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Analytical guide for routine beverage analysis



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### Introduction

#### Process Critical Parameters in Alcoholic Beverages

- Acetaldehyde
- Alpha-Amino Nitrogen (NOPA)
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- Bitterness
- Ethanol
- Glycerol
- Total Polyphenol
- Total Protein (Biuret)
- Alpha Amylase

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- Acetic Acid
- Citric Acid
- D-Gluconic Acid
- D-Isocitric Acid
- D-Lactic Acid
- L-Lactic Acid
- L-Malic Acid
- Oxalic Acid
- L-Ascorbic Acid

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- Potassium

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- D-Glucose
- D-Glucose & D-Fructose
- D-Glucose & D-Fructose & Sucrose
- Sucrose

#### Metals

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- pH and Conductivity by Electro chemistry
- Free and Total SO<sub>2</sub>
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#### Reagent Ordering Table





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For all beverages, the compositional quality and safety must be monitored to help track contamination, adulteration and product consistency, and to ensure regulatory compliance from raw ingredients (water, additives and fruits) to the final product.

Analytes of interest include ions, sugars, organic acids, alcohol, color, metals, protein and enzymes. Analytical testing solutions involved in beverage analysis encompass several types of chromatographic, elemental and traditional wet chemistry analysis techniques.

**Automated wet chemistry analysis**

Traditional wet chemistry methods include titration, colorimetry and pH and ion-selective measurements, as well as, Flow Injection Analysis (FIA), or Segmented Flow Analysis (SFA). However, traditional wet chemistry methods are labor-intensive and time-consuming.

The [Thermo Scientific™ Gallery™ Discrete Analyzer](#) provides an integrated platform for colorimetric, photometric and electrochemical analyses, which can be run in parallel. Discrete cell technology allows for simultaneous measurement of several different tests for the same sample, eliminating method changeover time. Each individual reaction cell is isolated and temperature-stabilized, thus providing highly controlled reaction conditions. The Gallery discrete analyzer is able to achieve very low detection levels, and its sophisticated dilution features help to manage a wide concentration range without user intervention. Results are ready within minutes, translating into a remarkable reduction of hands-on time. The wavelength range covers filter configurations from 340 nm up to 880 nm. An optional, integrated Electro Chemical Measurement (ECM) unit supports conductivity and pH measurements over a wide range.

**Technology comparison**

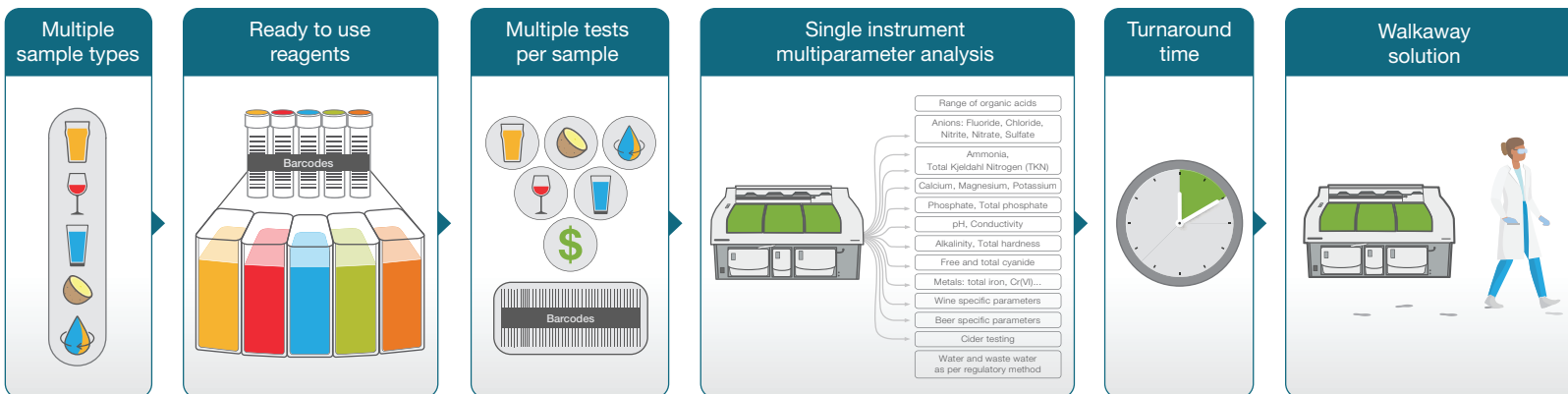
**Traditional wet chemistry**

- mL reagents
- 50-100 mL samples per test
- Liters of waste generation
- Multiple instruments
- Labor intensive
- Sequential or batch
- Typically single or max 4 parameters per sample
- High cost per analysis
- Low throughput – 20-80 tests/hr

**Gallery discrete analyzer platform**

- 2-120 µL reagents
- Max 300 µL per test
- Few mL of waste
- Single platform
- Easy to operate
- Fully automated
- Parallel and batch
- Up to 20 parameters per sample
- Low cost per analysis
- High throughput up to 200-350 tests/hr
- Integrated barcode reader for samples and reagents

**Multiparameter analysis – optimized workflow with the Gallery discrete analyzer**



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### Sample preparation

Sample preparation is an important step in beverage analysis, but it's not always necessary. Some samples (e.g., wine) often require no treatment. With other samples (e.g., juice), sometimes short centrifugation (e.g., 5 min., 15,000 rpm) is enough to make the sample homogeneous, or extraction, dilution or filtration is sufficient for sample preparation. Typical sample preparation methods are listed below.

Sample characteristics	Preparation method
Liquid, clear, colorless and practically neutral	Use samples directly, or after dilution, into the measuring range of the assay. The Gallery discrete analyzer can perform the dilutions automatically.
Turbid	Use filter or centrifuge.
Contains carbon dioxide	Degas samples by shaking and filtering with a folder filter paper or in an ultrasonic bath.
Contains protein	Deproteinize samples with Carrez reagents.
Solid or semi-solid, does not contain fat	Crush or homogenize the samples. Weigh sufficient quantity of the sample in a volumetric flask (take note of the measuring range); extract with water and filtrate; centrifuge or use Carrez clarification, if necessary.
Solid or semi-solid, contains fat	Weigh sufficient quantity of the sample into a volumetric flask (take note of the measuring range); extract with hot water. Cool to allow the fat to separate, make up to the mark, place the volumetric flask in an ice bath for 15-30 min. and filter. Discard the first few ml of filtrate, and use the clear supernatant (which may be slightly opalescent) for the assay. Alternatively, clarify with Carrez reagents.
Needs pH adjustment	Adjust the sample solution to suitable pH by adding Hydrochloric acid (1 M) or potassium hydroxide solution (2 M) and incubate for approx. 15-30 min.
Strongly colored	Treat sample solution with polyvinylpyrrolidone (PVPP) (e.g., 1 g/100 ml sample). Shake the tube vigorously for 5 min., then filter the sample.

### Download Smart Note:

[What is Better for Automating Wet Chemical Analysis? Integrated Discrete Analyzer or Flow Analyzers?](#)







Acetaldehyde is commonly found in food and beverages such as fruits, vegetables, and dairy products. Generally, fruits have more acetaldehyde than vegetables and other foods. Eating foods with significant amounts of acetaldehyde may increase the risk of cancers of the digestive tract.

Some foods are made through fermentation by microorganisms, such as yeast, fungi or bacteria. Acetaldehyde is a byproduct of fermentation, which means that fermented foods can have high amounts of acetaldehyde. Types of fermented foods that may be high in acetaldehyde include yogurt, vinegar, kombucha, fish products, fermented mushrooms, fermented soy products, pickled vegetables, canned vegetables and kimchi.

