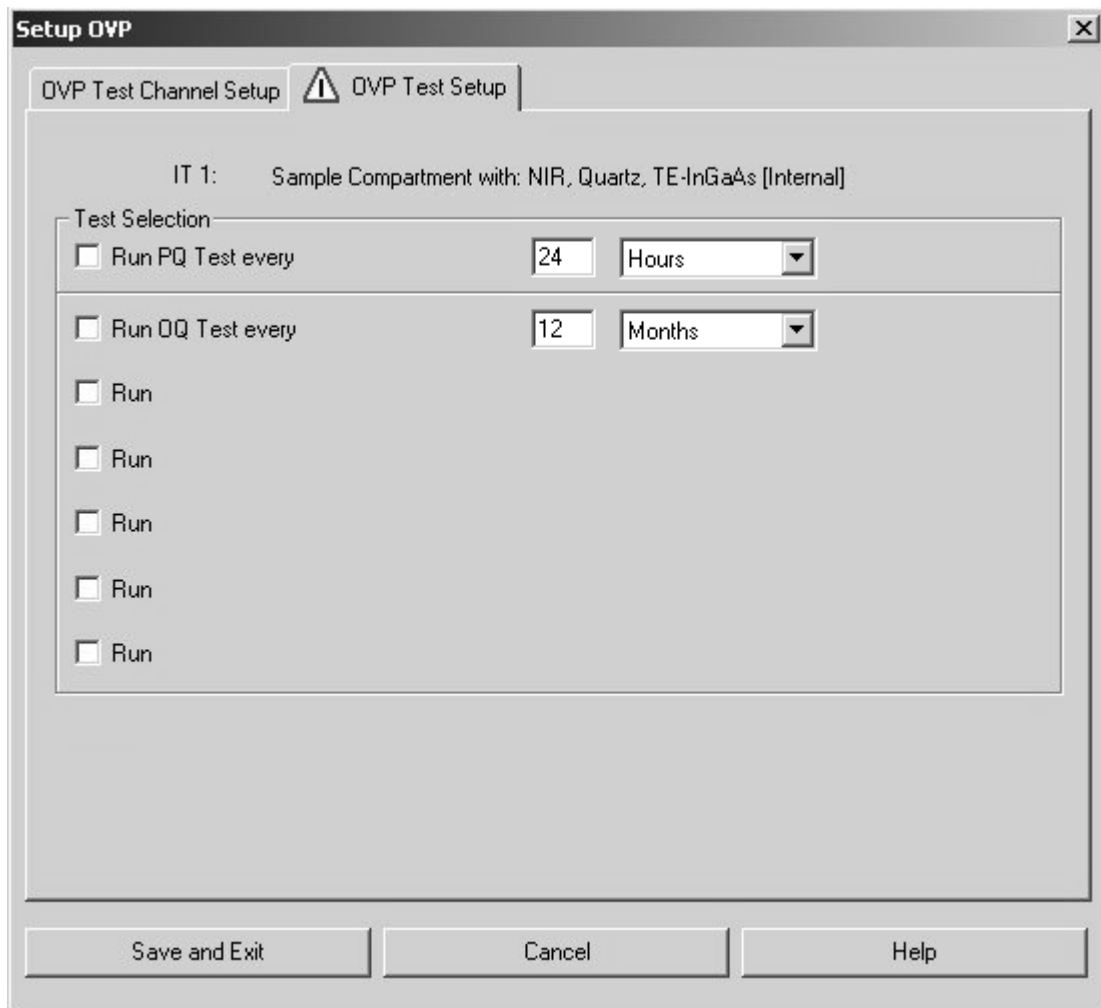
Figure 4: OVP Test Setup tab

The line on the very top shows the test channel selected on the *OVP Test Channel Setup* tab. If you activate the *Run* check box on the *OVP Test Setup* tab, the following line will be displayed:
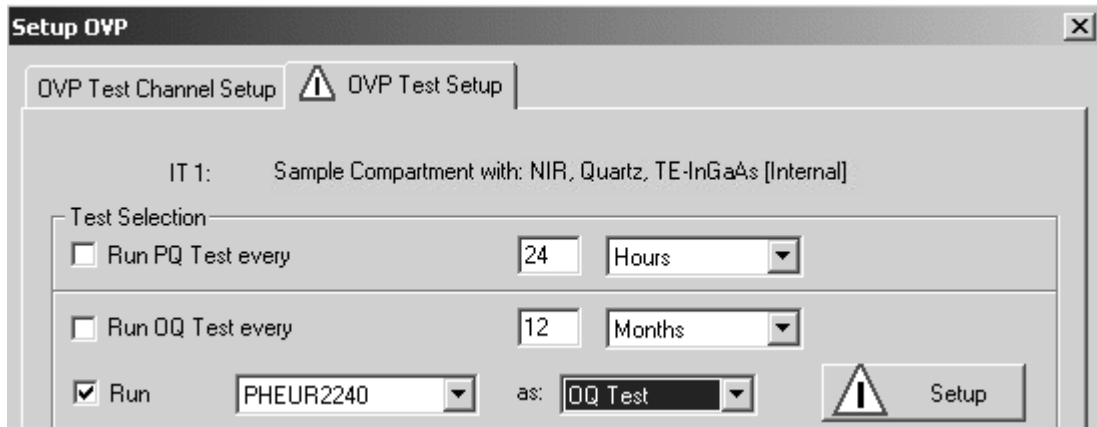
Figure 5: Configuring PhEur 2.2.40 test

Select *PhEur 2.2.40* from the first drop-down list and the *OQ* test category from the second. If the OQ test has not been setup, make sure that the expiration time for the test is properly defined in the OQ line. Type the interval period into the entry field and select the units from the drop-down list. Available units are hours, days, or months.

Click on the *Setup* button to open the *PhEur 2.2.40 Test Protocol* dialog. If all tests have been set up and you click on the *OK* button, the setup status is indicated by a green check mark.
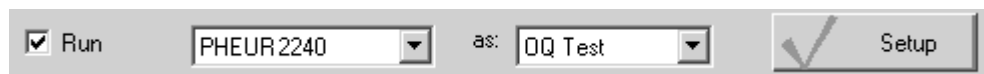
2.3   Reflection Measurement Mode
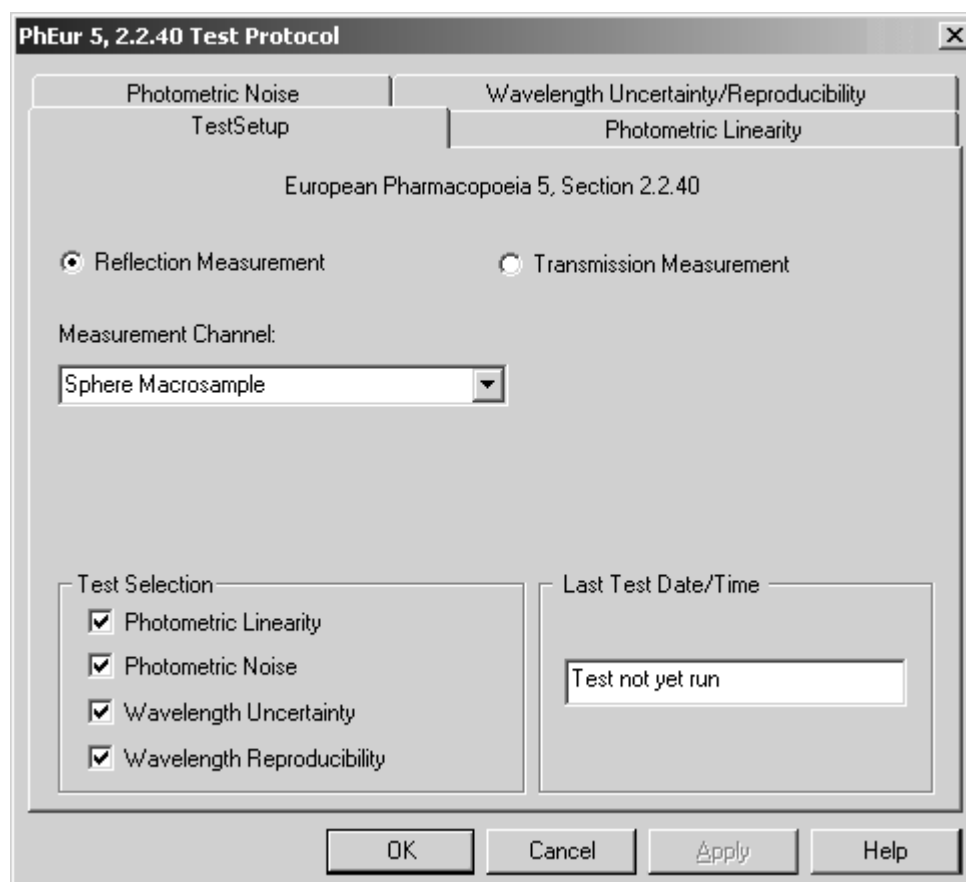
Figure 6: PhEur 2.2.40 Test Protocol - TestSetup for reflection

First, define the correct type of measurement, i.e. either reflection or transmission. Activate the corresponding option button.

Figure 6 shows the setup dialog for reflection measurement. Initially, all tests are selected in the *Test Selection* group field. When using a configuration with an integrating sphere the test channel for OVP will normally be set up with the internal reference position (e.g. Sphere Background for the MPA). In this case the *Measurement Channel* drop down list allows selecting the channel which shall be used for the linearity measurements.

To perform all four tests make sure that all test options are selected. There is a separate page for each test selected. Note that at least one test has to be selected.

2.3.1   Photometric Linearity Test

The photometric linearity test is performed using a set of reflectance standards of known relative reflectance. This test has been optimized for use with the Labsphere™ brand of NIR reflectance standards.

According to PhEur 2.2.40 the spectra obtained from reflectance standards are subject to variability due to the difference between the experiment conditions under which they are factory-calibrated and those under which they are subsequently used.

Hence, the percent reflectance values supplied with the calibration certificate may not be useful to establish an *absolute* calibration for a given instrument. However, as long as the standards have the same physical description, the same reference background is used, and the target instrument measures each under identical settings, the reproducibility of the photometric scale will be established over the range of standards used. Subsequent measurements give information on long-term stability.

