



ASSIMILABLE AMINO NITROGEN PROCEDURE

Rapid Method 1998

EQUIPMENT

Spectrophotometer, UV at 335nm, zero on DI water
Cuvettes, 10mm
Pipettor, 50 μ L
Adjustable micro-pipettor with disposable syringes
50 mL centrifuge tubes
Centrifuge

REAGENTS

NAC Buffer (Reagent buffer-No OPA)
OPA Solution, 5%
10mM Isoleucine Standard (10mM Ile)
DI Water

PROCEDURE

Mix reagents as needed:

NAC Buffer, pour premeasured crystalline NAC into premeasured borate solution.

Use within 2 days of mixing. Refrigerate for stability and use at room temperature.

5% OPA, add the contents of ethanol vial (5 mL) to the premeasured OPA. Use within 1 day.

STANDARD CURVE

1. Add 3 mL NAC buffer into cuvettes. Add 10mM Ile standard and water as follows:

Cuvette	1	2	3	4
10mM Ile	0	10 μ L	30 μ L	50 μ L
DI Water	50 μ L	40 μ L	20 μ L	0

2. Add 50 μ L 5% OPA solution to each cuvette.
3. Read Absorbance values and record in log book.

SAMPLES

1. Clarify juice by centrifuging.
2. Add 3 mL NAC buffer into cuvettes. Run each sample in duplicate.
3. Pipette 50 μ L sample into each cuvette and record Absorbance "blank" reading. For duplicate sample, we recommend running x2 dilution of sample since juice nitrogen values can be high. Use 25 μ L sample and 25 μ L water or dilute separately.
4. Add 50 μ L 5% OPA solution. Mix and incubate 10 minutes.
5. Read Absorbances @ 335nm and record in log book.

CALCULATIONS

Setting up calculations in EXCEL is most expedient.
Note: To convert to ppm Nitrogen, the multiplication factor is 14.

1. Key in (on HP scientific calculator)

f GSB (clears statistical register)

Concentration value 1 (y), Enter

Absorbance value 1 (x) $\Sigma+$

Concentration value 2, Enter

Absorbance value 2 $\Sigma+$

Etc..... (Enter all the concentration (y) and absorbance (x) values)

$f \Sigma+$ (Calculates the linear regression equation and displays the y-intercept)

$X \Leftrightarrow Y$ (Displays the slope)

$f \bullet$, then $X \Leftrightarrow Y$ (Displays r value)

Sample

Absorbance value $f \bullet$ (Displays concentration value)
(multiply by 14 to convert to ppm Nitrogen)

DISPOSAL

Dispose in sink.