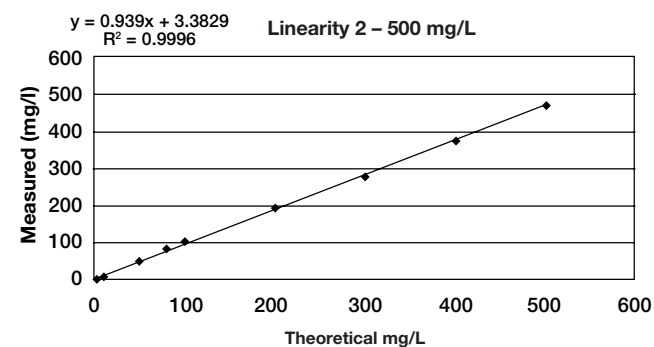
Acetaldehyde enters the human body when breathing air that contains it, like with cigarette smoke. It can also enter body when you ingest foods or drinks containing acetaldehyde. When drinking alcohol, for example, the body makes acetaldehyde to process the alcohol. Acetaldehyde enters the blood, damaging your membranes and possibly causing scar tissue. It may also cause a hangover, and can result in a faster heartbeat, a headache or an upset stomach.

**Method:** [Gas Chromatography](#) with Headspace is also used for acetaldehyde determination in several food products. Selective enzymatic reaction followed by a photometric measurement is highly selective and can be automated. Automated photometric measurement is rapid and can measure acetaldehyde down to 2 mg/L.

**Chemistry:** The method is based on the enzyme catalyzed conversion of acetaldehyde to acetic acid in the presence of NAD. Within that reaction, NAD is reduced to NADH, which causes a spectral shift in the absorbance of the solution. NADH has a distinct absorbance at 340 nm while NAD absorbs at 260 nm. The amount of NADH formed in this reaction is stoichiometric with the amount of acetaldehyde. The method could be extended to other food matrices with appropriate sample preparation.

**Table 1. Reagents**

Reagent Part Number	Maximum Number of Tests	Measuring Range (mg/L)
984347	250	2 – 500
984396 – Acetaldehyde Standard		



**Figure 1: Method performance linearity**

**Samples:** Method performance was tested for beer and white wine; however, the method can also be applied for other alcoholic beverages, citrus fruit juices and vegetable juices. Additional sample preparation may be necessary to remove interference, especially with colored samples like red wine, which needs to be decolorized. Please refer to the sample preparation section of the insert for more details. It is recommended to validate the method using spiked samples with a known amount of analyte to see the possible matrix effect of the sample.

**Table 2. Precision**

	Spiked Beer		White Wine	
	SD	CV%	SD	CV%
N	30		30	
Mean (mg/L)	33		36	
Within Run	0.226	0.7	0.246	0.7
Between Run	0.136	0.4	1.003	2.7
Total	0.263	0.8	1.032	2.8



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## Process Critical Parameters in Alcoholic Beverages

Acetaldehyde

### Alpha-Amino Nitrogen (NOPA)

Beta-Glucan (High MW)

Bitterness

Ethanol

Glycerol

Total Polyphenol

Total Protein (Biuret)

Alpha Amylase

### Organic Acids

### Important Cations

### Sugars

### Metals

### Titration Parameters

### Reagent Ordering Table

Nitrogen is a critical nutrient for yeast growth and fermentation activity, and affects the rate and completion of fermentation, fermentation bouquet and style of wine, beer and cider. Primary Amino Nitrogen is one of the two main sources of nitrogen for yeast. It's a measure of the concentration of individual amino acids and small peptides that can be used by yeast for cell growth. Together with ammonia, Primary Amino Nitrogen makes up the measurement of Yeast Assimilable Nitrogen (YAN) and can be measured prior to fermentation.

Testing for nitrogen before and during the fermentation is smart. YAN that is too low or too high can have negative effects on the winemaking and brewing process, and ultimately, the end product/beverage. Excessive inorganic nitrogen can increase the risk of ester taint formation. Excessive organic nitrogen levels may lead to increased formation of ethyl carbamate. YAN analysis provides information on the nitrogen status of grapes, musts and juices: specifically, the amount of nitrogen available for yeast to utilize during fermentation. Early detection of nitrogen deficiency can enable wine, beer and cider makers to make informed decisions regarding nitrogen additions using inorganic nitrogen or organic nitrogen. Rapid and fully automated analysis of nitrogen sources are important.

**Method:** A high performance liquid chromatography (HPLC) method based on ninhydrin post column reaction method could be used for Ammonia and NOPA analysis. Alternatively, a colorimetric method could be used. For NOPA analysis, a rapid two-reagent NOPA (alpha-amino nitrogen by OPA) method was developed for the automated discrete analyzer using a blank buffer to eliminate sample color interference.

**Chemistry:** o-phthaldialdehyde (OPA) and N-acetylcystine (NAC)

Primary Amino groups are derivated by OPA (o-Phthaldialdehyde) and NAC (N-acetyl cysteine) to form isoindoles. In optimized conditions, isoindoles form a chromogenic complex with max absorbance at 340 nm, proportional to the concentration of alpha-amino nitrogen in the sample.

Amino nitrogen + N-acetyl-L-cysteine + o-phthaldialdehyde

↓  
isoindole derivative

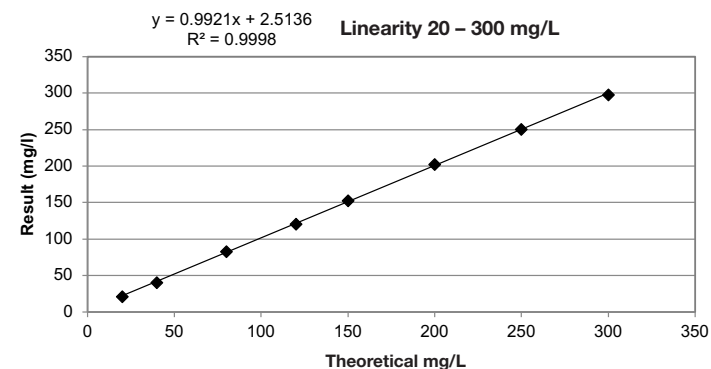
**Table 1. Reagents**

Reagent Part Number	Maximum Number of Tests	Measuring Range (mg/L)
984342	300	20 – 300
984394 – NOPA Standard		

**Samples:** Method precision is tested with red wine and two varieties of beer samples. Method can be applied to other wine and beer, as well as cider samples. The test has been developed to determine alpha-amino nitrogen concentrations within a measuring range of 20 to 300 mg/L.

**Table 2. Precision**

	Red Wine		Lager Beer 1		Lager Beer 2	
	SD	CV%	SD	CV%	SD	CV%
N	50		50		50	
Mean (mg/L)	46		88		196	
Within Run	0.665	1.5	1.451	1.6	3.182	1.6
Between Run	0.342	0.7	1.911	2.2	3.392	1.7
Total	0.745	1.6	2.400	2.7	4.651	2.4



**Figure 1. Method Performance Linearity**

### Download Application Note:

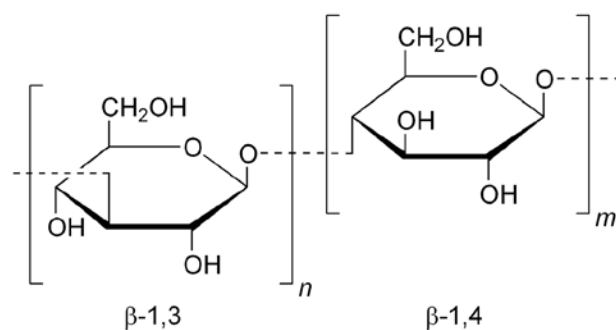
[Correlation of the free amino nitrogen and nitrogen by O-phthaldialdehyde methods in the assay of beer application note](#)





# Beta-Glucan (High MW)

In the malting and brewing process used for beer production, one important analyte is beta-glucan ( $\beta$ -glucan).  $\beta$ -glucans are polysaccharides of D-glucose monomers linked by beta-glycosidic bonds.  $\beta$ -glucans are present in the cell walls of various cereals and are capable of clogging process filters; therefore, an excess of  $\beta$ -glucans may cause haze in the end product and thus impact appearance of the beer. For this reason, it's important to determine the concentration of  $\beta$ -glucan, particularly the portion of the polymer with a molecular size of 10,000 daltons (Da) or more.