The photometric linearity test requires a set of certified reflectance standards with known reflectance values. PhEur 2.2.40 requires one standard (99%) for reference and at least four standards for the test itself (80%, 40%, 20% and 10%). When measuring samples of low reflectance an additional low reflecting standard (5%) is required. Enter the data for the certification table. These data are listed in tables in the calibration certificate, or are provided in digital form on a CD or diskette.

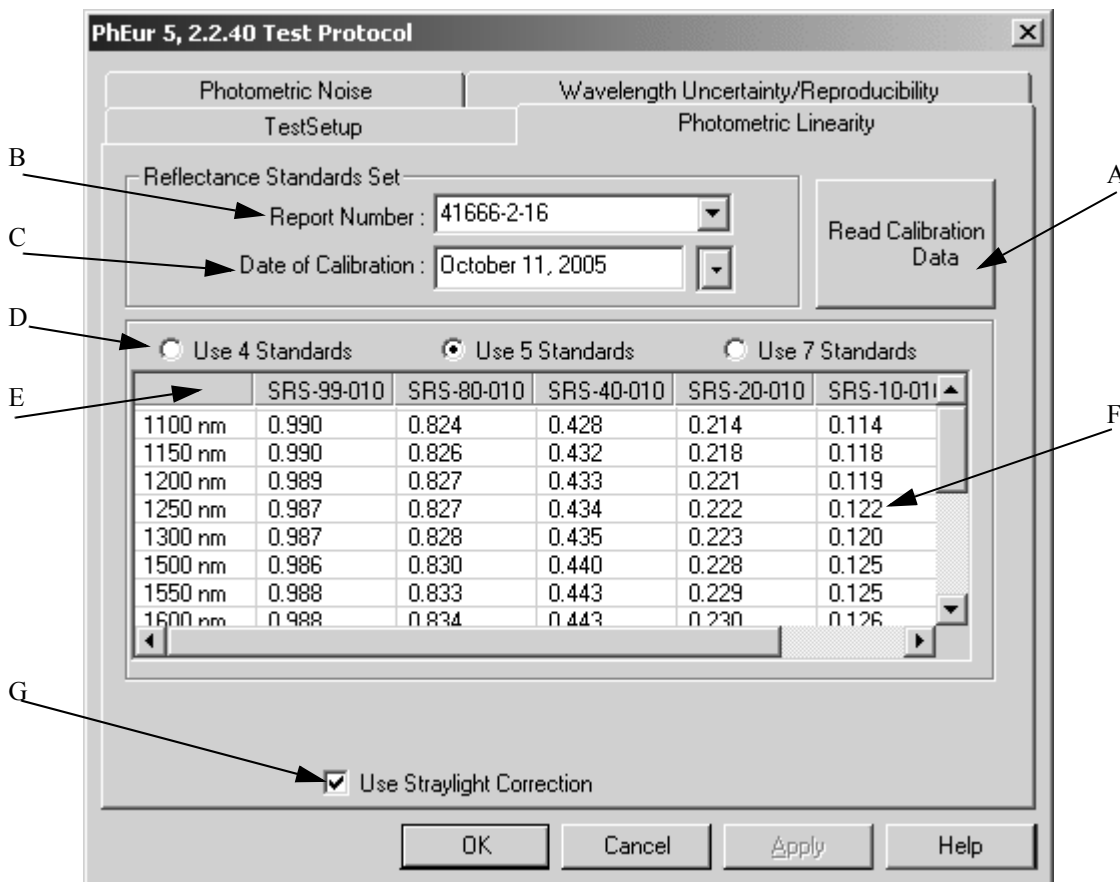Click on the *Photometric Linearity* tab to open the following dialog:

Figure 7: PhEur 2.2.40 Test Protocol - Photometric Linearity tab

A) If you click on this button, a load dialog opens. Load the calibration data file which allows to read the certificate values from a text file which is normally delivered with the set, provided the set was purchased from Bruker. Table F and the drop-down lists B and C correlate to the file data.

B) Enter the report number to identify a set of standards. The report number is listed in the certificate.

C) Enter the date of calibration to identify a set of standards. The date of calibration is listed in the certificate. If you click on the arrow key, a small calendar view pops up.

D) Select the number of standards to be measured for the test (excluding the 99% reference standard).

E) Depending on the number of standard selected the table displays only those columns for which an entry is required

F) You have to enter both the values for the three specified frequencies and two values left and two values right from these frequencies.

G) Activate this check box if a stray light correction has to be done prior to the evaluation of test spectra. If you measure by using an integrating sphere it is essential to use the stray light correction for this test. For details about the stray light correction and how to record the correction spectra refer to the OPUS Reference Manual.

Some hints for data entry

We highly recommend to use a text file for data input. If such a file has not been supplied, generate such a file. For details see chapter 4.

```
Spectralon Standards
----------------------------
Report Number: 41666-2-16
Date of Calibration: October 11, 2005
SRS-99-010
800      0.986
850      0.987
900      0.987
950      0.990
1000     0.989
1050     0.989
1100     0.990
1150     0.990
1200     0.989
1250     0.987
1300     0.987
1350     0.986
1400     0.986
1450     0.986
1500     0.986
1550     0.988
1600     0.988
1650     0.986
1700     0.983
1750     0.986
1800     0.984
1850     0.980
1900     0.980
1950     0.977
2000     0.972
2050     0.959
2100     0.952
2150     0.949
2200     0.965
2250     0.967
2300     0.959
2350     0.950
2450     0.943
2500     0.943

SRS-80-010
800      0.815
850      0.817
900      0.818
```

Figure 8: Text file example for data input

- You do not need to add factors for every column of reflection standard, only enter the data for standards you will be using.
- You have to enter a value for every frequency in the table.
- You have to navigate by using the mouse as the *Tab* and *Enter* keys cannot be used.

2.3.2 Photometric Noise Test

The photometric noise test involves measuring spectra of high and low light throughput which is achieved by using high and low reflectance material. The analysis is performed by measuring the peak-to-peak noise level in successive 100nm ($300cm^{-1}$) spectral segments, between 1200 and 2200nm (8300 and $4500cm^{-1}$). Depending on the measurement channel a different frequency range may be used.

The High Light Flux measurement uses the Labsphere[TM] 99% (SRS-99-010) standard.

The Low Light Flux measurement uses the Labsphere[TM] 10% (SRS-10-010) standard. This measures system performance at reduced light level. Noise from the source, detector, optics, and electronics make significant contributions at this level.
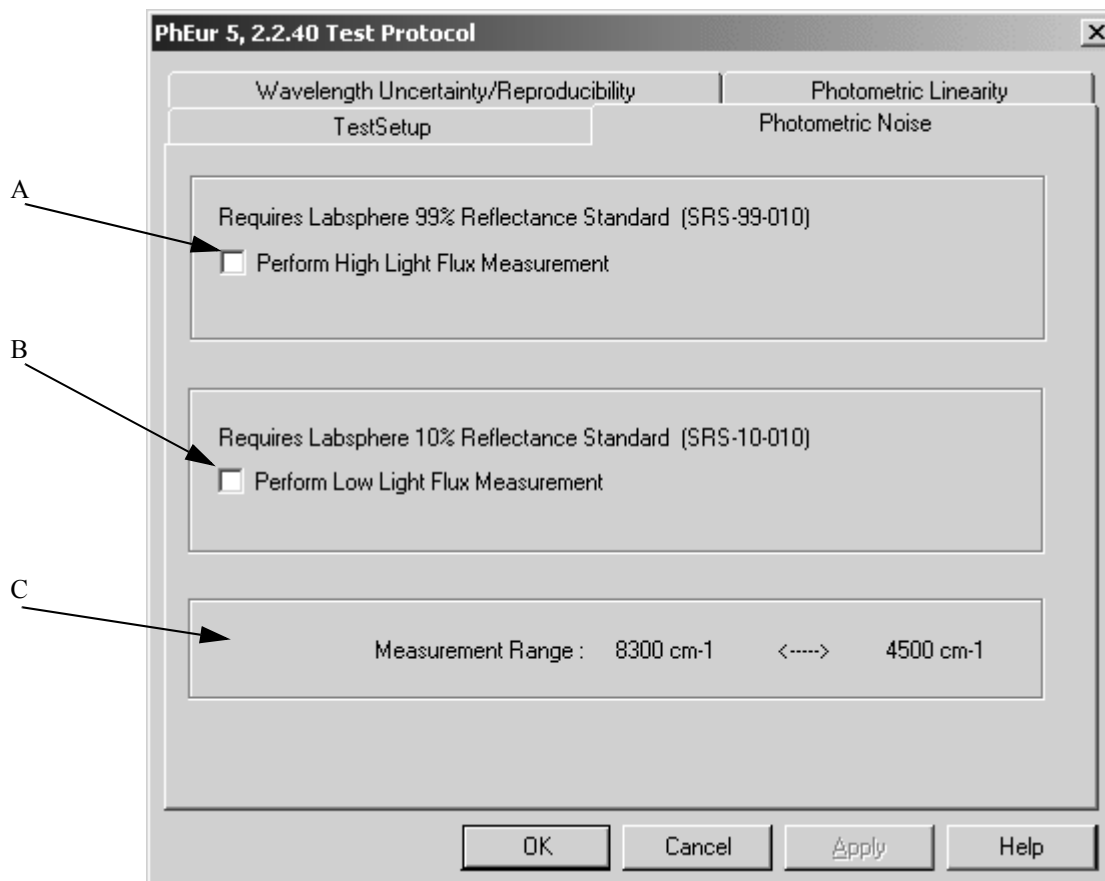

Figure 9: PhEur 2.2.40 Test Protocol - Photometric Noise tab

A) Activate this check box if you want to perform the High Flux Noise measurement.

B) Activate this check box if you want to select the Low Flux Noise measurement.

C) The measurement range used for the specified channel. The range is recommended by PhEur 2.2.40 but may be different if the instrument does not support this range.

