



## Identity Raman Reader Detection Limits for Common Compounds

### Introduction

A common question regarding instrument performance is the sensitivity or detection limits for trace components in mixtures. The question is difficult to answer unequivocally because the answer is; it depends. Detection limits are dependent on the Raman scattering cross-section of the analyte, as some compounds with high polarizability have high Raman cross-sections, whereas more polar compounds have weaker Raman signatures. Detection limits are also dependent on the solvent or matrix the analyte is to be measured in, because for an analyte to be detectable it generally must have Raman bands that are free from interference from matrix or solvent bands.

Lastly, detection limits are dependent on measurement conditions, which for Raman spectroscopy generally mean laser wavelength, laser power and integration time. Shorter wavelength lasers generate more intense Raman scattering due to the  $\nu^4$  relationship between laser frequency and scattering intensity. In addition, the Raman scattering intensity is linearly dependent on laser power and measurement time, meaning that with a given laser operating at full power, the analyst can improve detection limits by measuring for longer periods of time. Longer measurement times can be achieved by increasing the CCD integration time, or by co-adding scans from individual integrations, or combinations thereof. No matter how the integration time is increased, the Signal to Noise (S/N) of the measurement usually improves with the square root of the measurement time, so in the end there is a trade off between reasonable measurement times and achievable detection limits.

This application note will demonstrate detection limits for common chemical compounds in water; fructose, formaldehyde and di-methyl sulfoxide (DMSO) for reasonable measurement times. The analytes were chosen to be representative of food, environmental and biological applications, and water was chosen as the solvent because of its obvious use in these fields.

### Sample Preparation and Procedure

Formaldehyde in water solutions were prepared in concentrations ranging from 2.3 to 0.07 weight % in water. Similarly, DMSO in water solutions was prepared from 2.5 to 0.07 wt%, as were fructose in water from 5 to 0.07 wt%. 200 microliter aliquots of each solution were pipetted into a Corning clear, flat bottom 96-well microtiter plate, from which Raman spectra of all solutions were recorded on the Identity Raman plate reader using 532 nm laser excitation at 60 mW laser power while integrating the spectra for a total of 10 minutes per sample, in one plate scan.

All spectra were pre-processed by spectral subtraction of a reference spectrum of water collected under the same conditions using Panorama (LabCognition GMBH, Cologne, Germany). Quantitative analysis calibration curves were generated from the three sets of spectra, also using Panorama, from which the Limit of Detection (LOD) for each compound was estimated.

## APPLICATION NOTE

**Results**

The Raman spectra for the formaldehyde dilution series is shown in Figure 1. A quantitative analysis calibration curve, displayed in Figure 2, was generated from the peak height of the strongest band at  $1046\text{ cm}^{-1}$ , baseline corrected to baseline points at  $822$  and  $1176\text{ cm}^{-1}$ .

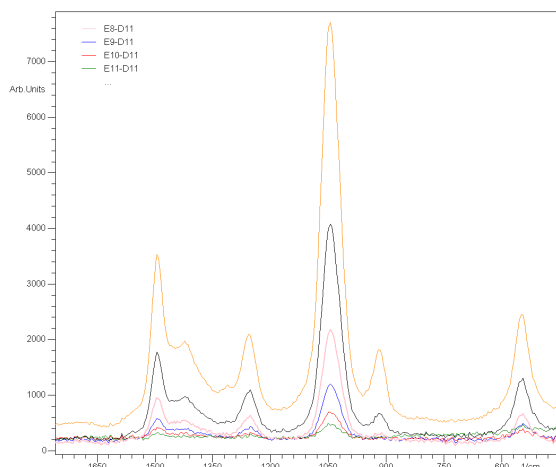


Figure 1. Calibration spectra of formaldehyde in water solutions for concentrations of 2.3, 1.2, 0.6, 0.3, 0.15 and 0.07% (w/w), top to bottom, respectively.

