

Quantitative analysis using ATR-FTIR Spectroscopy

Application Note

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Introduction

The use of attenuated total reflection (ATR) accessories in conjunction with Fourier transform infrared (FTIR) spectrometers is now commonplace. This accessory provides for the non-destructive measurement of samples with little or no preparation. Most samples can be directly applied to the internal reflection element (IRE) of an ATR without time-consuming dilution with matrices such as Nujol or KBr. The ATR accessory also allows for easy analysis of liquid samples with just a single drop required, applied directly to the IRE crystal. However, by the nature of their design, ATR accessories absorb infrared radiation and consequently reduce the amount of energy that reaches the infrared detector. The attenuation caused by these accessories typically varies from 70–90%.

Agilent Cary 600 FTIR series spectrometers provide the ideal platform for this accessory by delivering up to four times more IR power at the sample focus than most competitive products.¹ They have been designed to offset accessory attenuation of the IR beam and to provide exceptional performance and sensitivity, which are typically compromised with the use of the accessory.

Infrared spectroscopy is particularly well-suited to quantitative analyses. The determination of sample concentration is made simple with the *Quant Calibration* application in the Resolutions Pro software. This Application Note highlights the benefits of using Agilent's Cary 600 FTIR spectrometer series and software for simple quantitative analysis by using a model system of ethanol (EtOH) in water. The limit of detection is measured using a PIKE MIRacle single bounce diamond ATR in conjunction with an Agilent Cary 660 FTIR (mechanical-bearing) and a Cary 670 FTIR (air-bearing) spectrometer is also determined.



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Instrumentation

The Agilent Cary 660 FTIR and Cary 670 FTIR spectrometers were used in conjunction with a PIKE MIRacle single bounce diamond ATR accessory. The spectrometer settings used in all spectral collections are listed in Table 1. The instrument and accessory were purged for five minutes with nitrogen prior to spectral collection.

Table 1. Instrument parameters used in all collections

	Instrument Parameters	Settings
Detector	DLaTGS	
	Speed (kHz)	5.0
Source	MIR	Normal
ATR	PIKE Diamond MIRacle	
Collection	Sample scans	16
	Background scans	64
	Resolution (cm ⁻¹)	4
	Aperture (cm ⁻¹)	4
	Symmetry	sym
Computation	Apodisation type	BH4
	Zero filling factor	2
	UDR	2

Materials and reagents

Standard solutions for the quantitative calibration curve were sequentially diluted from an aliquot of ethanol (5.00mL, Merck, HPLC grade) in deionized water (100.0mL, 18 MΩ cm, Millipore Milli-Q, Billerica, MA, USA).



Figure 1. The PIKE MIRacle ATR used in this study. Energy-limiting accessories such as an ATR require a high energy FTIR spectrometer to offset accessory attenuation

Results and discussion

Quant Calibration - Ethanol in Water

A calibration curve was prepared from a range of solutions of known concentration of ethanol in water. An aliquot of each solution was placed on the IRE of the ATR and spectra were recorded (with both the Cary 660 FTIR and Cary 670 FTIR) using the parameters in Table 1. The spectrum of each solution was ratioed against a background spectrum of water. Figure 2 (Appendix) is a screen capture of the Resolutions Pro software (labeled for clarity) that displays: (a) a selected spectrum of ethanol in water, (b) a calibration curve based on the prepared solutions, and (c) a customizable spectral spreadsheet of important spectral parameters. Solutions in the range 1.0 – 5.0 %v/v EtOH/H₂O were used and the known concentration value for each solution was entered into Resolutions Pro software (Figure 2c, column 4 of spectral spreadsheet). The spectra that are included in the calibration are listed in column 3 of the spreadsheet. Multiple replicates for each concentration can also be included. A data point in the calibration curve can be included/excluded from the calibration by double-clicking on this column to change its status. The calibration can be based on a user-defined peak height or peak area (as in this case), which can be changed by simply dragging the handles at the left and right of the peak area². Likewise, the baseline definition can be changed by simply dragging the handles to the left and right of the baseline with a live update to the calibration curve.

The concentration of ethanol in an unknown solution with the same matrix can be readily determined from the calibration curve. Depending on the experiment, it may be advantageous to include solutions of known concentrations periodically to verify the robustness of the model as well as to ensure the quality of the predictions of unknown concentrations³. The calibration curve can be conveniently stored for later use, meaning that a new set of calibration solutions does not need to be prepared each time a determination is required (Figure 3).