2.3.3 Wavelength Uncertainty Test

In case of the wavelength uncertainty test you can choose between two test methods. The recommended method measures a specific peak of water vapor. This has the advantage of not requiring a reflectance standard and also grants a much higher precision. The appendix describes the advantages of using water vapor for this test.
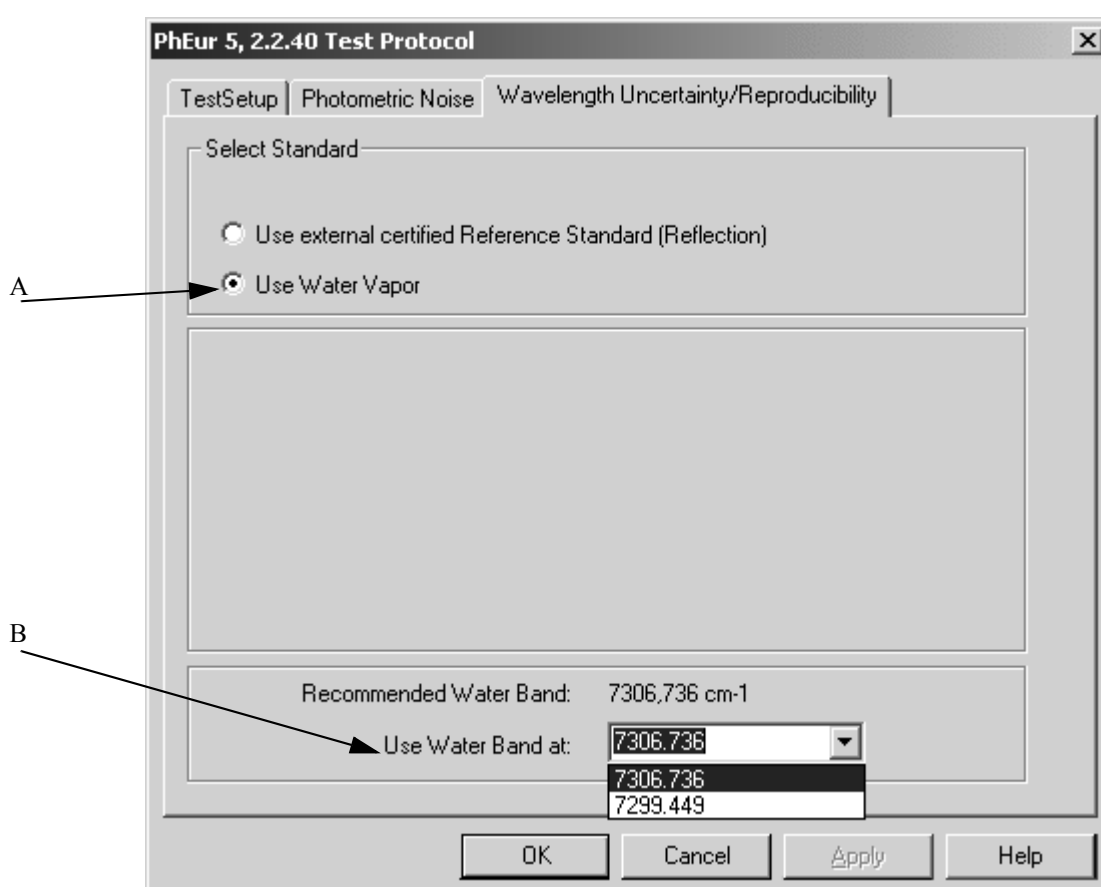
Figure 10: PhEur 2.2.40 Test Protocol - Wavelength Uncertainty test

A) Activating the *Use Water Vapor* option button measures a water vapor peak using the standard peak-picking algorithm.

B) According to PhEur 2.2.40 two water bands are suited for this test. Select either the band at 7306.736cm^{-1} (recommended) or the band at 7299.449cm^{-1}.

The alternative method requires the use of a certified reference standard like NIST 1920a, NIST SRM 2065, KTA 1920x or CH_2Cl_2 (TiO_2 suspension). A standard background measurement is performed, and then the standard is measured. The specific peaks for the reflectance standards are measured with their values determined using the center of gravity algorithm. This test also provides information for long-term system stability.

The band frequencies are listed in the Certificate of Analysis provided with the reference standard. Some certificates list frequencies for a different resolution. Make sure that you choose the frequency values from the column with the specified resolution of 4cm^{-1}.
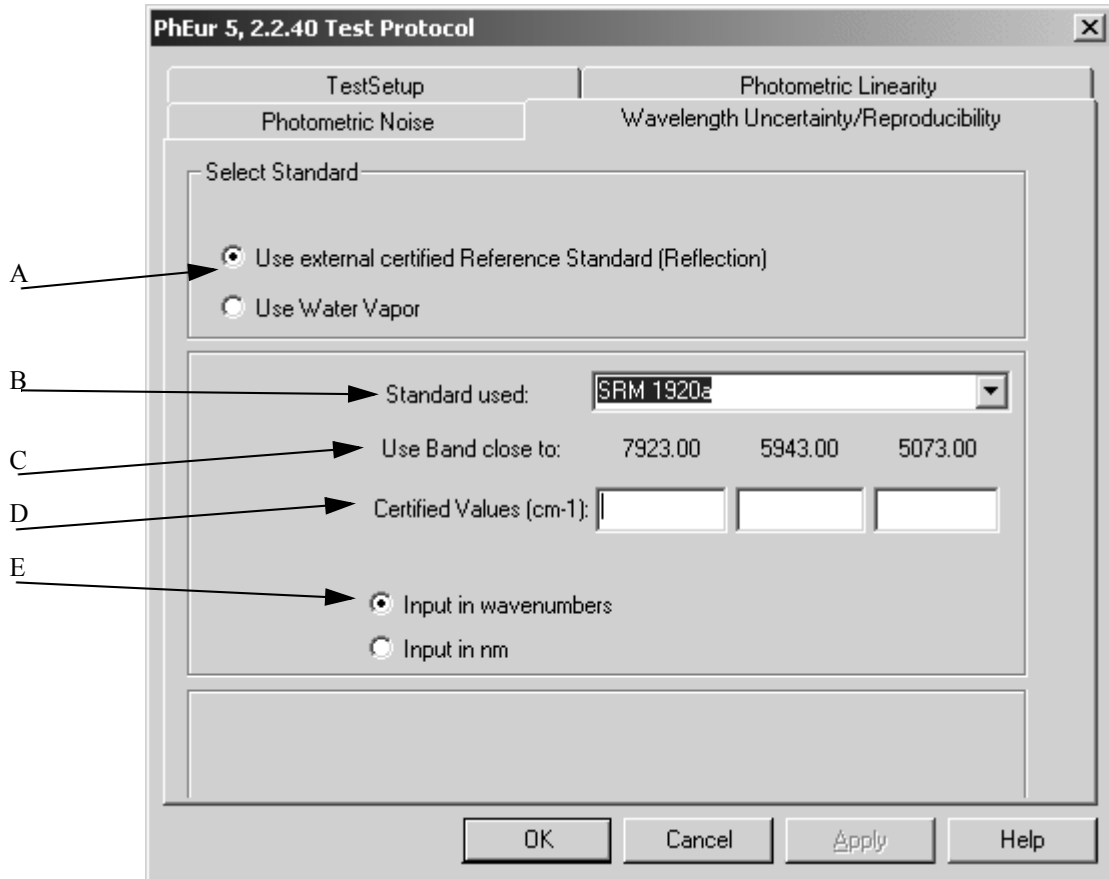
Figure 11: PhEur 2.2.40 Test Protocol - Wavelength Uncertainty test

A) Select the standard to be used for the test. When using water vapor B to E will not be visible.

B) Select the standard to be used from the drop-down list.

C) This line shows the recommended frequencies to be used for evaluation. Select bands from the certificate which are close to these frequencies.

D) The three entry fields allow to enter the bands as provided in the Certificate of Analysis.

E) Depending on the standard the frequencies in the certificate can be listed in nanometers or wavenumbers. Thus, the values can either be entered in nanometers or as wavenumbers. Select the required units by activating the respective option button.

2.3.4   Wavelength Reproducibility Test

This test uses the parameters, settings and standards as specified for the wavelength uncertainty test, except that only a single band is used for evaluation.

Ten sample measurements are performed and the band location of the specified band is evaluated for all ten spectra. Based on the band location of these 10 single measurements the average is calculated. The maximum deviation determined from this average. The deviation must not exceed a specified limit, which depends on the type of standard used. The table below shows the evaluated band and maximum deviation of a few standards.

Standard	Band used (cm^{-1})	Maximum Deviation (cm^{-1})
Water vapor	7306.736	0.05
NIST SRM 2036	10245	0.1
Ch_2Cl_2	6064	0.1