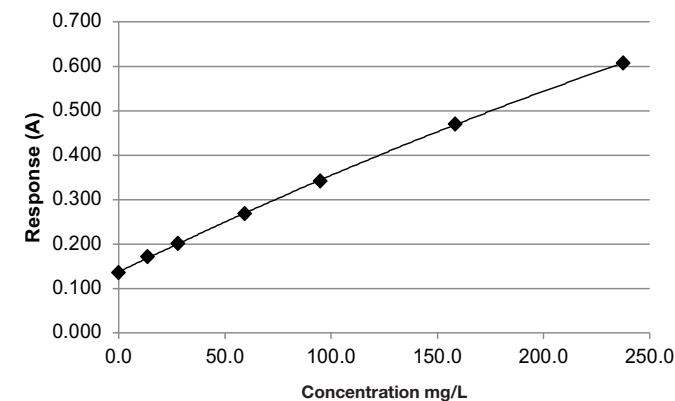


**Method:** A rapid two-reagent method was developed for Beta-Glucan (High MW) in wort and beer samples. The method was designed with the use of a blank buffer to eliminate possible sample color interference. The Beta-Glucan (High MW) method can also be performed using a manual spectrophotometer at 405 nm with a one cm cuvette path length.

In this novel method, high molecular weight barley (1,3/1,4)- $\beta$ -glucan forms a complex with reagent R2 in buffered conditions at pH 8 that is proportional to the concentration of high molecular weight  $\beta$ -glucan in the sample. The reaction is measured photometrically at a 405 nm wavelength. It's an end-point method with a reaction time of 10 min.

**Table 1. Reagents**

Reagent Part Number	Maximum Number of Tests	Measuring Range (mg/L)
984305	350	15 – 500
984383 – Beta-Glucan Standard		



**Figure 1.** A calibrator solution containing 250 mg/L of  $\beta$ -glucan and deionized water was used for calibrating the test, and was automatically diluted by the analyzer.



**Samples:** Beer and wort samples were analyzed for method comparison and performance studies. Turbid wort samples were centrifuged. Beer samples were degassed by manual shaking for 10 minutes.

**Table 2. Precision**

	Wort 1		Wort 2		Wort 3	
	SD	CV%	SD	CV%	SD	CV%
N	20		20		20	
Mean (mg/L)	195		245		91	
Within Run	0.79	0.4	1.34	0.5	0.96	1.1
Between Run	0.17	0.1	0.55	0.2	0.13	0.1
Total	0.81	0.4	1.45	0.6	0.97	1.1

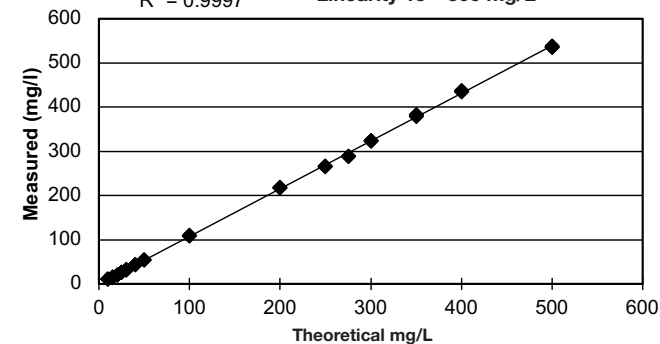
**Download Application Note:**

[Rapid determination of high molecular weight beta-glucan using a photometric method](#)

$$y = 1.0793x - 0.5303$$

$$R^2 = 0.9997$$

Linearity 15 – 500 mg/L



**Figure 2.** Method linearity was tested with pure chemicals dissolved in deionized water. The primary measurement was determined to range from 15 to 300 mg/L and was extended with an automatic secondary dilution (1:3) up to 500 mg/L. All linearity samples were measured in triplicate.







Bitterness

Bitterness is one of the most fundamental characteristics of beer and a chief component in its flavor quality. The bitterness of beer is imparted from the use of hops, primarily through the extraction of iso- $\alpha$ -acids from the hop cones during brewing. The International Bitterness Unit (IBU), developed in association with the American Society of Brewing Chemists (ASBC), is a scale that measures the overall bitterness of beer. It's a direct measurement of the concentration of iso-alpha acids, isohumulones as main component, the bitterness-causing chemical component of beer. A light lager beer would result in an IBU between 5 and 15, whereas a dark one would be between 18 and 25. Extremely bitter beers can return readings upwards of 100 IBUs. The final BU of beer is a result of both the amount and type of hops used during the brewing process. In order to maintain consistency in quality, bitterness needs to be tightly monitored and controlled.

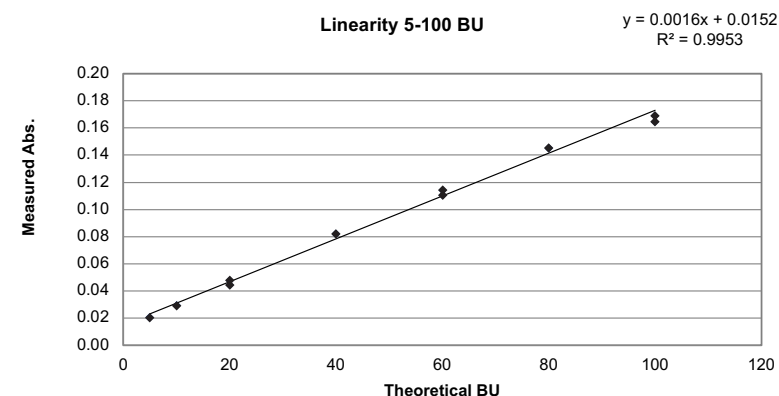
**Method:** The overall bitterness of beer can be determined by extracting an acidified beer solution into isooctane followed by measuring the absorbance at 275 nm using [Thermo Scientific™ Genesys™ UV-VIS Spectrophotometer](#). The absorbance is then multiplied by 50 to obtain the bitterness in BU. A fully automated method of bitterness measurement is developed using inline solid-phase extraction followed by UV detection at 275 nm. The bitterness test used with the [Thermo Scientific™ Gallery™ Plus Beermaster Discrete Analyzer](#) is based on binding iso-alpha acids onto the surface of a solid phase extraction column, which is integrated into the photometric analyzer.

**Chemistry:** Automated Bitterness Testing

The Bitterness unit (BCM), an integrated coated capillary column within the Gallery Plus Beermaster discrete analyzer, includes unique automated pretreatment and measurement for bitterness. The traditional manual test for bitterness is slow and laborious, and uses liquid-liquid extraction with isooctane, which must be disposed of properly. The system's bitterness module, on the other hand, uses 'green' solid-phase extraction ahead of a fully-automated testing protocol. During the automated process, the beer bittering substances are first extracted from interfering compounds present in the sample matrix, and then measured at 275 nm approximately eight bitterness tests per hour. With the BCM in use, the Gallery Plus Beermaster discrete analyzer can accommodate 72 samples.

**Table 1. Reagents (all reagents are required.)**

Reagent Part Number	Maximum Number of Tests	Measuring Range (IBU)
984353 – BC System Liquid	360	5 – 100
984354 – BC Diluent	360	5 – 100
984355 – BC Eluent	360	5 – 100



**Figure 1. Method Performance Linearity: Linearity curve from one single diluted beer sample.**



Ethanol

In normal fermentation, yeast converts sugar to ethanol and carbon dioxide. A simple, reliable and accurate method for the determination of low ethanol concentrations is typically required for quality and labeling purposes. Ethanol in wine, beverages, foodstuffs and other materials based on alcohol dehydrogenase is highly specific, accurate, reliable and simple.

