

# Vicinal diketone (Diacetyl and 2,3-Pentanedione) Measurement in Beer using an Eppendorf BioSpectrometer®

Jessica Geisler<sup>1</sup>, Natascha Weiß<sup>2</sup>

<sup>1</sup>Eppendorf North America; <sup>2</sup>Eppendorf AG

## Introduction

### Objective

Vicinal diketones (VDKs) include diacetyl and 2,3-pentanedione. VDK measurements are critical because VDK content can greatly alter the flavor of beer.

VDKs are produced during the fermentation process and are considered unbeneficial to the taste in the vast majority of brews. This is a quick method to measure VDK using a steam distillation device and

the Eppendorf BioSpectrometer instead of time-consuming and expensive gas chromatography.

### Principle

The VDKs in beer reacts with 1,2-phenylenediamine to form 2,3-dimethylquinoxaline which absorbs at 335 nm.

## Material and Methods

### Protocol

1. Weigh 100 g of non-decarbonized beer into a distillation apparatus.
2. Add one drop of an antifoam emulsion.
3. The steam supply of the distiller should be set to obtain 25 mL of distillate in 2 minutes.
4. Place 10 mL distillate in each of two 50 mL tubes: one for the blank and one for the sample.  
Blank: Add 2.5 mL 4 N hydrochloric acid.  
Sample: Add 0.5 mL 1,2-phenylenediamine (diluted in 1% 4 N hydrochloric acid, freshly prepared), mix and incubate at room temperature in a dark place for 30 minutes. After the incubation, add 2 mL 4 N hydrochloric acid. Measure sample against blank value within 20 minutes.
5. On the BioSpectrometer: Under MAIN GROUPS select the Basic folder. Under SUB GROUPS select Factor, standard. Under METHODS select FACTOR.
6. Select the soft key EDIT to change the parameters.  
Parameters  
Cuvette: 10 mm  
Wavelength: 335 nm  
Unit: mg/kg  
Factor: 2.373  
Decimal Places: 2
7. Save parameters using the soft key SAVE or SAVE AS.
8. Select the soft key NEXT.
9. Transfer blank to a cuvette (e.g. Eppendorf Vis Cuvettes), insert the cuvette and measure by pressing the round BLANK key.
10. Transfer sample to cuvette, insert cuvette in the same orientation as the blank and measure by pressing the round SAMPLE key.

### VDK calculation:

The calculation can be implemented in the parameters of the BioSpectrometer via "FACTOR" (as described in step 6).  
VDKs (mg/kg) = Absorbance at 335 nm × 2.373

## Literature

[1] American Society of Brewing Chemists. Methods of Analysis. 8th ed. St. Paul, MN: The Society; 1992.

## Ordering information

| Description  | Order no. international | Order no. North America |
|--|-------------------------|-------------------------|
| <b>Eppendorf BioSpectrometer® basic</b><br>230 V / 50-60 Hz, mains/power plug Europe,<br>120 V / 50-60 Hz, mains/power plug North America        | 6135 000.009            | 6135000017              |
| <b>Eppendorf BioSpectrometer® kinetic</b><br>230 V / 50-60 Hz, mains/power plug Europe,<br>120 V / 50-60 Hz, mains/power plug North America      | 6136 000.002            | 6136000010              |
| <b>Eppendorf BioSpectrometer® fluorescence</b><br>230 V / 50-60 Hz, mains/power plug Europe,<br>120 V / 50-60 Hz, mains/power plug North America | 6137 000.006            | 6137000014              |
| <b>Eppendorf® macro Vis Cuvette</b><br>plastic cuvette for measurements in the Vis range,<br>max. filling volume 4,500 µL, 10 x box of 100       | 0030 079.345            | 0030079345              |
| <b>Eppendorf® semi-micro Vis Cuvette</b><br>plastic cuvette for measurements in the Vis range,<br>max. filling volume 3,000 µL, 10 x box of 100  | 0030 079.353            | 0030079353              |



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