2.4   Transmission Measurement Mode
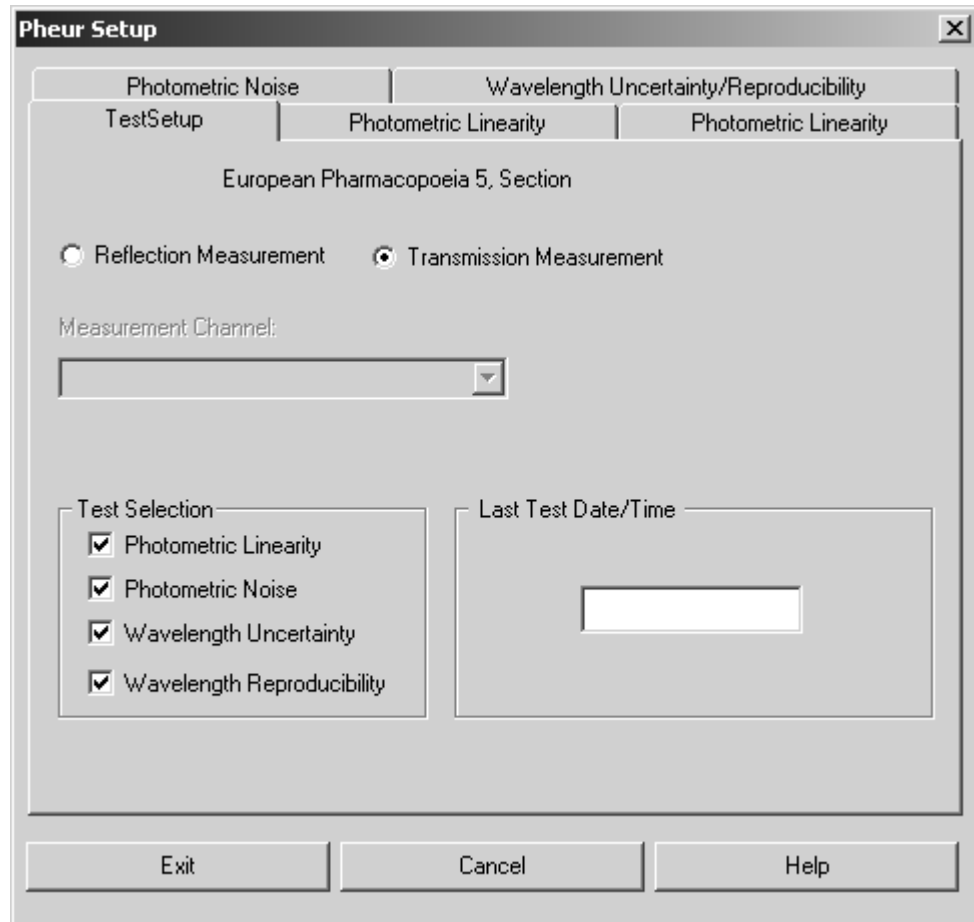
Figure 12: PhEur 2.2.40 Test Protocol - TestSetup for transmission

The test setup for the transmission mode is very similar to the one for reflection mode. Initially all tests are selected. The *Test Selection* group field contains all tests available for transmission measurement.

To perform all four tests, select all the test options. There is a separate page for each test selected. Note that at least one test has to be selected.

2.4.1   Photometric Linearity Test

The photometric linearity test is performed using the open channel and three different glass filters which are mounted on the internal validation unit (IVU) of your spectrometer. Currently no external certified transmission standards with known transmission values are available.

Similar to the reflectance measurements the evaluation uses three different frequencies over the measurement range. The frequencies have been selected with respect to the filter material which does not provide a linear transmission

over the full range. The open channel and three filters have to be measured to cover an as large as possible range of transmission values.

According to PhEur 2.2.40 the measurements are not possible to establish an *absolute* calibration for a given instrument. However, as long as the standards have the same physical description, the same reference background is used, and the target instrument measures each under identical settings, the reproducibility of the photometric scale will be established over the range of standards used. Subsequent measurements give information on long-term stability.

To set up a photometric linearity test you have to measure reference values before you can run the test. Reference measurements should only be repeated after replacement of optical components or major repair.

> **NOTE:** In case of instruments without the internal NG11 glass filter you will be asked during the reference measurement to insert the external NG11 standard which is available at Bruker.
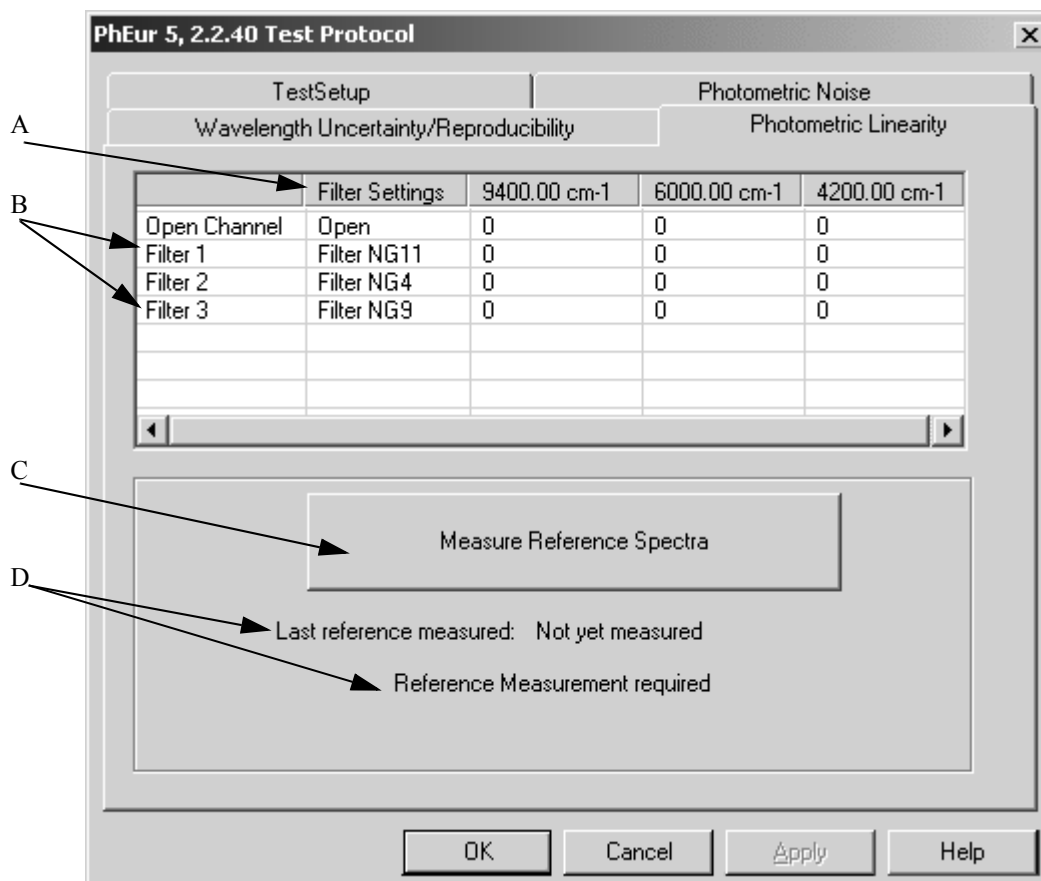
Figure 13: PhEur 2.2.40 Test Protocol - Photometric Linearity tab

A) The *Filter Setting* column shows the filters used for testing. The filters are automatically determined by the program

B) The values for the reference measurement are displayed in the table.

C) Click on this button to measure the reference spectra.

D) These lines show if and when the last reference has been measured. In the latter case the date and time of the measurement is also displayed. These lines also show whether a reference measurement is in progress.

The transmission values are retrieved from the spectra and filled in the grid. Typical filter spectra and the frequencies used for evaluation are shown in figure 14.
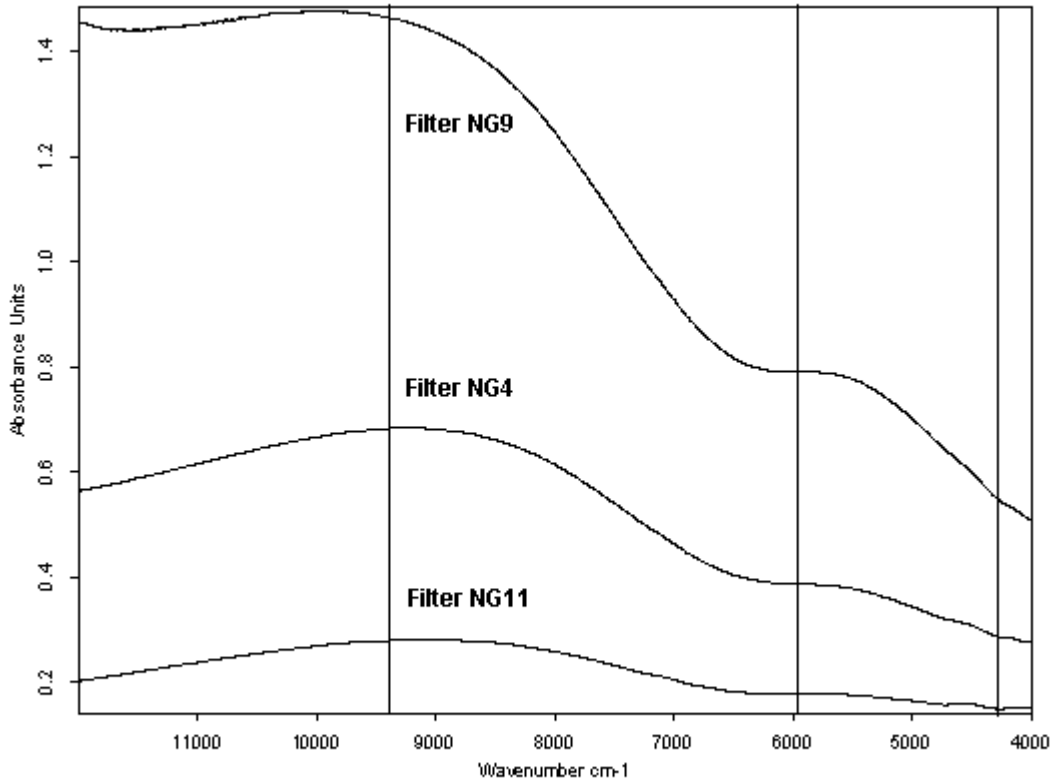
Figure 14: Typical filter spectra

2.4.2 Photometric Noise Test

The photometric noise test involves measuring spectra using the open channel and one of the glass filters on the IVU as low-transmission material. The analysis is performed by tabulating the Peak to Peak (PP) noise level in successive 100nm ($300cm^{-1}$) spectral segments between 1200 and 2200nm (8300 and $4500cm^{-1}$) and the average of these values. Depending on the measurement channel a different frequency range may be used.

