



## Correlation of the free amino nitrogen and nitrogen by O-phthaldialdehyde methods in the assay of beer

### Authors

Otama, Liisa,<sup>1</sup> Tikanoja, Sari,<sup>1</sup> Kane, Hilary,<sup>2</sup> Hartikainen, Sari,<sup>1</sup> Kaski, Leena,<sup>1</sup> and Suoniemi-Kähärä, Annu<sup>1</sup>

<sup>1</sup>Thermo Fisher Scientific, Vantaa Finland

<sup>2</sup>Thermo Fisher Scientific, Hemel Hempstead, UK

### Keywords

Discrete Analyzer, Photometric Analyzer, Gallery Analyzer, Fermentation, Beer, Wort, FAN, Free Amino Nitrogen

### Goal

To demonstrate that the automated discrete analyzer method for analysis of free amino nitrogen (FAN) provides comparable results to traditional ninhydrin methods, while also allowing multiple automated tests to be performed on the same sample.

### Introduction

In normal fermentation, the yeast *Saccharomyces*, converts sugar to ethanol and carbon dioxide. Yeast synthesizes the proteins required for healthy growth from amino acids that may be created from ammonia or by removal of the amino group from other alpha amino acids. The alpha amino acids available to yeast during fermentation are known as Free Amino Nitrogen (FAN). Levels of FAN in wort influence the formation of higher alcohols, specifically ethanol, which contributes to the flavor of beer and influences the flavor contribution other alcohols will make. Alcohols will be present at either excessively high or insufficiently low levels based on amounts of assimilable nitrogen available to the yeast from the wort. If levels are low, yeast growth is limited. If levels of nitrogen (N) are high, amino acids inhibit further synthesis increasing yeast growth and promoting higher alcohol formation. A FAN level of 140–150 mg/L for wort of 10° Plato (a measurement of the density of beer wort as a percent of extracted sucrose by weight) has generally been recommended to place the ratio of carbon to nitrogen in the range of favorable flavor compounds.

The ninhydrin method estimates amino acids, ammonia, and the terminal nitrogen groups of peptides and proteins. The European Brewery Convention (EBC), MEBAK, and American Society of Brewing Chemists (ASBC) describe FAN measurement using the ninhydrin based assay.

The correlation between the Brewing Analytes Proficiency Testing Scheme (BAPS) beer samples measured according to the EBC FAN protocol and the Thermo Scientific™ Gallery™ system alpha-amino nitrogen by o-Phthaldialdehyde (NOPA) method are examined. This rapid 2-reagent method was developed for a multipurpose discrete analyzer and the method is easily adapted for use with a manual spectrophotometer. A blank buffer eliminates possible sample color interference. The total analysis time for six samples and 60 test requests is approximately 45 minutes.

## Experimental

### Materials and methods

#### Instrument

The NOPA method was tested using a Thermo Scientific™ Gallery discrete analyzer. Similar results were obtained with the Thermo Scientific™ Gallery™ Plus discrete analyzer, Thermo Scientific™ Arena™ discrete analyzer, and Thermo Scientific™ Gallery™ Plus Beermaster discrete analyzer.

#### Reagents

A Thermo Scientific™ Gallery™ system reagent based on the alpha amino nitrogen by O-phthaldialdehyde (NOPA) method was used which provides ready-to-use non-hazardous reagents for 300 tests when performed using the automated discrete analyzer.

#### Method principle

Primary amino groups are derived by a reaction of o-Phthaldialdehyde (OPA) and N-acetyl cysteine (NAC) to form isoindoles. In optimized conditions isoindoles form a chromogenic complex proportional to the concentration of alpha-amino nitrogen in the sample. The reaction is measured photometrically at a 340 nm wavelength using a side wavelength of 700 nm.

#### Samples

Six commercial BAPS reference samples were purchased from LGC Standards. Assigned values from the proficiency test average result from the analysis of samples according to the official EBC method in different laboratories. Beer and wort samples were analyzed for method comparison and performance studies.

In addition to the BAPS samples, 16 commercial beer samples and two wort samples were analyzed. Reference values were assigned by the ninhydrin method. For NOPA analysis, beer samples were degassed by shaking for 10 minutes. Wort samples were centrifuged.

In an industry setting, 33 malt or malt extract samples were analyzed. In-house ninhydrin results were assigned as reference values. Malt samples were prepared as either an Institute of Brewing (IoB) or an EBC congress mash extract. Malt extract samples were diluted 1:4.