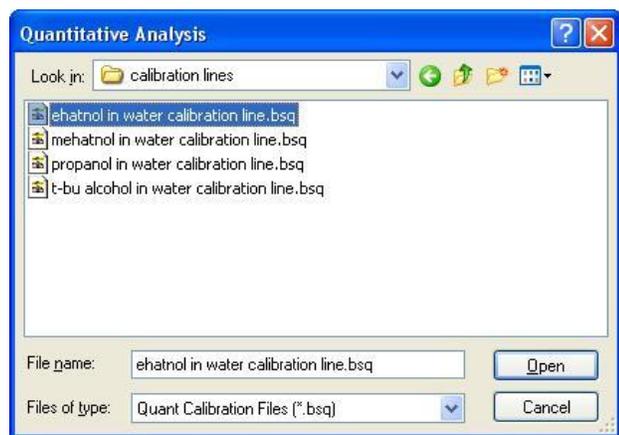


Figure 3. The simplicity of storing a calibration curve (and associated spectra) permits the determination of unknown concentrations of relevant samples at a later date

Using this method, the concentration of a solution, made up to be 0.500% v/v EtOH/H₂O (see materials and reagents) was determined to be 0.504% v/v EtOH/H₂O from the average of three replicate measurements. This represents an accuracy of better than 0.5%, demonstrating the performance of the Agilent Cary 670 FTIR spectrometer, even at low concentrations of solute.

Limit of Detection - Agilent Cary 660 FTIR with a PIKE MIRacle Diamond ATR Accessory

Instrument sensitivity can be defined by the limit of detection (LOD). The LOD is normally defined as the point at which the peak height of a sample is three times that of the peak-to-peak (p-p) noise in an adjacent region. This is a better representation of instrument sensitivity than a S/N specification number, as it provides practical experimental conditions based on measurements in the spectral region of interest. For instruments incorporating an ATR accessory this can be quoted as % volume of solute divided by % volume of solvent, in this case, solution of ethanol (solute) diluted in water (solvent), %(v/v) EtOH/H₂O.

The p-p noise of water was measured between 1220–1120 cm⁻¹ on an Agilent Cary 660 FTIR and Cary 670 FTIR spectrometer (Table 2). Using the commonly accepted definition of LOD, a sample of EtOH in H₂O is detectable if it has a peak height ~3 times the p-p noise. Using the calibration curve previously collected,

this translated to an LOD of 0.04% on a Cary 670 FTIR and 0.08% on a Cary 660 FTIR.

To validate the above calculations, a solution of 0.04% v/v EtOH/H₂O was made up and measured on an Agilent Cary 670 FTIR spectrometer. Figure 4 shows the IR spectrum overlaid with a spectrum of a 1.0% v/v EtOH/H₂O solution for reference. Even at this very low concentration the C-O stretch of ethanol at 1045 cm⁻¹ is visible. The height of this peak was compared with the p-p noise in the region 1220–1120 cm⁻¹ using the *Noise* application within the *Resolutions Pro* software package (Table 2). This data shows the peak height to be three times that of the noise in the adjacent region, indicating the limit of detection for this spectrometer/ATR configuration.

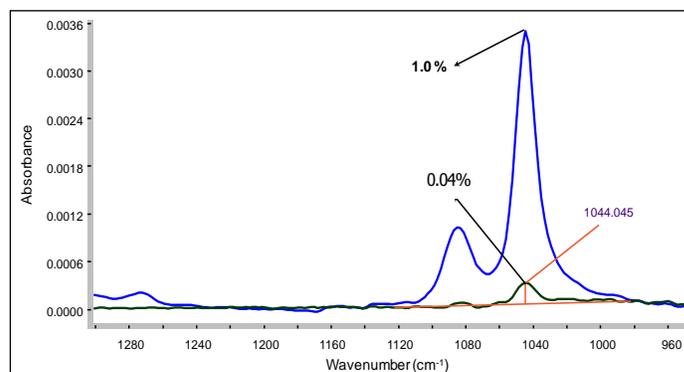


Figure 4. Overlaid spectra of 1.0% (v/v-) and 0.04% (v/v) EtOH/H₂O acquired using an Agilent Cary 670 FTIR spectrometer with a PIKE MIRacle ATR showing the C-O stretch of EtOH at 1045cm⁻¹

Table 2. Measured LOD for two Agilent Cary 600 FTIR series spectrometers configured with a PIKE MIRacle Diamond ATR accessory

	LOD % v/v EtOH/H ₂ O	Peak Height 1045 cm ⁻¹	P-P Noise 1220 - 1120 cm ⁻¹
Cary 670 FTIR	0.04	2.5 x 10 ⁻⁴ abs	0.8 x 10 ⁻⁴ abs
Cary 660 FTIR	0.08	4.6 x 10 ⁻⁴ abs	1.4 x 10 ⁻⁴ abs

Conclusion

The PIKE MIRacle ATR accessory, in conjunction with an Agilent Cary 670 FTIR or 660 FTIR spectrometer achieves a limit of detection of 0.04% and 0.08% v/v EtOH/H₂O respectively. These results reflect the high energy throughput of the Agilent Cary 600 FTIR series of FTIR spectrometers and demonstrate the instruments to be a powerful tool in the identification and quantitation of even the most dilute samples.