The High Flux Noise measurement uses the open channel. The Low Flux Noise measurement uses the glass filter NG9 or the internal Spectralon. This measures system performance at reduced light levels. Noise from the source, detector, optics, and electronics make significant contributions at this level. If one of the tests is not available for a certain instrument configuration, this test will be disabled.
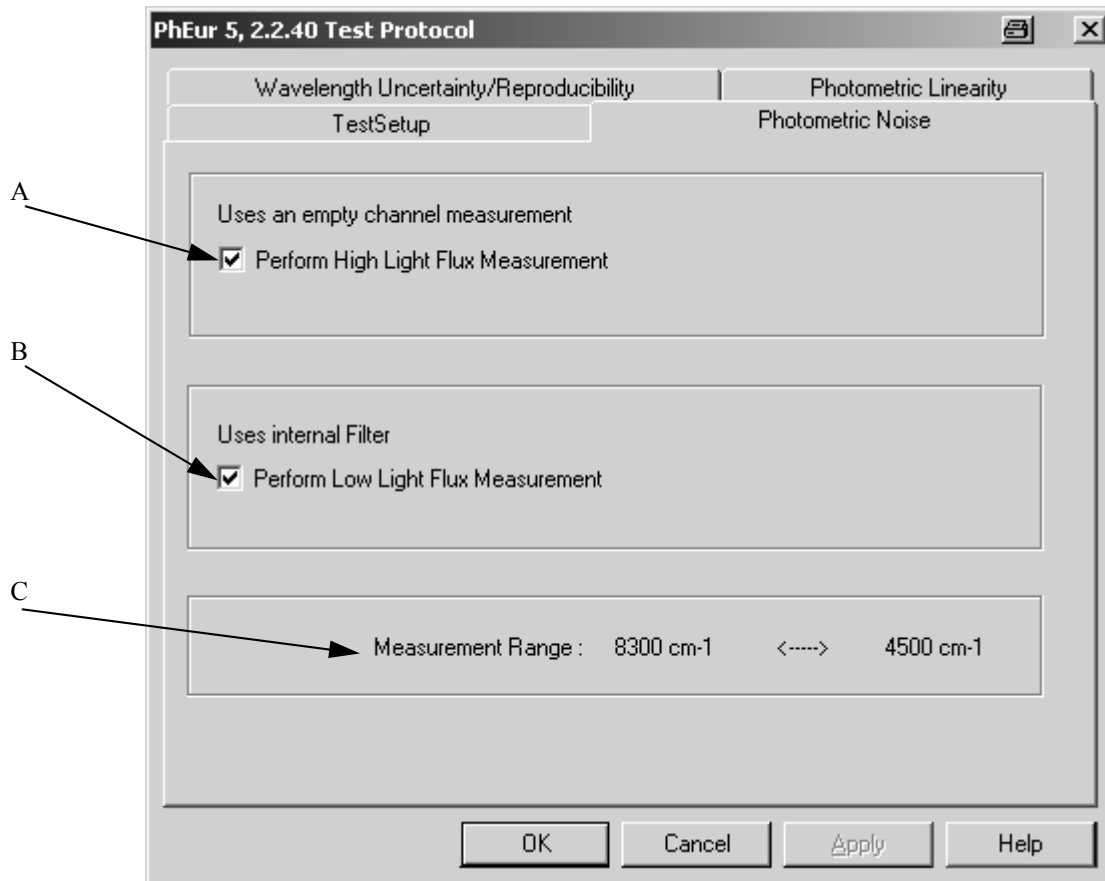
Figure 15: PhEur 2.2.40 Test Protocol - Photometric Noise tab

A) Activate this check box if you want to perform the High Flux Noise measurement.
B) Activate this check box if you want to perform the Low Flux Noise measurement.
C) The measurement range used for the specified channel. The range is recommended by PhEur 2.2.40 but may be different if the instrument channel does not support this range.

2.4.3 Wavelength Uncertainty Test

In case of the wavelength uncertainty test you can choose between two test methods. The method recommended by Bruker measures a specific peak of water vapor. This has the advantage of not requiring a transmission standard and also grants a much higher precision. The appendix describes the advantages of using water vapor for this test.
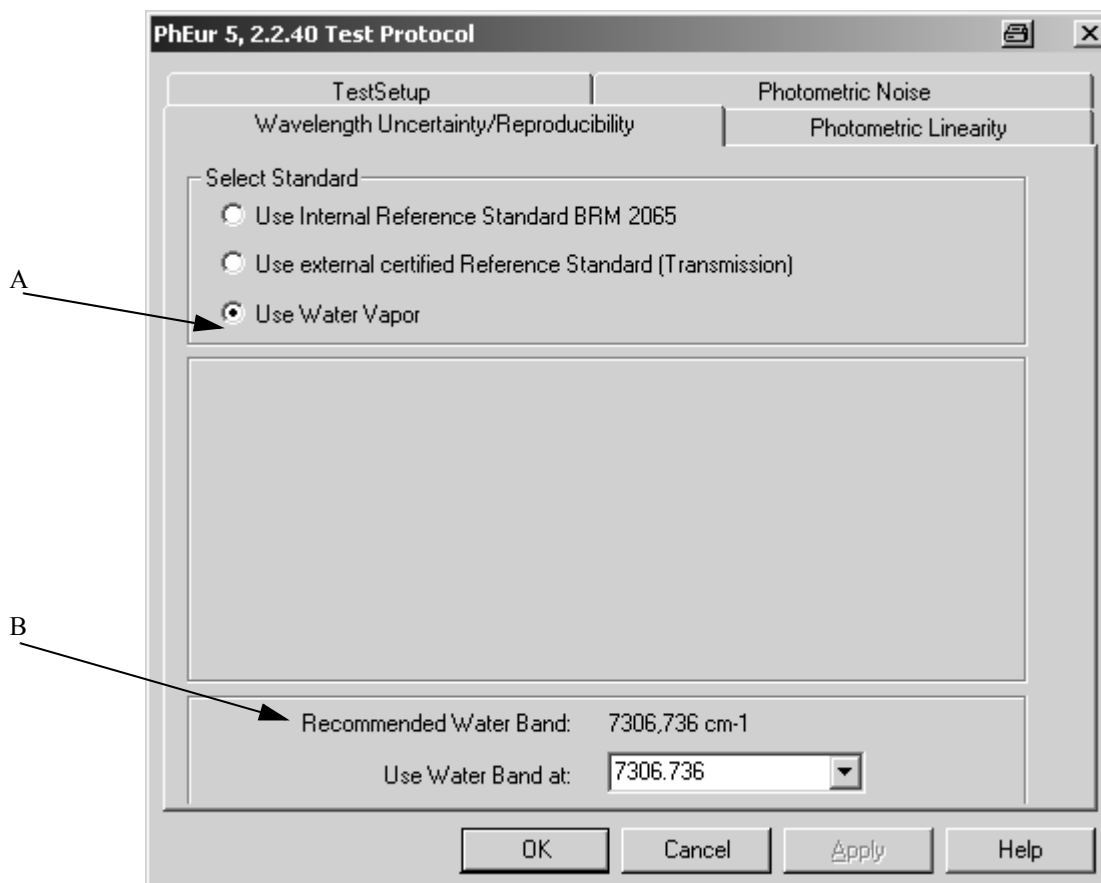
Figure 16: PhEur 2.2.40 Test Protocol - Wavelength Uncertainty test

  A) Activating the *Use Water Vapor* option button measures a water vapor peak using the standard peak-picking algorithm.
  B) Two water bands are suited for this test. Select either the band at 7306.736cm^{-1} (recommended) or the band at 7299.449cm^{-1}.

External standards like NIST SRM 2065, SRM 2035 or CH_2Cl_2 can be purchased and used as well. Bruker offers a traceable BRM 2065 reference standard which is mounted on the IVU and which allows to run all tests fully automatically. A certificate for this standard is available.

Note: For instruments without the internal BRM 2065 an external transmission standard (BRM 2065 or SRM 2065) is required if you want to use a standard. This standard is available from Bruker.

First, a background measurement is performed. Then, the standard is measured. The specific frequency peaks for the reflectance standards are measured with their values determined using the center of gravity algorithm. This test also provides information for long-term system stability.

The band frequencies are listed in the Certificate of Analysis provided with the reference standard. Make sure that you select the frequency values from the column with the specified resolution of 4cm^{-1}. Recommended band frequencies vary for different standards and are shown on the *Wavelength Uncertainty* page.

Thus, before entering the reference values from the certificate make sure the proper reference standard has been selected from the standards drop-down list.
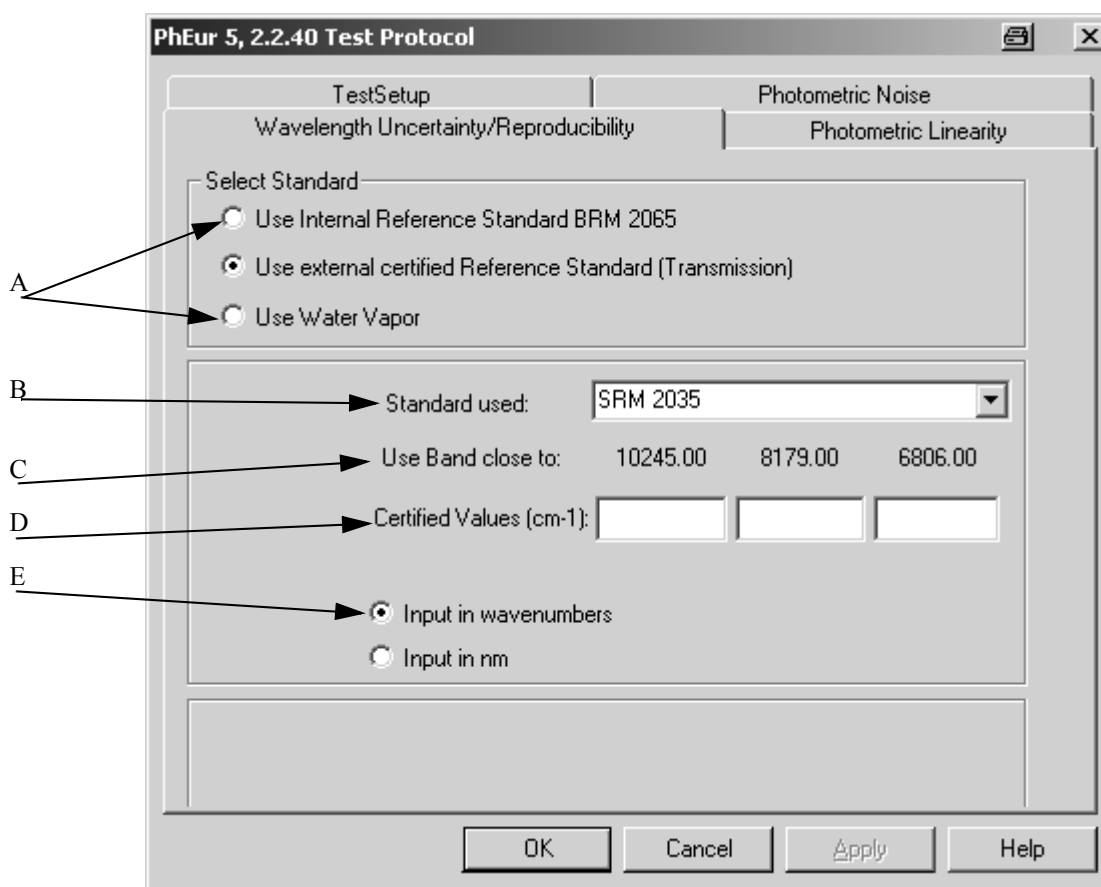
Figure 17: PhEur 2.2.40 Test Protocol - Wavelength Uncertainty test

A) Select the standard to be used for the test. When selecting the internal or external reference standard using water vapor B to E will not be visible.
B) Select the standard to be used from the drop-down list.
C) This line shows the recommended peaks to be used for evaluation. First, select the standard as described in A.
D) Three entry fields are provided to enter the peak frequencies as provided in the Certificate of Analysis.
E) Depending on the standard the frequencies in the certificate can be listed in nanometers or wavenumbers. The values can thus either be entered in nanometers or as wavenumbers. Select the required units by activating the respective option button.