#### Method calibration and quality control

The method was calibrated with a NOPA standard from Thermo Fisher Scientific. The NOPA standard has an alpha-amino nitrogen concentration of 150 mg/L.

A glycine standard (Sigma-Aldrich™, Cat # G7403) dissolved in water was used as a quality control sample. The standard stock was prepared by weighing 0.1085 g of pure glycine standard (C<sub>2</sub>H<sub>5</sub>NO<sub>2</sub>, MW = 75.07 g/mol, purity 99%) into a volumetric flask and diluted to 100 ml with deionized water. The solution has an alpha-amino nitrogen concentration of 200 mg/L. QC samples of 20, 50, and 125 mg/L were diluted from stock with deionized water.

#### Application for automation

The automated Gallery discrete analyzer NOPA application consists of two reagents, an end-point measurement with a sample blank, and a linear calibration curve used for result calculation. Measuring ranges were tested from 20 to 300 mg/L with a secondary dilution of 1:3 at 150 mg/L. First, 200 µl of NOPA R1 reagent and 2 µL of sample are incubated for 120 seconds, then the reaction is blanked. After the addition of 20 µL of R2 reagent and 300 seconds of incubation, the reaction is measured at a wavelength of 340 nm. An additional side wavelength of 700 nm or 750 nm can be used to remove the effect of bubbles which may appear in cuvettes. The side wavelength is determined from the spectrum area where no reaction occurs. The method is performed at 37 °C.

#### Method calibration

Results were calculated automatically by the discrete analyzer using a linear calibration curve. A calibrator solution with 150 mg/L of alpha-amino nitrogen and deionized water was used to calibrate the test.

## Results and discussion

### Repeatability and reproducibility

Repeatability was performed by analyzing BAPS samples with the Gallery discrete analyzer in three batches. Six different beer samples were analyzed with the number of measurements at  $n = 30$ . The test was calibrated prior to the analysis of each batch.

The NOPA method showed excellent repeatability both within and between runs, and the typical within run coefficient of variation (CV %) was below 1.5%. Total repeatability of the measurement for the samples was between 2.2 and 3.2%. Repeatability results are shown in Table 1.

**Table 1. Method repeatability ( $n = 30$ ).**

	Sample 206-2L		Sample 207-2L		Sample 209-2L	
	Results (mg/L)	Assign value	92.50	Assign value	73.00	Assign value
	N	30	N	30	N	30
	Mean	98.63	Mean	74.07	Mean	29.83
	z score	1.23	z score	0.21	z score	-0.03
	SD	CV %	SD	CV %	SD	CV %
Within run	1.14	1.2	0.79	1.1	0.75	2.5
Between run	1.86	1.9	1.85	2.5	0.21	0.7
Total	2.19	2.2	2.02	2.7	0.78	2.6
	Sample 206-2B		Sample 209-2B		Sample 210-2L	
	Results (mg/L)	Assign value	52.74	Assign value	29.00	Assign value
	N	30	N	30	N	30
	Mean	54.36	Mean	29.10	Mean	126.49
	z score	0.32	z score	0.02	z score	2.46
	SD	CV %	SD	CV %	SD	CV %
Within run	0.44	0.8	0.32	1.1	1.52	1.2
Between run	1.66	3.1	0.62	2.1	3.24	2.6
Total	1.72	3.2	0.69	2.4	3.58	2.8

### Calculating the Z-score

Instructions from the BAPS were followed. A Z-score reports the statistical relationship to the mean of a group of scores.

Interpretation of the Z-score calculation results are shown below:

#### Z score

$|Z| \leq 2.00$

$2.00 < |Z|$  and  $< 3.00$

$|Z| \geq 3.00$

#### Interpretation

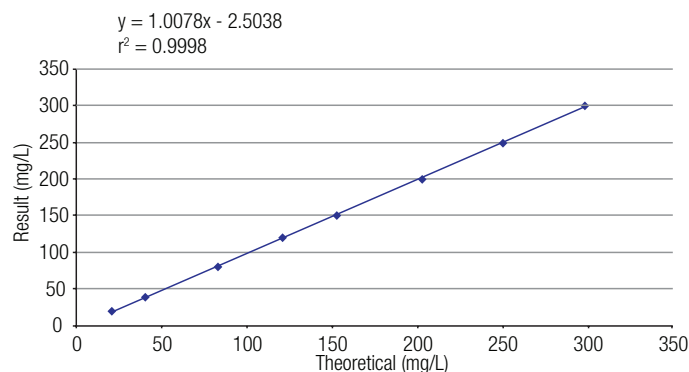
Satisfactory result

Questionable result

Unsatisfactory result

### Linearity

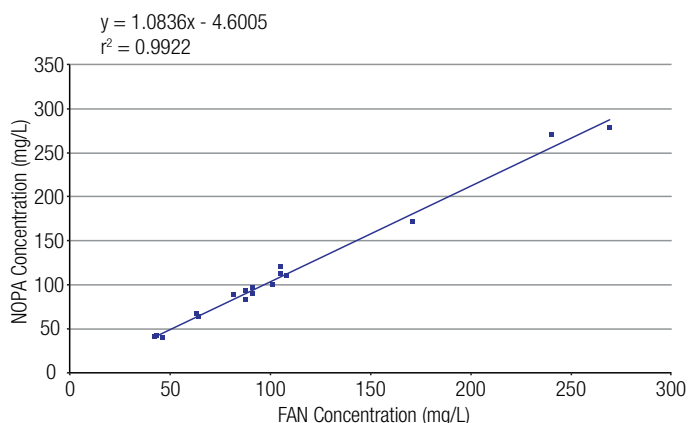
Method linearity was tested with pure chemicals dissolved in deionized water. The primary measurement was designed for a range of 20–150 mg/L and extended with a automatic secondary dilution (1:3) up to 300 mg/L. All linearity samples were measured as triplicates. An example of method linearity is shown in Figure 1.



**Figure 1. Method linearity for 20–300 mg/L samples.**

## Method comparison to FAN

For the method comparison study, 16 commercial beer samples and two wort samples were analyzed. Values used as a reference were assigned by the FAN ninhydrin method. For the NOPA analysis, beer samples were degassed by shaking for 10 minutes. Wort samples were also centrifuged. Recovery rates varied from 88–114%. For most samples, the recovery rates were 96–109% demonstrating excellent correlation between the two methods. Based on this preliminary study, this method shows good correlation to FAN ( $r^2=0.99$ ). A method comparison curve is shown in Figure 2.



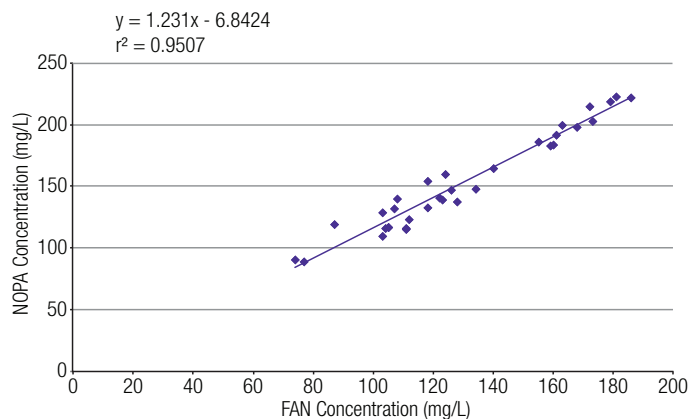
**Figure 2. Method comparison.** Two wort samples have the highest NOPA concentration.

## Manual method

The Gallery system reagent based on the alpha amino nitrogen by O-phthaldialdehyde (NOPA) method can also be performed manually using a spectrophotometer at 340 nm with a 1 cm cuvette path length. A baseline measurement is done against air or deionized water at 37 °C. The method was designed as an end-point method with a reaction time of 5 minutes. The sample/R1/R2 ratio is 1/100/10. Manual method linearity was in a range from 0 to 200 mg/L.

## Malting industry comparison to FAN

For a within-industry comparison, two malt extract samples and 31 malt samples (either loB or congress mash extracts) were analyzed. NOPA results using the Gallery discrete analyzer were compared to in-house FAN ninhydrin results. The calibration was completed using a 200 mg/L glycine standard. Results of the comparison demonstrate good correlation to the FAN method ( $r^2=0.95$ ) and are shown in Figure 3.



**Figure 3. A comparison of the FAN and NOPA methods.**

## Conclusion

All samples tested by the Gallery system reagent based on the alpha amino nitrogen by O-phthaldialdehyde (NOPA) method demonstrate result levels similar to samples tested according to the EBC FAN protocol for beer. Furthermore, no additional ammonia measurements are needed. The Gallery system reagent based on the alpha amino nitrogen by O-phthaldialdehyde (NOPA) method is an easy to use, robust method for the measurement of FAN in beer and wort. The advantage of using an automated discrete analyzer is its ability to measure multiple analytes like beta-glucan and  $SO_2$  in addition to performing the NOPA measurement on the same sample. The results for all measured analytes can be reported together.

## Acknowledgements

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