

## Diacetyl Analyses method – Using i-Lab

Diacetyl determination in Beer has been adapted to from the American Society of Brewing Chemists (ASBC) Broad Spectrum method for VDK (vicinal diketones) found in the Beer-25.pfd document.

The procedure is based on the development of calibration curve from known calibration samples. Calibration standards of diacetyl were prepared by first blending a Stock Solution of 500 mg/L diacetyl in distilled water. This Stock Solution was then diluted to prepare standards in the range of 0.05 to 1.6 mg/L with distilled water immediately before using. These standards were used to follow the procedure below to define a concentration calibration curve for diacetyl using the i-Lab.

### Solutions

- (a)  $\alpha$ -Naphthol solution. Dissolve 4 g  $\alpha$ -naphthol ( $C_{10}H_7OH$ ) in 100 mL isopropanol, 99.6%. Add ca. 0.5 g vegetable carbon and shake mixture for about 0.5 hr, then filter. Store filtrate in the dark in an amber bottle.
- (b) KOH-creatine solution. Dissolve 0.3 g creatine in 80 mL 40% KOH solution (aqueous) and filter. Store solution in a polyethylene container under refrigeration.

### Method

1. Distill 100 mL decarbonated beer into 50-mL graduated cylinder containing 5 mL water. Collect ca. 15 mL distillate and make to 25 mL with water. Pipet a 5-mL aliquot into a 10-mL volumetric flask.
2. Color development. Add 1 mL  $\alpha$ -naphthol solution (reagent a) to each flask and swirl. Add 0.5 mL KOH/creatine solution (reagent b) to not more than 4–5 flasks at a time. Make to mark and shake vigorously for exactly 1 min. Let stand, and measure absorbance at 530 nm against the reagent blank between 5 and 6 min after shaking. Repeat this procedure until all samples have been measured.

### Concentration Calibration Curve

A concentration calibration curve was developed using the i-Lab by obtaining the spectra for the calibration samples. This was done by first treating the calibration standards according to step-2 of the method above to develop a “color” related to the concentration of diacetyl. This color was measured at 530 nm in the spectra of these standards. Plotting the concentration versus absorbance measured at 530 nm produced the calibration curve.

The calibration samples were analyzed by the i-Lab using the round vial adaptor. Since there are currently two firmware defined collection methods for spectra (2-step and n-

step) which are known to have different instrumental extension coefficients, both methods were used to develop calibration curve data. The figures below show these two calibration curves.

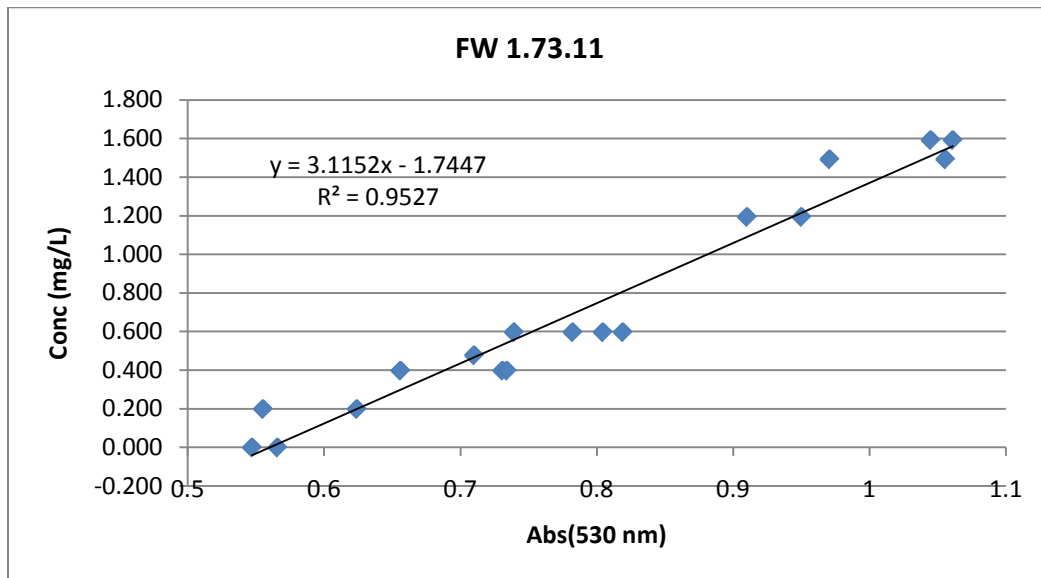


Fig. 1 2-Step Spectral Collection.

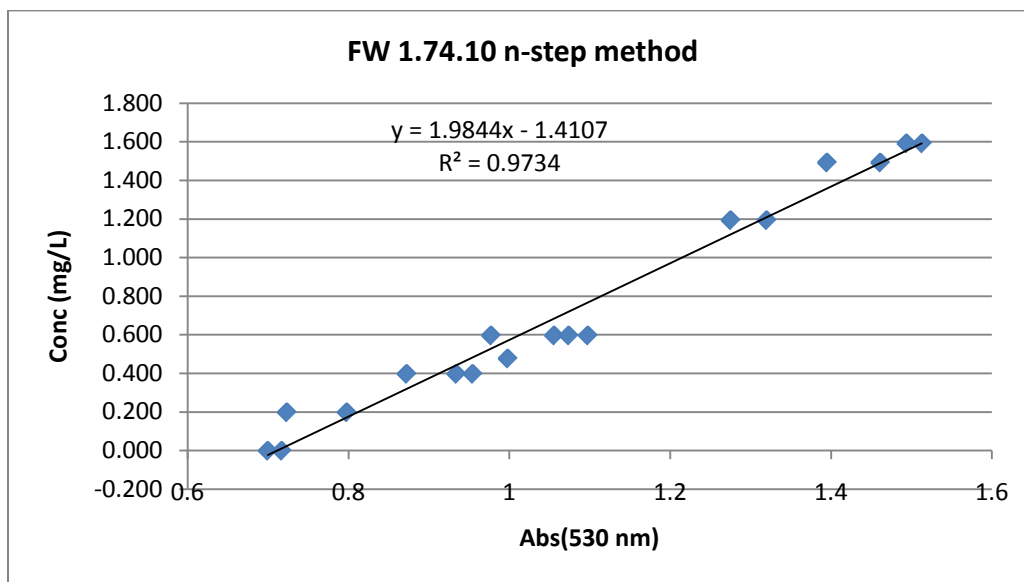


Fig. 1 N-Step Spectral Collection.

Since the calibrations were linear ( $R^2=0.95$  and  $R^2=0.97$ ) methods were built based on a collection of the spectrum, measuring the 530 nm absorbance, multiplying it by the calibration curve slope and adding its intercept. The sample collection is based similarly to all Round Vial where a background and calibration of the system is first performed. For the 2-step process this is achieved by first running the Backg\_RV program – which uses

Bypass method for reflection correction. For the n-step process this is achieved through the programs nCalibrate\_All (to first achieve the adapter bypass calibration) and nBackg\_All (to obtain a background spectrum). The calculation methods for diacetyl are then used for the determination – Diacetyl\_RV (for 2-step) and Diacetyl\_All (for n-Step).