The high energy throughput means that the performance of the instrument is maintained even when using energy limiting accessories such as an ATR or when analyzing highly attenuating samples. This permits faster spectral acquisition times as the number of co-added scans required to meet signal-to-noise requirements is significantly reduced. Coupled with the convenience of using an ATR accessory, where little or no sample preparation is required, the Agilent Cary 600 FTIR series allows users to obtain high-quality spectra faster than ever before. In addition, the *Quant Analysis* application within the *Resolutions Pro* software package offers a simple, convenient and accurate means of quantifying the concentration of infrared-active compounds.

References

1. Boyd S. and Kansiz M. *Highest Available Signal-To-Noise Performance, Delivering Superior Sensitivity and Analytical Performance*, Advantage Note SI-01353
2. Resolutions Pro Software 'Help' files.
3. Harris Daniel C., *Quantitative Chemical Analysis*, W. H. Freeman, 2006, ISBN 071677041

Appendix

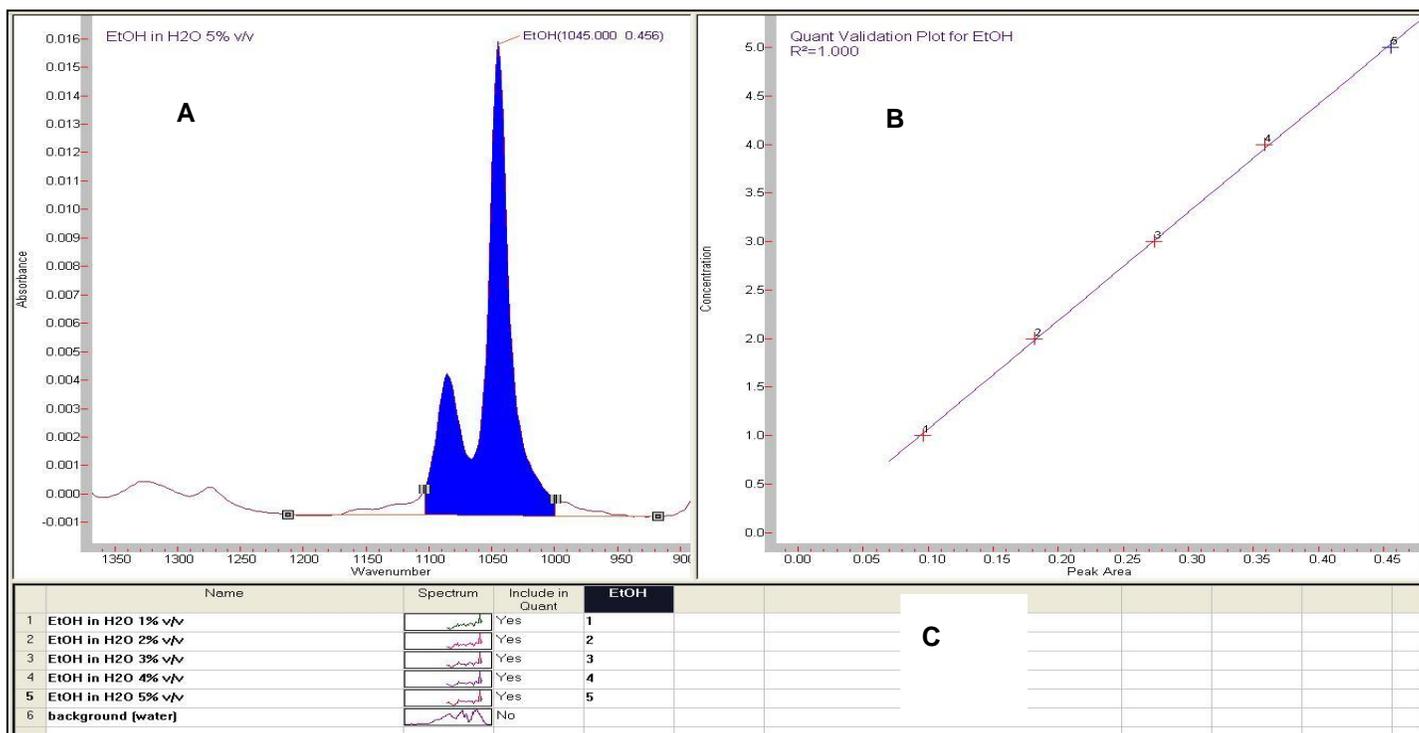


Figure 2. Resolutions Pro software illustrating the *Quant Calibration* application. Agilent's FTIR software simplifies quantitative analysis

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