**Method:** An enzymatic test with Alcohol-Dehydrogenase (ADH). Method is performed at 37 °C, using a 340 nm filter. Conversion of ethanol via the following reactions is directly proportional to the coupled formation of NADH. This method is best suitable for low concentration of Ethanol, the typical measuring range is from 0.01 to 10 g/L.

**Chemistry:** Alcohol-dehydrogenase

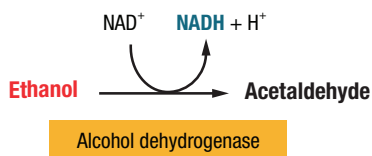


Table 1. Reagents

Reagent Part Number	Maximum Number of Tests	Measuring Range (g/L)
984300	300	0.01 – 10
984384 – Alcohol Standard		

Table 2. Precision: low calibration range

	Sample 1		Sample 2		Sample 3	
N	50		50		50	
Mean (mg/L)	0.50		1.0		3.0	
	SD	CV%	SD	CV%	SD	CV%
Within Run	0.007	1.5	0.010	1.0	0.030	1.0
Between Run	0.009	1.9	0.012	1.2	0.042	1.4
Total	0.012	2.4	0.015	1.5	0.051	1.7

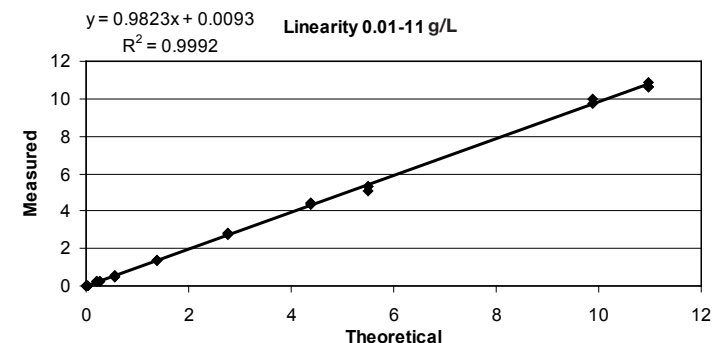


Figure 1. Method Performance Linearity: The test has been developed to determine ethanol concentrations within a measuring range of 0.01 to 10 g/L.







During fermentation, glycerol is synthesized from the glucose within yeast cells. Glucose is converted to glyceraldehyde-3-phosphate and dehydroxyacetone phosphate. Most of the produced dehydroxyacetone phosphate converts to glyceraldehyde-3-phosphate, eventually producing ethanol. The remainder produces glycerol.

Glycerol is the third most common chemical compound in wines and an important by-product of alcoholic fermentation. Usually the glycerol concentration in wines is about 5 g/L, but concentrations can be as high as 15-20 g/L and depend upon fermentation conditions, especially the level of sulfur dioxide. The influence of glycerol in finished wine is usually at or below the level of sensory perception. Wines with elevated levels of alcohol tend to have more body and viscosity and a sweet taste, which has often been attributed to the presence of glycerol.

Glycerol is an indicator of quality and the most important by-product of alcoholic fermentation. The concentration of glycerol depends on sulfur dioxide levels during fermentation conditions. It is monitored in the wine industry, where it occurs at concentrations of approximately 1% (v/v).

**Method:** The Glycerol kit is a simple, reliable, rapid and accurate method for the measurement and analysis of glycerol in beverages, foodstuffs and other materials. Method is performed at 37 °C, using 340 nm filter.

**Chemistry:** ADP dependent hexokinase and glucose-6-phosphate-dehydrogenase.

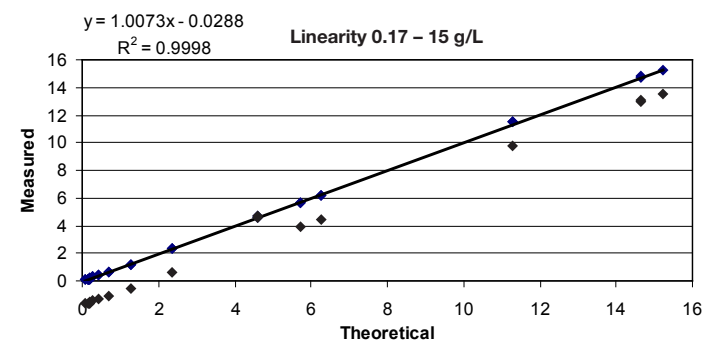
- (1) **Glycerol + ATP**  $\xrightarrow{GK}$  L-Glycerol-3-phosphate + ADP
- (2) **ADP + D-Glucose**  $\xrightarrow{ADP-HK}$  D-Glucose-6-phosphate + AMP
- (3) **D-Glucose-6-phosphate + NAD<sup>+</sup>**  $\xrightarrow{G6P-DH}$  phospho-D-glucono-1,5-lactone + NADH

**Table 1. Reagents**

Reagent Part Number	Maximum Number of Tests	Measuring Range (g/L)
984316	300	0.07 – 30
984386 – Glycerol Standard		

**Table 2. Precision**

	Sample 1		Sample 2		Sample 3	
N	50		50		50	
Mean (g/L)	2.41		8.20		12.10	
	SD	CV%	SD	CV%	SD	CV%
Within Run	0.034	1.4	0.108	1.3	0.140	1.2
Between Run	0.060	2.5	0.189	2.3	0.274	2.3
Total	0.069	2.9	0.218	2.7	0.308	2.5


**Figure 1. Method Performance Linearity**

Polyphenols are components of wine, particularly red wine, that do not exist in spirits, and exist in low concentrations in beer and malt whiskey.

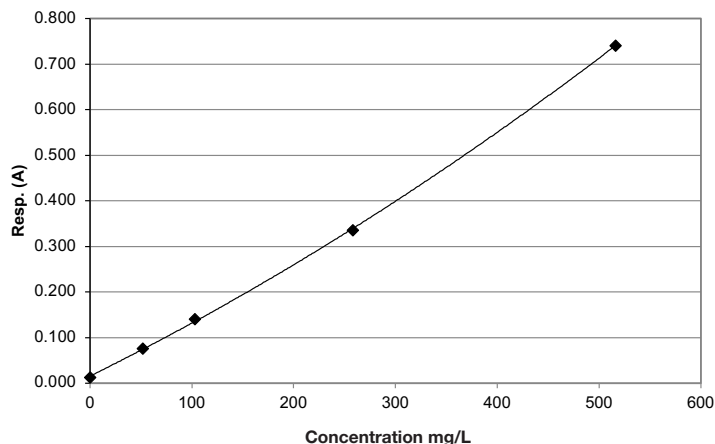
Polyphenolic composition varies among different wines according to the type of grape used, vivification process used, type of yeast that participates in the fermentation process.

**Chemistry:** Folin-Ciocalteu

Polyphenols react in basic environment with Folin-Ciocalteu Reagent optimized and modified. The chromogenic complex is proportional to the concentration of polyphenols in the sample, measured at 700 nm wavelength. For beer, self made reagents are used (ferric method).