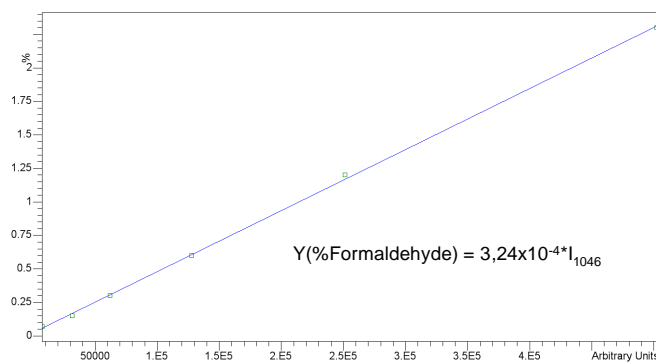


Figure 2. Quantitative calibration curve for the formaldehyde in water solutions displayed in Figure 1, measuring the peak height of the Raman band centered at  $1046\text{ cm}^{-1}$  relative to baseline points at  $822$  and  $1176\text{ cm}^{-1}$ .

## APPLICATION NOTE

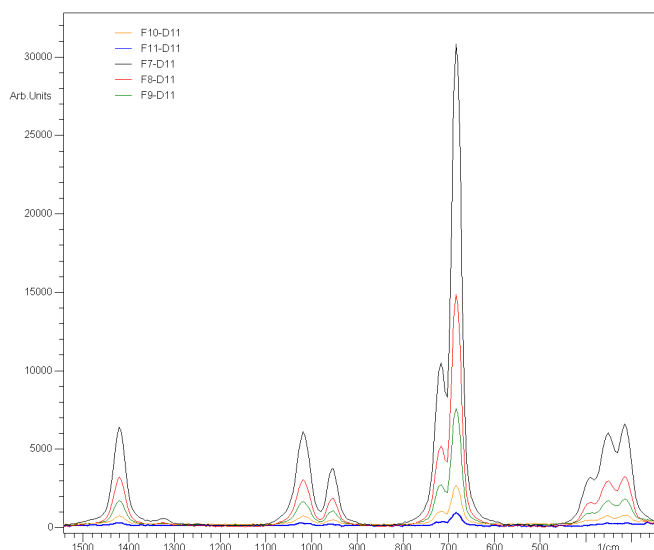


Figure 3 (left). Calibration spectra of DMSO in water solutions for concentrations of 2.5, 1.25, 0.62, 0.213, and 0.07% (v/v), top to bottom, respectively.

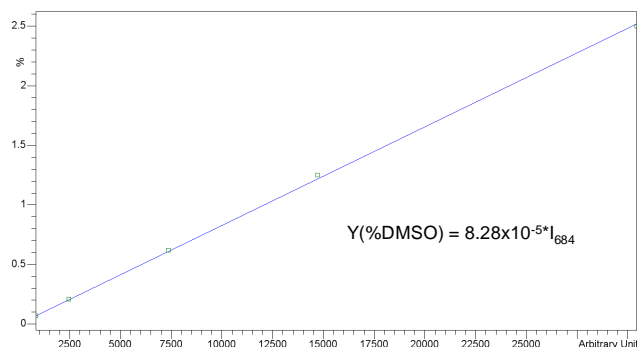


Figure 4 (right). Quantitative calibration curve for the DMSO in water solutions displayed in Figure 3, measuring the peak height of the Raman band centered at  $684\text{ cm}^{-1}$  relative to baseline points at  $595$  and  $787\text{ cm}^{-1}$ .