If you use an internal standard and in case of instruments previously designed, the standard data are read from the instrument and entered automatically. In this case you cannot enter any data into the entry fields. Additionally, a temperature control of the band position is automatically performed in the subsequent test.

2.4.4   Wavelength Reproducibility Test

This test uses the same parameters, settings and standards as specified for the wavelength uncertainty test. If a reference standard is selected for the wavelength reproducibility test, the frequency specified in the first entry field is used (D in figure 17).

A reference measurement is only required if the wavelength uncertainty test had not been run before. Ten sample measurements are performed and the band location of the specified band is evaluated for all ten spectra. Based on the band location of these 10 single measurements the average is calculated. The maximum deviation determined from this average. The deviation must not exceed a specified limit, which depends on the type of standard used. The table below shows the evaluated band and maximum deviation of a few standards.

Standard	Band used (cm^{-1})	Maximum Deviation (cm^{-1})
Water vapor	7306.736	0.05
NIST SRM 2036	10245	0.1
Ch_2Cl_2	6064	0.1

3 Run OVP Tests

There are two possibilities to run OVP tests. Either by selecting the Run *OVP Tests* command in the *Validation* menu or by clicking on the status light ( ) and using the *Instrument Status* dialog. For details on the letter case refer to the OPUS Reference Manual, chapter 13.

To start running the tests click on the *Run OVP Tests* command in the *Validation* menu. The following dialog opens:
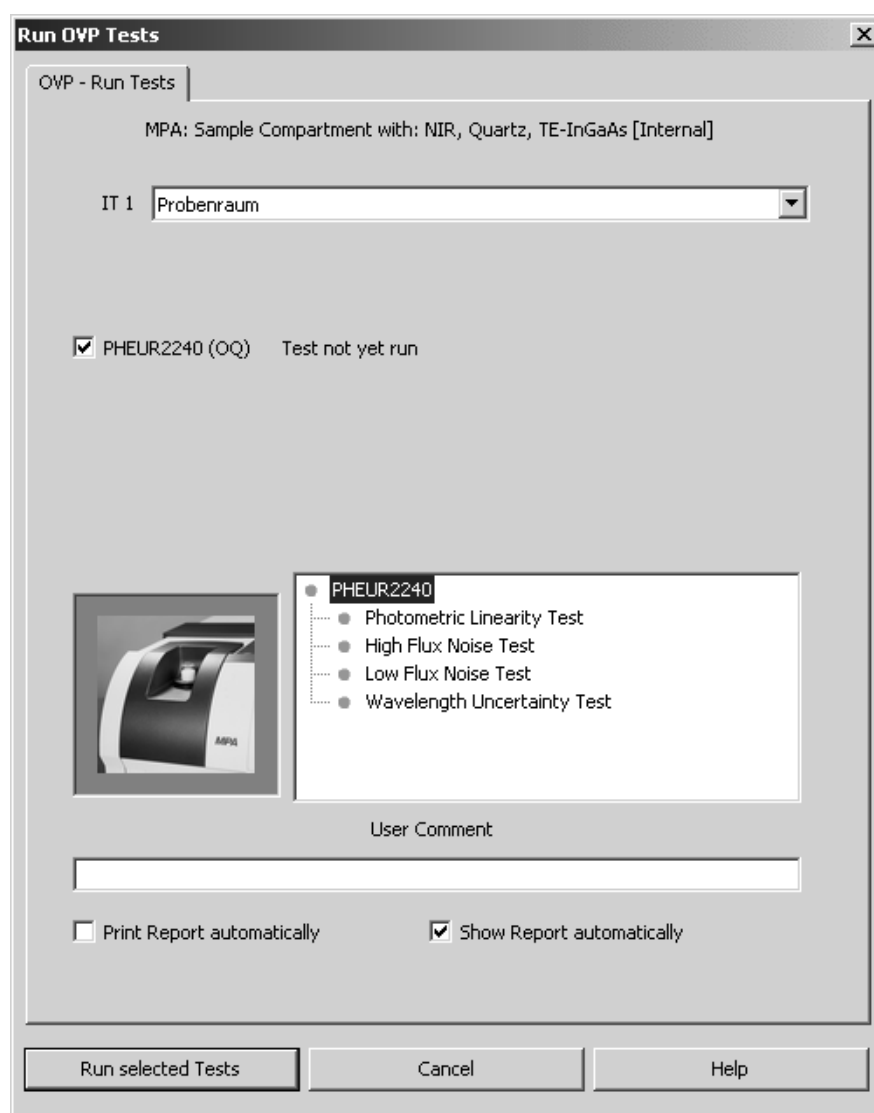
Figure 18: Run OVP Tests

The *IT n* drop-down list allows to select the appropriate IT test channel. The lines below show the tests set up for the IT test channel selected, as well as the test status. Tests which have expired, or which have not yet been performed are automatically activated for measurement. Tests which interval has not yet expired are not activated, and their remaining time is indicated as a reminder.

If a test has not expired, but the interval has only e.g. 2 hours left you can activate the check box for that test and run the test earlier.

If a test has expired, but you are not prepared to perform that test (external standards required), you can deactivate the check box for the corresponding test.
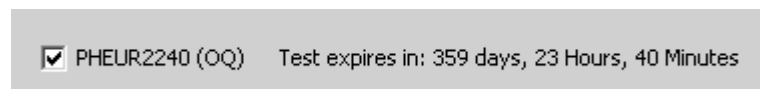
Figure 19: OVP test status

As soon as you have activated the check box for the respective test protocol you can see which test(s) will be performed by the browser-like display next to the bitmap.

All measurement results are stored in a report (PDF file) and in the *UserData* database. To append a comment to the current test protocol entry write a comment into the *User Comment* entry field.

Click on the *Run selected Tests* button to start the respective tests. If you do not want to perform any test, click on the *Cancel* button.

3.1   Photometric Linearity Test

3.1.1   Reflection Measurement

Prerequisites:

- The photometric linearity test for reflection measurements requires the use of the **99% reflectance standard (SRS-99-010) for reference** (background) measurement.
- For an integrating sphere the **straylight correction spectra** must have been measured using a Bruker certified gold standard with the certified reflection factor entered to measure the straylight correction spectra.
- For an integrating sphere the **straylight correction check box** in the PhEur setup has to be set and the proper channel selected.
- For an integrating sphere an **8 degree sample holder** has to be used.
- For a fiber solid probe the straylight correction must <u>not</u> be used. Instead, use an appropriate sample holder.

Follow the wizard instructions and insert the standard using whatever method is appropriate for the instrument configuration you are using.

   Note: The measurement requires specialized holders for the reference standards.

After the reference measurement has completed, you will be prompted for each of the reflectance standards selected in the *PhEur Setup* dialog. Insert each standard as requested, and follow the instructions to perform the measurements. If each of the standards has been measured, the final results will be computed.

3.1.2   Transmission Measurement

Prerequisites:

The Photometric Linearity test for transmission measurements uses the glass filters mounted on the IVU (Internal Validation Unit). The test itself runs fully automatically and needs no user interaction.

- For instruments with only two glass filters on the IVU an **external NG 11 glass filter** is required.

3.2   Photometric Noise Test

3.2.1   Reflection Measurement

Prerequisites:

- The Photometric High Light Flux Noise test for reflection measurements requires the Labsphere **99% reflectance standard** (SRS-99-010).
- The Photometric Low Light Flux Noise test for reflectance measurements requires the Labsphere **10% reflectance standard** (SRS-10-010).
- For an integrating sphere an **8 degree sample holder** must be used.

Follow the wizard instructions and insert the standard using whatever method is appropriate for the instrument configuration you are using. You will be prompted for each of the standards.

> **Note:** The measurement requires specialized holders for the reference standards.

For each test (High or Low Light Flux) a reference and sample measurement are made using the reflectance standard. At the end of the test the final results are computed.

3.2.2   Transmission Measurement

Prerequisites:

- None

The photometric noise test for transmission measurements is performed by using the open channel and a filter on the IVU. The test itself runs fully automatically and needs no user interaction.

3.3 Wavelength Uncertainty/Reproducibility Test

3.3.1 Reflection Measurement

Prerequisites:

- The wavelength uncertainty and wavelength reproducibility test for reflection measurements require the use of either the **99% reflectance standard** (SRS-99-010) or a **gold standard for the reference** (background) measurement.
- Unless water vapor is used for these tests an **external certified reference standard** (see table in chapter 2.1) is required for the sample measurement.

The reference measurement for these tests can be performed using the standard method used for your spectrometer and configuration (e.g. gold standard for integrating sphere, reference position for fiber optics solid probe etc.) Instead of the gold standard, the 99% Labsphere Reference standard can be used. If both tests are run, a reference is only measured once.