**Table 1. Reagents**

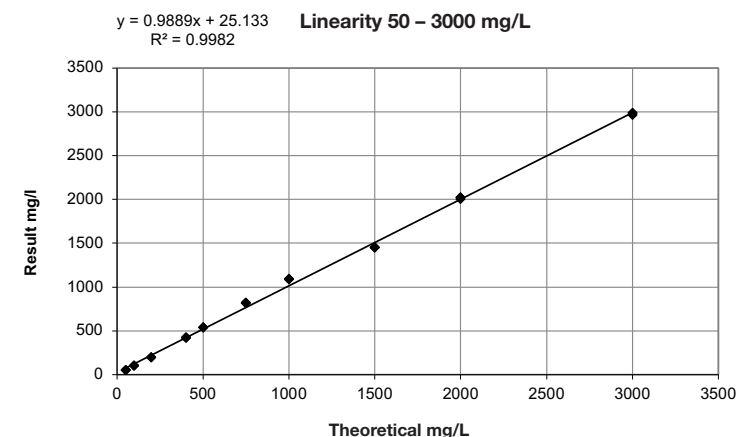
Reagent Part Number	Maximum Number of Tests	Measuring Range (mg/L)
984346	280	50 – 3000



**Figure 1. Calibration is second order using 500 mg/L gallic acid standard dilutions.**

**Table 2. Precision**

	White Wine		Dark Beer		Red Wine	
N	30		30		30	
Mean (mg/L)	318		825		2452	
	SD	CV%	SD	CV%	SD	CV%
Within Run	1.80	0.6	5.58	0.7	36.45	1.5
Between Run	5.00	1.6	12.31	1.5	23.35	1.0
Total	5.31	1.7	13.51	1.7	43.29	1.8



**Figure 2. Method Performance Linearity**





Total Protein (Biuret)

In beer, proteins are analyzed to control haze formation, which primarily results from protein-polyphenol interactions within the product.

**Chemistry:** Biuret

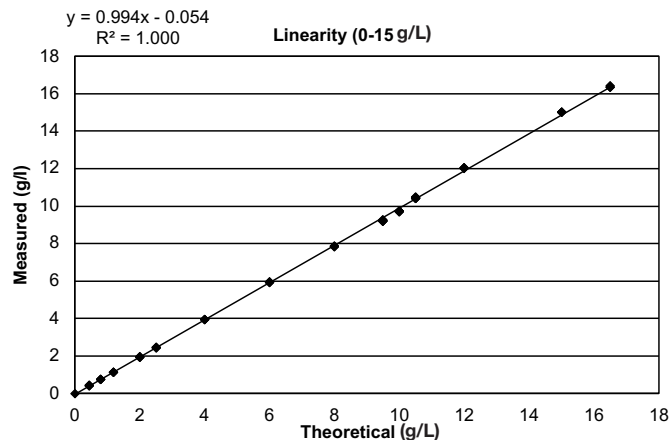
Protein forms a colored complex with cupric ions in alkaline solutions. The formation of the complex is measured at 540 nm. The method employs EDTA as a chelating and stabilizing agent for cupric ions.

**Table 1. Reagents**

Reagent Part Number	Maximum Number of Tests	Measuring Range (g/L)
984328	450	0.5 – 15

**Table 2. Precision**

	Dark Beer		Lager Beer 1		Lager Beer 2	
N	50		50		50	
Mean (g/L)	3.5		4.5		8.1	
	SD	CV%	SD	CV%	SD	CV%
Within Run	0.032	0.9	0.035	0.8	0.062	0.8
Between Run	0.076	2.1	0.110	2.4	0.158	1.9
Total	0.083	2.3	0.115	2.6	0.170	2.1



**Figure 1. Method Performance Linearity**







## Alpha Amylase

Alpha Amylase is a major mash enzyme of critical concern to brewers in their production of fermentable wort. It digests starch, a large polymer of glucose, into smaller units, exposing it to further digestion by beta amylase. Together these two amylases produce the spectrum of wort sugars essential in the production of a beer. Levels of alpha amylase are typically high in pale malt but are virtually zero in roasted malt due to heat degradation. Levels vary according to malt variety and to malting conditions.

Alpha-amylase activity is measured by monitoring the color change of the reaction of a buffered extract of malt with a dextrinized starch substrate and iodine. Reactions are performed at 37 °C and a photometric endpoint measurement at 660 nm.

**Third party application available with self-made reagents.**

### **Download Poster:**

[Rapid Automated Method to Measure Alpha-Amylase Activity in Malt](#)



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Tartaric Acid

Acetic Acid

Citric Acid

D-Gluconic Acid

D-Isocitric Acid

D-Lactic Acid

L-Lactic Acid

L-Malic Acid

Oxalic Acid

L-Ascorbic Acid

## Important Cations

Sugars

Metals

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Acids are important components in wine, beer, and cider contributing to its crisp, tart flavor. Alcohol, sugars, minerals and other components balance the sourness of the acids (tartaric, malic and citric acids), which are naturally present in grapes and provide the freshest, purest tastes. Lactic, acetic and other acids also play a minor role in fermentation and contribute milder, more complex flavors.

Tartaric and malic acid comprise over 90% of the total acids within must or wine in a tartaric-to-malic-acid ratio of 1:1 to 1:3. The actual acid concentration is influenced by varietal, region and farming practices. Since the presence of acids contributes to the flavor, stability, color and pH of wine, knowledge of acid concentrations can provide the winemaker with valuable information to help optimize flavor and stability.

During the growing phase, acids accumulate. When grapes are ripe, total acidity in the fruit decreases because of a reduction in malic acid. At harvest, more tartaric acid is present. Grapes are among the few fruits that naturally contain tartaric acid, which is microbially stable and normally present at a concentration between 2.5 to 5 g/L at harvest. Malic acid is metabolized during ripening and at harvest, its concentration declines to between 1 to 4 g/L.

Citric acid often acts as a preservative in commercial juice production. Levels can be measured to authenticate commercially produced juices in order to meet international standards. Alone or in combination with tartaric or malic acids, citric acid may be added to adjust the profile of grapes deficient in organic acids by increasing acidity, complementing the flavor, and preventing ferric haze in the finished product.

Tartaric acid is the predominant acid in grapes and controls the effective acidity (pH) of wine. An insufficient amount could affect the color, stability and taste of a particular wine. L-Lactic acid is produced during the fermentation process by lactate dehydrogenase. In the wine industry, the course of malolactic fermentation is monitored by following the declining level of L-Malic acid and the increasing level of L-Lactic acid. Monitoring L-Lactic acid is critical for wine making. When the bacteria introduced into the wine converts sugars into D-lactic acid and acetic acid, instead of converting malic acid into L-lactic acid, the wine spoilage occurs. Monitoring D-Lactic acid is important for preventing wine spoilage. The presence of acetic acid is recognized as a vinegary smell and is an indication of spoilage.

**Method:** Organic acids can be determined by [Ion Chromatography](#). Most carboxylic acids ionize sufficiently, making ion chromatography with suppressed conductivity detection the technique of choice to separate a large variety of organic acids with inorganic anions and detect them with high sensitivity while minimizing the sugar interferences. Since these organic acids do not contain chromophore, ion chromatography with suppressed conductivity detection is a preferred method of analysis when a wide range of organic acids need to be determined in wine, beer, cider or other fruit juices and food products.

Fully automated discrete analysis based on enzymatic/colorimetric analysis is rapid and simple to perform. Selective enzymatic reaction in complex food matrices ensures the selectivity of the measurement. Many of the enzymatic test kits are official methods of prestigious organizations, such as the Association of Official Analytical Chemicals (AOAC), International Standardization Organization (ISO), and the American Association of Cereal Chemists (AACC). [Gallery discrete analyzers](#) can automate the enzymatic, colorimetric and electrochemical wet chemical measurements in a single platform.





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- D-Isocitric Acid
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- L-Lactic Acid
- L-Malic Acid
- Oxalic Acid
- L-Ascorbic Acid

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Titration Parameters

Reagent Ordering Table

Tartaric acid, not found in most other fruits, is the primary acid in grapes and thus controls the acidity of a wine. It plays a critical role in the taste, feel and color of wine. More importantly, it lowers the pH to a level that improves resistance to bacterial contamination, acting as a preservative. Tartaric acid together with calcium contributes the formation of wine stones.

**Method:** Method is based on a complex formation between tartrate and vanadate. Color of the formed complex is measured at a wavelength of 540 nm.