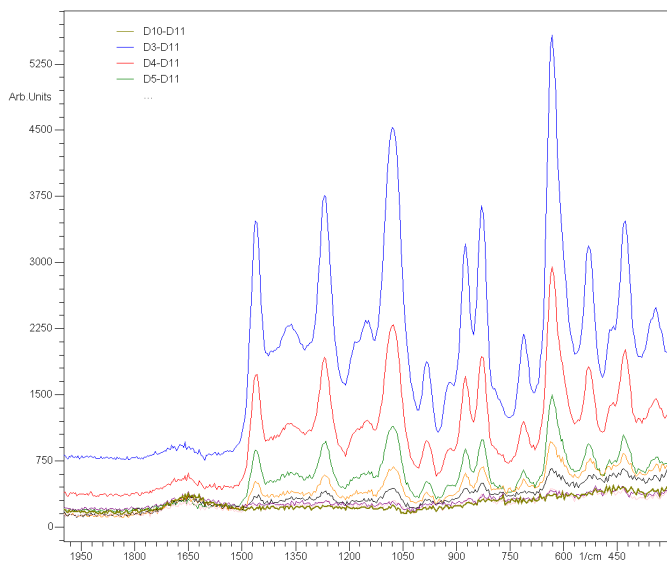


Figure 5 (left). Calibration spectra of fructose in water solutions for concentrations of 4.0, 2.0, 1.0, 0.5, 0.25, 0.12, 0.06 and 0.03% (v/v), top to bottom, respectively.

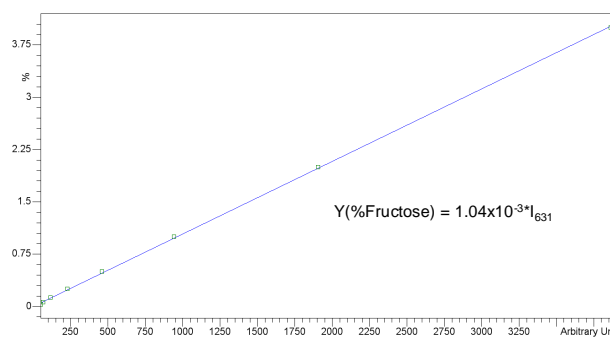


Figure 6 (right). Quantitative calibration curve for the fructose in water solutions displayed in Figure 5, measuring the peak height of the Raman band centered at  $633\text{ cm}^{-1}$  relative to baseline points at  $563$  and  $683\text{ cm}^{-1}$ .

## APPLICATION NOTE

The Limit of Detection (LOD) for an analysis is typically reached when the Signal to Noise ratio of the intensity of the analytical band measured relative to the peak to peak noise in the baseline in the region of the band reaches a value of 3:1. Under this definition, the LOD for formaldehyde in water is 0.04%.

Similarly, the calibration spectra and calibration curves for DMSO in water and fructose in water are displayed in Figures 3 through 6, and the LOD's for DMSO and fructose in water are 0.02% and 0.12%, respectively, as summarized in Table 1. The LOD's of the three compounds vary because of their relative Raman scattering cross sections.

Compound	Peak Center (cm <sup>-1</sup> )	LOD (% w/w)
Formaldehyde	1176	0.04
DMSO	684	0.02
Fructose	633	0.12

Table 1. Limits of Detection (defined as the intensity of the analytical peak equal to three times the peak to peak noise) for Raman spectra of aqueous solutions of formaldehyde, di-methyl sulfoxide (DMSO) and fructose, collected on the Identity Raman plate reader with 532 nm laser excitation, 60 mW of laser power, and 10 minute measurement time.

## Conclusions

While Raman spectroscopy is generally not considered to be an analytical technique with high sensitivity without the employment of enhancement techniques, low detection limits in spontaneous Raman scattering are achievable in conditions where the intensity of a strong analytical band can be measured free from interference of the solvent bands. Here we demonstrated detection limits in the low part per thousand and high parts per billion ranges for common solvents and food products, showing that Raman spectroscopy is a method that can be used not just for qualitative analysis, but for reliable quantitative measurements as well.

**WORLDWIDE OFFICE**  
84 October Hill Road  
Holliston, MA 01746  
United States  
Phone: 508 893 3130  
Fax: 508 893 8011

**EUROPEAN OFFICE**  
18 Blackstone Road  
Huntingdon, Cambridgeshire  
PE29 6EF United Kingdom  
Phone: [+44] 1480 426 700  
Fax: [+44] 1480 426 767

**ASIAN PACIFIC OFFICE**  
6th Fl. Yokohama World Porters  
2-2-1 Shinkou, Nakaku  
Yokohama, Japan 231-0001  
Phone/Fax: 045 651 6252