Follow the wizard instructions and insert the standard using whatever method is appropriate for the instrument configuration you are using. You will be prompted for each of the standards.

> **Note:** This may require specialized holders for the reference standard.

At the end of the test the final results will be computed.

3.3.2 Transmission Measurement

Prerequisites:

- In case of transmission measurements the reference (background) measurement for the wavelength uncertainty test is run with the open channel.
- Unless water vapor is used for this test either an **external certified reference standard** (see table in chapter 2.1) or the **internal reference standard** (BRM 2065) on the IVU with certificate is required for the sample measurement.

You will be prompted for the standard to be inserted only if an external reference standard has been selected.

> **Note:** This may require specialized holders for the reference standard. If the internal BRM 2065 standard or water vapor has been selected, the test will run without any further user interaction.

3.4   Printing Reports

The results of test measurements are stored in a report (PDF format). The test report contains a summarized test result page and subsequent pages for each test with all the test spectra displayed. Reports are stored in the separate *VALIDATION\REPORTS* directory and can automatically be displayed and/or printed. To have the report displayed and printed you require the Adobe Acrobat Reader which is available on the OPUS installation CD.

4 Appendix - Wavelength Uncertainty Test using Water Vapor

4.1 References

[1]Second Supplement, USP-NF <1119> Near-Infrared Spectrophotometry

[2]H2O vapor calibration-engl.doc, Dr. Axel Keens, 31 May 2001

[3]2035.pdf, National Institute of Standards & Technology, Certificate, Standard Reference Material® 2035

[4]C. Kradjel, U. Grummisch, R. Rachlis, in: "Making Light Work: Advantages in Near Infrared Spectroscopy", Developed from the 4th International Conference on Near Infrared Spectroscopy, Aberdeen, Scotland, August 19-23, 1991; edited by I. Murray and I.A. Cowe, VCH Weinheim, 1992, Page 470.

[5]H. Günzler, H.M. Heise, "IR-Spektroskopie, Eine Einführung"; 3. Auflage, VCH Weinheim, 1996, Seite 84.

[6]J. Gronholz, W. Herres, "Understanding FT-IR Data Processing", I&C, Dr. Alfred Huethig Publishers, 1984, Page 2.

[7]H. Gremlich, Infrared and Raman Spectroscopy (§2.1),Ullmann's Encyclopedia of Industrial Chemistry, 1994, VCH.

[8]P. Griffiths, Fourier Transform Infrared Spectrometry (§1 VII), 1986, Wiley, ISBN 0-471-09902-3

4.2 Document History

ISSUE	DATE	AUTHOR	Description	File name
REVISION 1	10/09/2002	Andrea Giehl	Wavelength uncertainty test for Bruker FT-NIR spectrometer	Wavelength accuracy.doc

4.3 Introduction

Wavelength uncertainty test is part of the instrumental tests needed for the qualification of an NIR spectrometer in accordance with:

- USP Pharmacopoeia Forum <1119> 26(2000) 237-247
- Ph. Eur. III, 2.2.40 Near-Infrared Spectrometry, 43-44

This test is to ensure that the measured wavelengths are accurate and remain accurate for measurements in diffuse reflectance or transmittance mode.

4.4 Purpose

The purpose of this document is to provide detailed information on the use of water vapor absorbance bands for the calibration and validation of Fourier Transform NIR spectrometers. Measuring the water vapor absorbance band at a single wavenumber shall replace the wavelength uncertainty test of Michelson-type FT-NIR spectrometers required by USP, for both diffuse reflection and transmission measurements.

    NOTE: wavenumber = 1/wavelength

4.5 USP Requirements

In [1] the regulations are set for the *Instrument Operational Qualification* with regards to *Wavelength Uncertainty*. They are as follows:

Wavelength Uncertainty: [Note: The method used in developing wavelength uncertainty values is based on the NIST Center of Gravity algorithm.] Potential problems with internal calibration schemes are avoided by specifying appropriate independent external wavelength standards. For the reflectance mode, the NIST SRM 1920a reflectance standard is preferred. Standards containing dysprosium or holmium oxide are available. The nature and type of

background reference standard must also be specified. In transmittance measurements, the corresponding rare earth solution[1] or glass standards can be used. Solid or sealed solutions can be maintained similarly to a set of calibration weights to provide information on long term stability[2].

Table 1: Recommended Near-Infrared Instrument specifications[a]

SRM 1920a peaks[b] occur at 1261, 1681 and 1935nm		
Wavelength Uncertainty		
Tolerances	$\pm$1nm at 1200nm or $\pm$8cm^{-1} at 8300cm^{-1} $\pm$1nm at 1600nm or $\pm$4cm^{-1} at 6250cm^{-1} $\pm$1.5nm at 2000nm or $\pm$4cm^{-1} at 5000cm^{-1}	

a A maximum nominal instrument resolution of 10nm at 2500nm or 16cm^{-1} at 4000cm^{-1} is appropriate for most applications.

b The nominal 1935nm peak depends on the instrument resolution. Use the wavelength value from the SRM 1920a certificate, which comes close to the resolution used.

4.6 Spectra Comparison of SRM 2035 and Water Vapor

The NIST SRM 2035 transmittance standard is recommended by USP to be used to determine the wavelength accuracy for transmission measurements. Using the Center of Gravity (COG) of the absorbance peaks (instead of the peak maximum), the instrument can be considered as being qualified in the region from 10300cm^{-1} (971nm) to 5130cm^{-1} (1949nm). Spectra of this standard are shown in figure 20 and figure 21 (in wavenumbers).

For the NIST SRM 2035 transmittance standard, the maximum allowed deviations specified in the certificate depend on the investigated absorbance band and the applied resolution. The NIST certificate [3] applies to the COG of 7 wavelength bands (certified in nm and cm^{-1}).

Examples for an FT instrument with a resolution of 4cm^{-1}:

5138.45cm^{-1}, 6804.65cm^{-1}, 7313.46cm^{-1}, 8178.58cm^{-1}, 8681.56cm^{-1}, 9293.38cm^{-1}, 10245.43cm^{-1}

---

1. Holmium oxide solution, NIST SRM 2034, (wavelength standard from 240 to 650nm) may be used currently in the 650 to 1100nm region, although bands are not certified in this region.

2. A rare earth oxide in glass transmission wavelength standard, SRM 2035, has been certified recently by NIST. This standard may be used in transflectance mode, but is not currently certified for such use.

The NIST certificate provides the uncertainty on the measurements which are said to be about equivalent to a 95% confidence interval. The actual limits for the allowed deviations are, however, not defined in the certificate or the USP guideline. When determining the band position the same center of algorithm (COG) has to be used as the algorithm applied by NIST.
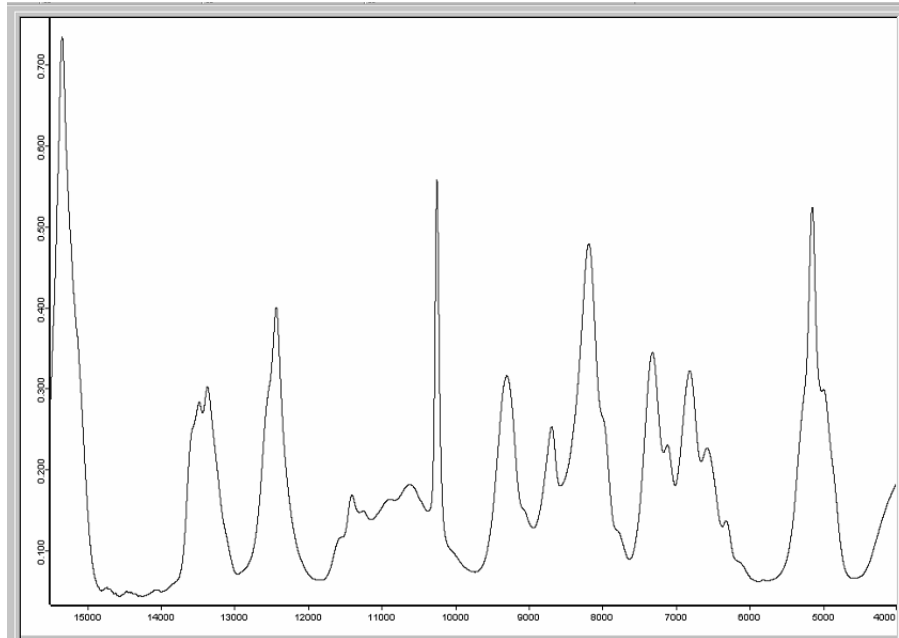

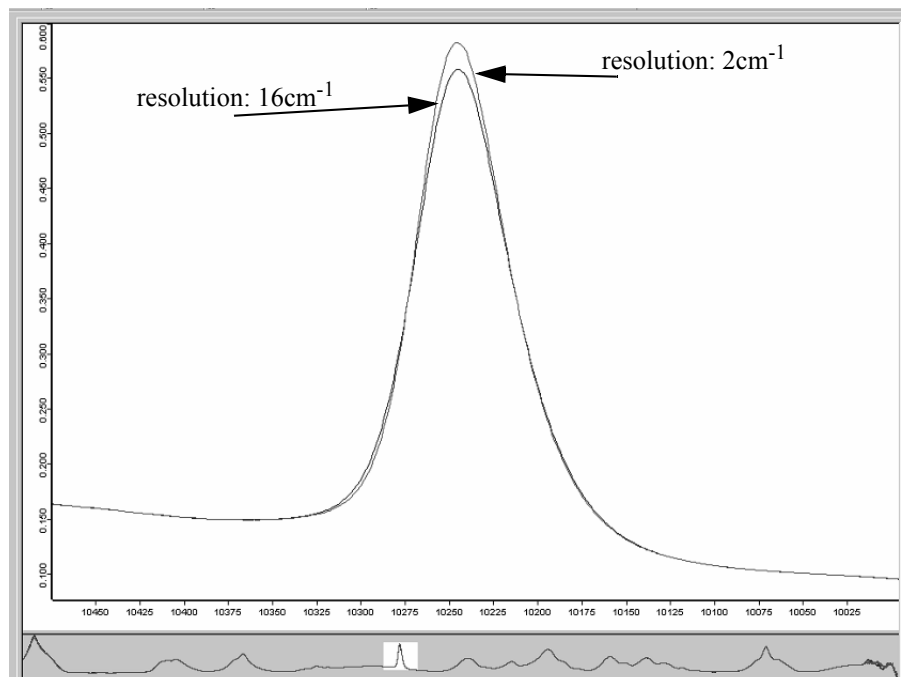Figure 20: SRM 2035 spectrum, transmission wavelength standard, mixture of rare earth oxides