**Chemistry:** Vanadium-tartrate complex

Table 1. Reagents

Reagent Part Number	Maximum Number of Tests	Measuring Range (g/L)
984309	480	0.5 – 12 g/L

Standard included in kit.

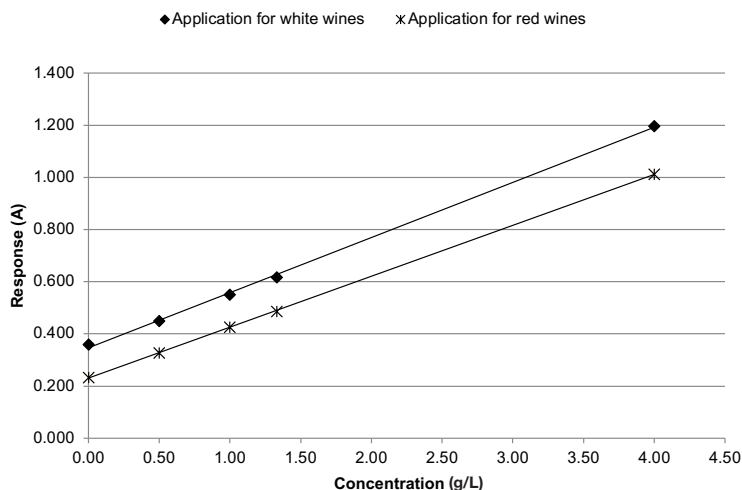


Figure 1. Calibration was performed using a standard included in the reagent kit. The concentration of the standard is 4 g/L. The calibration points were automatically diluted by the analyzer.

Table 2. Precision

	White Wine		Red Wine	
	SD	CV%	SD	CV%
N	30		30	
Mean (g/L)	2.76		2.11	
Within Run	0.045	1.6%	0.052	2.5%
Between Run	0.043	1.5%	0.042	2.0%
Total	0.062	2.2%	0.067	3.2%

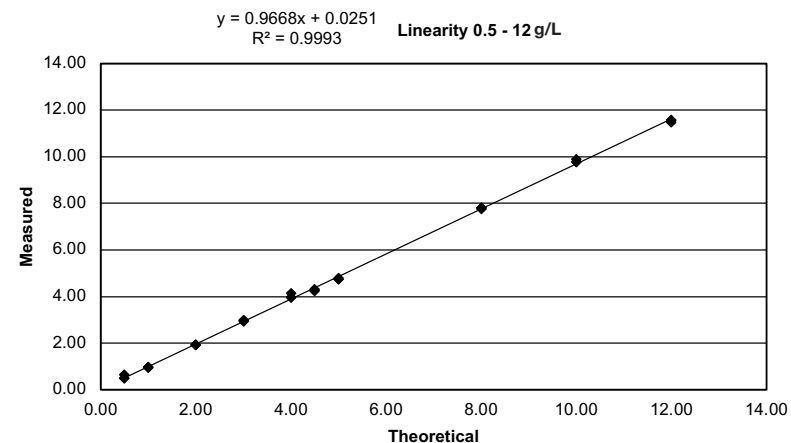


Figure 2. Method Performance Linearity: The test has been developed to determine tartaric acid within a measuring range from 0.5 to 12 g/L.

[Download: Poster](#)

[Rapid Automatic Analysis of Acids in Juice Using a Discrete Analyzer](#)





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Reagent Ordering Table

Acetic acid is a very important metabolite since it's the end product of fermentation processes, and the oxidation product of acetaldehyde and ethanol. Acetic acid is the main component of "volatile acids" in wine and one of the most important parameters of its quality control. A high concentration of acetic acid in wine results in spoilage of the product. Thus, there may be legal limitations in the acetic acid content. Acetic acid is used in food production as a preservative and a taste enhancer. Acetic acid is the compound determining the monetary value of vinegar.

Acetic acid determination is also important in cider products, beer, fruit, juices, soft drinks, and other food and beverage products.

**Chemistry:** Method 1 Acetate kinase  
 Enzymatic test with acetate kinase (AK), coenzyme A (CoA), phosphotransacetylase (PTA), ADP-dependent hexokinase (ADP-HK) and glucose-6-phosphat dehydrogenase (G6P-DH).

Method 2 Acetyl-coenzyme A synthetase (ACS)  
 Enzymatic test with acetyl-CoA synthetase (ACS), citrate synthetase (CS) and L-malate dehydrogenase (L-MDH).

**Samples:** Three different wine samples were tested by both the methods, with results summarized below. Method is suitable for other beverage and food samples for selective acetic acid determination. Samples of fruit juices and vinegar containing higher concentration of acetic acid, require dilution to fit within the linear working range. The built-in auto-dilution function in Gallery instruments can handle this need. Samples containing dissolved gases, such as beer or carbonated drinks, require degassing before sample analysis. Colored samples, such as red wine, need to be decolorized with PVPP before analysis.

Table 1. Method 1 based on Acetate Kinase

Reagent Part Number	Maximum Number of Tests	Measuring Range (g/L)
984318 – method 1	300	0.04 – 3.00

- (1) **Acetic acid** + ATP  $\xrightarrow{\text{acetate kinase}}$  acetyl-phosphate + ADP
- (2) **Acetylphosphate** + CoA  $\xrightarrow{\text{PTA}}$  Acetyl-CoA + Phosphate
- (3) **D-Glucose** + ADP  $\xrightarrow{\text{ADP-HK}}$  D-Glucose-6-phosphate + AMP
- (4) **D-Glucose-6-phosphate** + NAD<sup>+</sup>  $\xrightarrow{\text{G6P-DH}}$  D-Glucono-δ-lactone-6-phosphate + NADH + H<sup>+</sup>

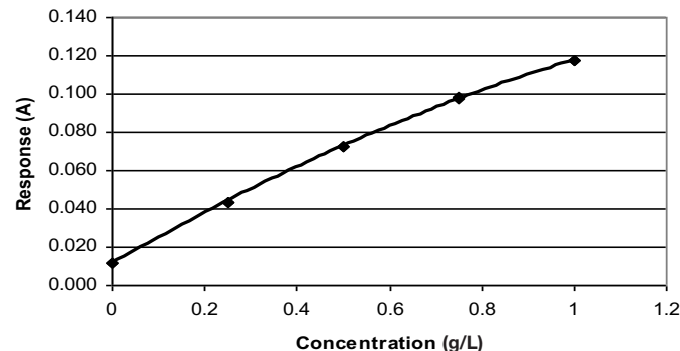


Figure 1. Calibration for method 1 is second order using 10 g/L acetic acid in standard dilutions.

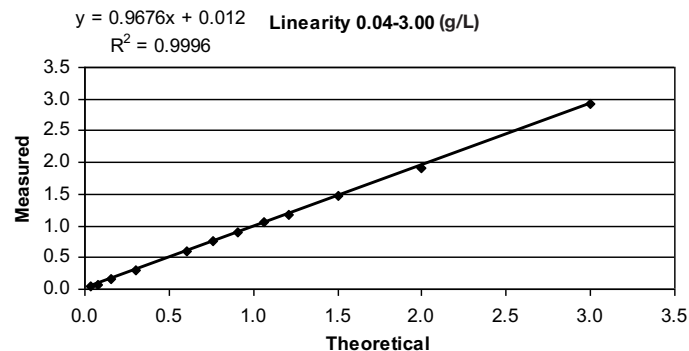


Figure 2. Method performance linearity for method 1. The test has been developed to determine acetic acid concentrations within a measuring range from 0.04 to 3.00 g/L.



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Table 2. Precision for method 1 Acetate Kinase

	Sample 1		Sample 2		Sample 3	
N	50		50		50	
Mean (g/L)	0.25		0.42		0.94	
	SD	CV%	SD	CV%	SD	CV%
Within Run	0.003	1.3	0.005	1.1	0.018	2.0
Between Run	0.002	0.9	0.003	0.8	0.013	1.4
Total	0.004	1.6	0.006	1.4	0.023	2.4

Table 3. Method 2 based on Acetyl-coenzyme A synthetase (ACS)

Reagent Part Number	Maximum Number of Tests	Measuring Range (g/L)
984356	150	0.04 – 3.00

Standard included in kit.

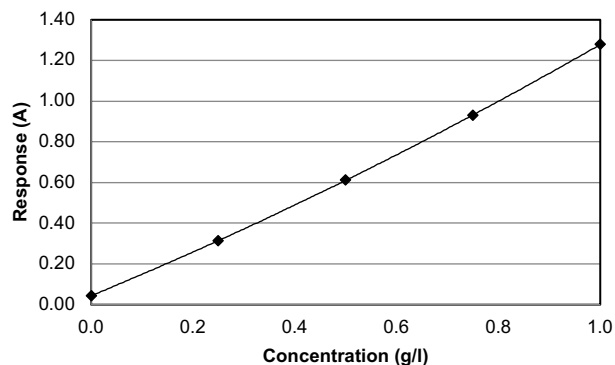
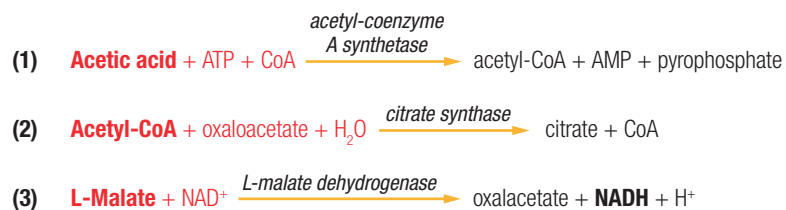


Figure 3. Calibration curve for method 2 (ACS).

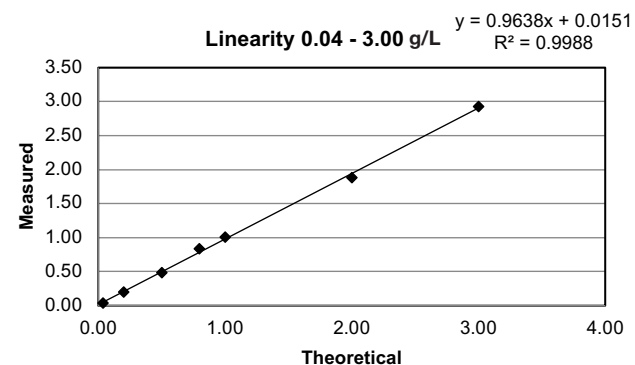


Figure 4. Method performance linearity for method 2 (ACS). The test has been developed to determine acetic acid concentrations within a measuring range from 0.04 to 3.00 g/L.

Table 4. Precision for method 2 (ACS).

	Red Wine		Spiked White Wine	
N	50		50	
Mean (g/L)	0.44		0.92	
	SD	CV%	SD	CV%
Within Run	0.003	0.7	0.005	0.6
Between Run	0.010	2.3	0.013	1.5
Total	0.010	2.4	0.014	1.6

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Citric acid is a flavoring agent, a pH adjustor, a metabolite and a food additive. In wine, it increases acidity, complements a specific flavor or prevents ferric hazes. The ratio of citric acid to D-Isocitric acid is commonly used as a marker to detect the authenticity and quality of fruit products, most often citrus juices.

**Method:** In the first reaction, citric acid (citrate) is converted to oxaloacetate and acetate in the reaction catalyzed by the enzyme citrate lyase (CL). In the presence of the enzymes L-malate dehydrogenase (L-MDH) and L-lactate dehydrogenase (L-LDH), oxaloacetate and its decarboxylation product pyruvate are reduced to L-malate and L-lactate, respectively, by reduced Nicotinamide-Adenine Dinucleotide (NADH). The amount of NADH oxidized in reactions is stoichiometric to the amount of citrate. NADH is determined by absorbance at 340 nm.

**Chemistry:** Citrate lyase

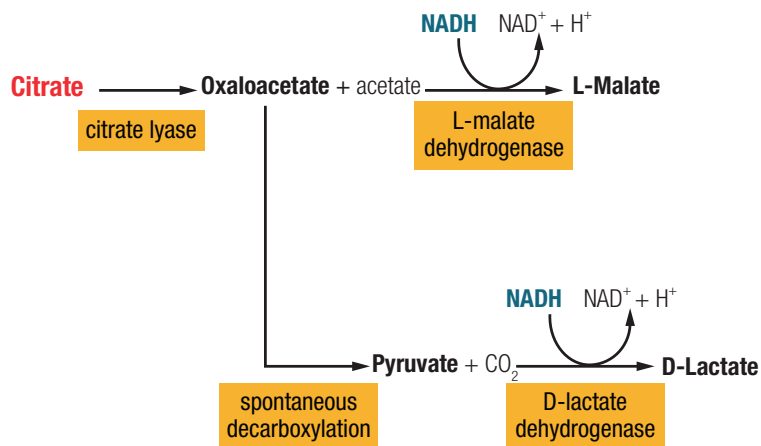


Table 1. Reagents

Reagent Part Number	Maximum Number of Tests	Measuring Range (mg/L)
984327	250	15 – 3000

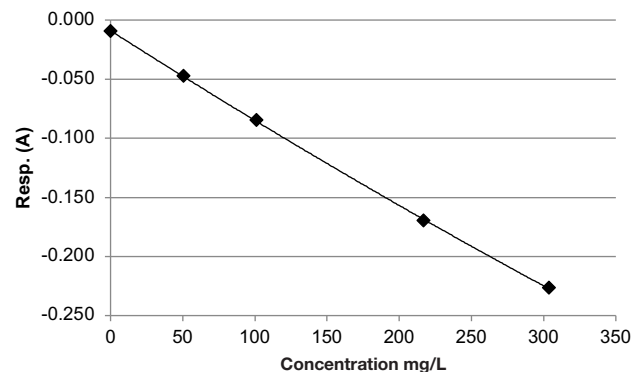


Figure 1. Calibration is 2nd order using dilutions of 1500 mg/L standard.

Table 2. Precision

	Red Wine		Lager Beer		Juice	
N	20		20		20	
Mean (mg/L)	61		126		3779	
	SD	CV%	SD	CV%	SD	CV%
Within Run	1.4	2.2	1.2	1.0	20.8	0.5
Between Run	0.4	0.6	1.0	0.8	60.8	1.6
Total	1.4	2.3	1.6	1.3	64.3	1.7

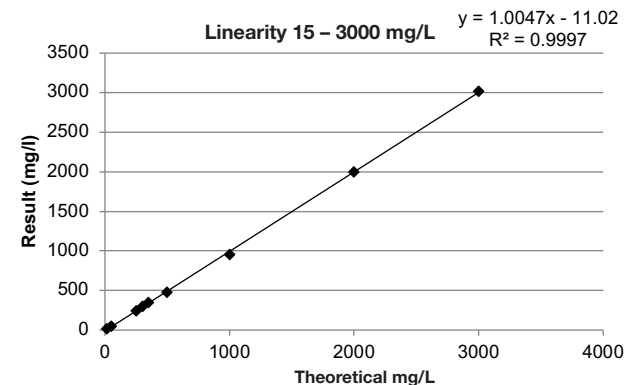


Figure 2. Method Performance Linearity: the test has been developed to determine citric acid concentrations within a measuring range of 15 to 3000 mg/L.





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Reagent Ordering Table

Gluconic acid is a non-volatile organic acid produced in grapes from enzymatic action of mould on grapes results in the generation of gluconic acid due to the oxidation of glucose.