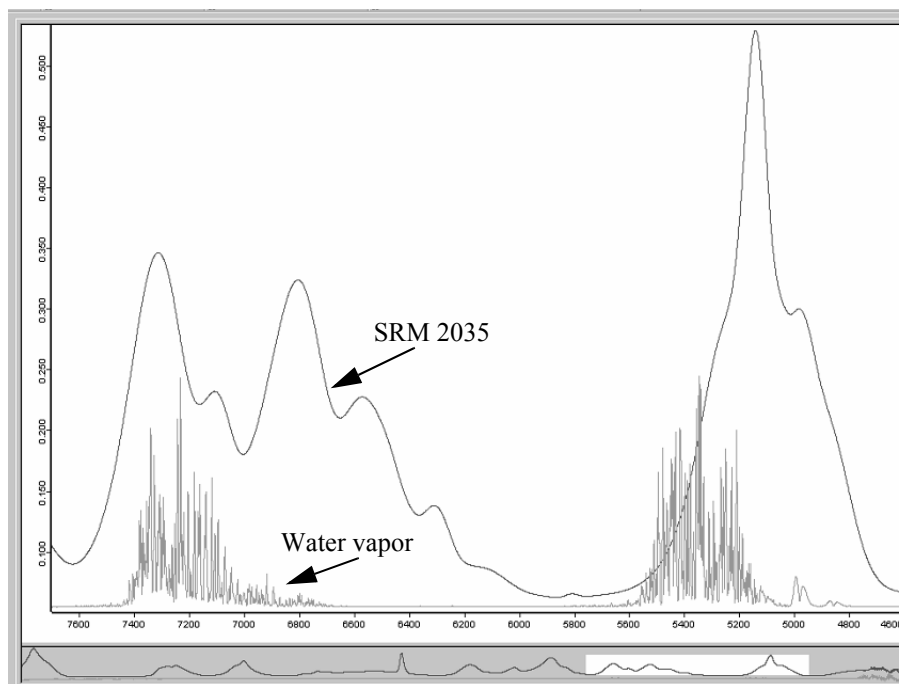Figure 21: The sharpest absorption band in the SRM 2035 spectrum

Figure 22: Comparing SRM 2035 and water vapor at a resolution of 2cm^{-1}

The measurement of water vapor can be used to check the wavelength accuracy for Michelson-type FT instruments. The water vapor absorbance peak at e.g. 7306.74cm^{-1} measured with a high resolution can be applied. Water vapor has the advantage of having an extremely narrow band, and the wavelength position can be measured to a very high degree of accuracy. This gives a more precise measurement of the wavelength accuracy than would be in case of NIST SRM 2035 standard (see figure 22). The NIST SRM 2035 standard itself is calibrated against the water vapor band (see [3]).

In addition, using only one point (e.g. 7306.74cm^{-1} for water vapor) to determine the wavelength accuracy test is sufficient for a Michelson-type FT instrument. The wavelength in an FT- instrument is calibrated by an internal He/Ne laser with a wavelength of 633nm. The value of the He/Ne wavenumber is entered in the control software of the instrument and slightly adjusted so that this specific water line is accurately registered in wavenumber - and hence wavelength - by taking the reciprocal.

Theoretically, for an FT instrument, all wavelengths across the entire spectrum will be accurately determined from this single setting. Repeated measurements of the water vapor line with a calibrated FT instrument will show residual fluctuations due to spectrometer noise. These shifts, however, are extremely small and can be neglected. The residual fluctuations at all other wavelengths will be of a similar negligible amount.
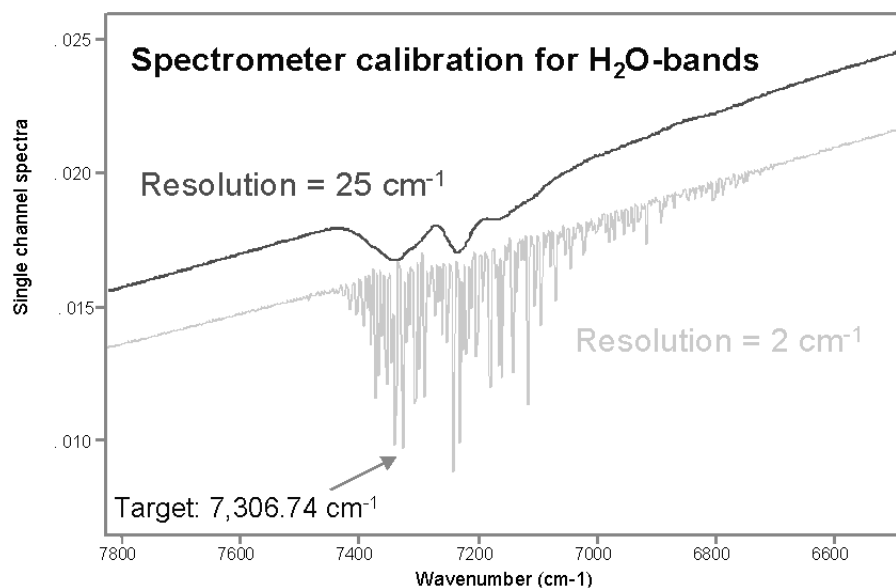
4.7   Reference for Water Vapor Absorbance

Figure 23: Spectrometer calibration using the water vapor band at 7,306.74cm^{-1}

There is no certification needed for water vapor as it represents a natural constant and is always the same substance present in the ambient air. It can be used for both transmittance and diffuse reflectance measurements. Details on the reference values of the water vapor absorbance peaks are given below [2]:

Ref.: Reference values for the calibration of Bruker FT-NIR spectrometers with reference to the water band at 7,306.74cm^{-1}

The data is obtained (data source is) from the HITRAN 1992 database, published by:

Air Force Phillips Laboratory
29 Randolph Road
Hanscom AFB, MA, 01731-3010

Dr. Laurence Rothman, PL/GPOS

M	*I*	*wn[cm-1]*	*S*	*E"*	*v"*	*v'*	*Q'*	*Q"*
H_2O	1	7299.44900	1.300E-20	42.372	000	101	211	110
H_2O	1	7303.23140	6.889E-22	95.176	000	200	322	211
H_2O	1	7305.08049	3.149E-22	326.625	000	200	606	515
H_2O	1	**7306.73600**	2.000E-20	79.496	000	101	313	212
H_2O	1	7306.74700	1.000E-21	325.348	000	200	616	505
H_2O	1	7308.81782	6.179E-22	136.762	000	101	322	303
H_2O	1	7310.34759	1.949E-22	326.625	000	101	514	515
H_2O	1	7310.67785	5.959E-21	134.902	000	101	322	221
H_2O	1	7312.27100	7.400E-21	70.091	000	101	303	202

M: Molecule
I: Isotope; 1= ^1H ^{16}O ^1H
wn: Vacuum wavenumber [cm^{-1}]
S: Intensity [cm^{-1}/(Molecule · cm^{-2}) at 296K]
E":   Energy of the lower state [cm^{-1}]
v":   Global Quantum numbers of the lower state
v':   Global Quantum numbers of the upper state
Q':   Local Quantum numbers of the upper state
Q":   Local Quantum numbers of the lower state

The reference band is the one with the largest intensity S (row 4). The wavenumber is usually regarded as a constant as it is independent of the refractive index of the surrounding medium.

A Fourier Transform Spectrometer (FTS), however, measures wavelengths and thus depends on the refractive index of the medium. The wavenumber (=1/λ) is calculated from the wavelength by FFT (Fast Fourier Transformation).

As the refractive index of the air is subject to a dispersion (at 15800cm^{-1} it is higher than at 7300cm^{-1}), the wavenumber range becomes distorted in a non-linear way (the effect, however, is extremely small). Therefore, it is possible to exactly calibrate an FTS under standard pressure only over a small range. The spectra which are calibrated in this way are indicated in vacuum wavenumbers.

When converting vacuum wavenumbers into wavelengths, you have to divide by the refractive index of the air. This index amounts to 1.0002735 for dry standard atmosphere at 7300cm^{-1}.

4.8 Connes' Advantage of Fourier Transform Interferometry

The fact that only one point (e.g. 7306.74cm^{-1} for water vapor) is required for the wavelength accuracy test is due to the *Connes' advantage* of the Fourier Transform Infrared technique (see [4] to [8]).

"In an FT spectrum, the accuracy of each wavenumber is coupled to the accuracy with which the position of the moving mirror is determined; by using an additional HeNe laser interferometer the position of the mirror can be determined to an accuracy better than 0.005nm. This means that the wavenumbers of an FT-NIR spectrum can be determined with high accuracy (<0.01cm^{-1}). In other words, FT-IR spectrometers have a built-in wavenumber calibration of high precision. This advantage is known as Connes' advantage" (see [7]).

Due to the *Connes' advantage* there is no distortion of the frequency in the spectrum and the x-axis may be calibrated exactly by a single frequency.

4.9 Conclusion

We believe, that for Michelson-type FT instruments (e.g.: Bruker MATRIX series, Bruker VECTOR series, Bomem BM160 series, Nicolet Antaris series, Perkin Elmer IdentiCheck series) using water vapor for the wavelength uncertainty test is the most optimal solution available for both diffuse reflection and transmission measurements.

Due to the *Connes' advantage* only a single absorption peak is required for the test.

The water vapor absorbance bands are very sharp and allow determination of the position to a very high accuracy.

For a Bruker MATRIX spectrometer, for example, the wavelength uncertainty test is specified with a maximum deviation of 0.1cm^{-1}. A higher deviation would be regarded as failing the test. This limit is much tighter than the requirements by USP (tolerances of 4cm^{-1}).

Index