**Method:** In presence of GK, gluconic acid is phosphorylated by ATP. Formed Dgluconate-6-phosphate is oxidatively decarboxylated by NADP in presence of 6PGDH. The amount of NADPH formed is proportional to the concentration of gluconic acid in the sample. The increase in NADPH is measured at 340 nm.

**Chemistry:** 6PGDH (6-P-gluconate-dehydrogenase) and GK (gluconate kinase)

Table 1. Reagents

Reagent Part Number	Maximum Number of Tests	Measuring Range (mg/L)
984322	300	10 – 600

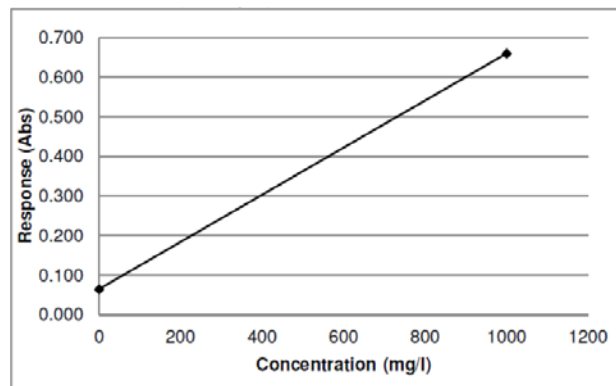


Figure 1. Calibration example.

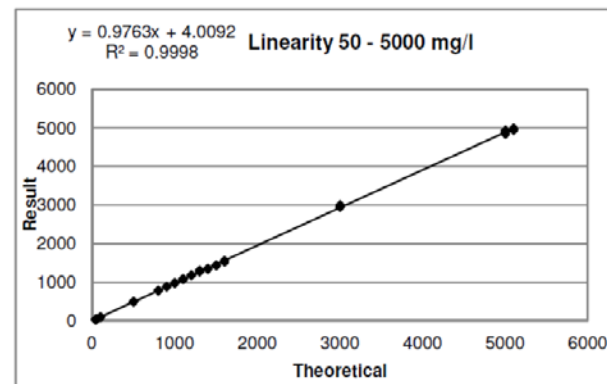


Figure 2. Method performance linearity

Table 2: Precision

	Mean 162 mg/l Sparkling wine		Mean 319 mg/l White wine		Mean 275 mg/l Red wine	
	SD	CV%	SD	CV%	SD	CV%
N	50		50		50	
Mean (mg/L)	162		319		275	
Within run	2.33	1.4	4.23	1.3	5.59	2.2
Between run	3.71	2.3	7.01	2.2	7.89	2.9
Total	4.39	2.7	8.19	2.6	9.88	3.6





# D-Isocitric Acid

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D-Isocitric acid is an organic acid found in most fruit juices. It's an important marker in multicomponent procedures for the evaluation of authenticity and quality of fruit products. High citric/isocitric acid ratios can be used as an indicator of citric acid addition in some juices.

**Method:** An enzymatic test with Isocitrate-Dehydrogenase (ICDH). The bound D-Isocitric acid is determined after alkaline hydrolysis. Method is performed at 37 °C using a 340 nm filter. A 700 nm or 750 nm filter is used as a side wavelength filter.

**Chemistry:** Isocitrate-Dehydrogenase

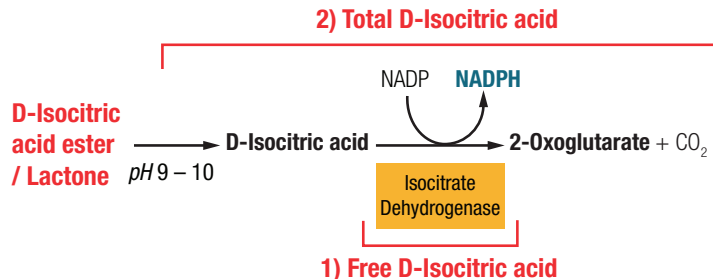


Table 1. Reagents

Reagent Part Number	Maximum Number of Tests	Measuring Range (g/L)
984322	300	10 – 600

Table 1. A precision study was performed using the Gallery analyzer for five days, with the number of measurements being n = 50.

	Sample 1		Sample 2		Sample 3	
N	50		50		50	
Mean (mg/L)	39		168		554	
	SD	CV%	SD	CV%	SD	CV%
Within Run	0.222	0.6	0.816	0.5	4.918	0.9
Between Run	0.712	1.8	0.625	0.4	2.286	0.4
Total	0.745	1.9	1.028	0.6	5.424	1.0

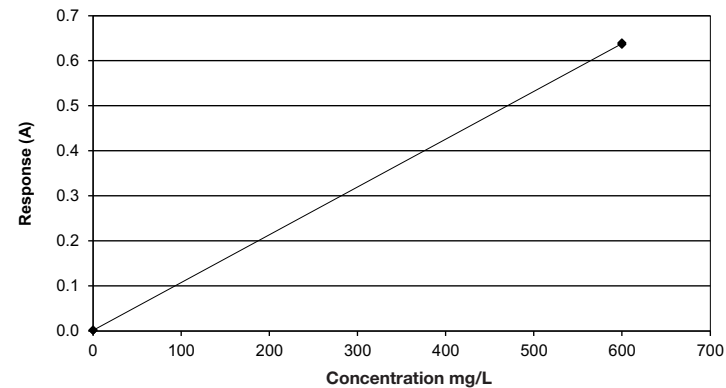


Figure 1. Calibration

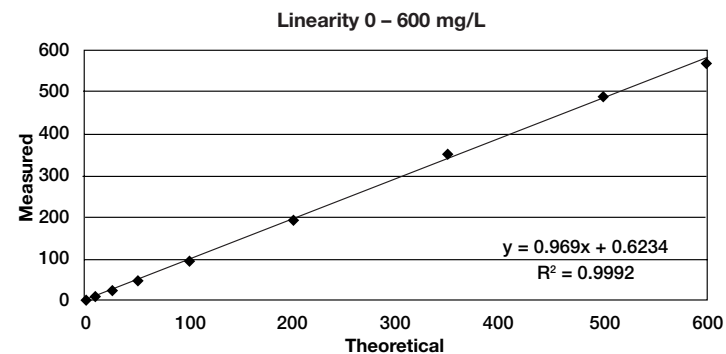


Figure 2. Method Performance Linearity: the test has been developed to determine D-Isocitric acid concentrations within a measuring range of 10 to 600 mg/L.





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Reagent Ordering Table

Two types of lactic acid are produced during the winemaking process: L-lactic acid, produced through malolactic fermentation; and D-Lactic acid, produced through wine spoilage. Both types of lactic acid can be produced by yeast or bacteria. Many winemakers will prevent the creation of lactic acid, for fear that "lactic souring" will occur. This is what happens when the bacteria introduced into the wine converts sugars into lactic acid and acetic acid (instead of converting malic acid into lactic acid). The end wine will have a sour milk smell that is entirely unpleasant. In the wine industry, the production of D-Lactic acid can indicate wine spoilage by lactic acid bacteria (e.g., lactobacillus). Conversely, it can be prevented by treating the wine with sulfur dioxide and/or keeping the wine cool. Some white grape varieties undergo MLF, whereas others may not be improved if they are valued for their crisp acidity.

**Method:** Enzymatic test with D-Lactate-Dehydrogenase (D-LDH). Method is performed at 37 °C, using 340 nm filter.

**Chemistry:** D-Lactate-dehydrogenase

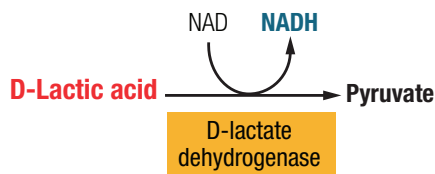


Table 1. Reagents

Reagent Part Number	Maximum Number of Tests	Measuring Range (mg/L)
984306	300	25 – 1600
984382 – Acid Combination Standard		

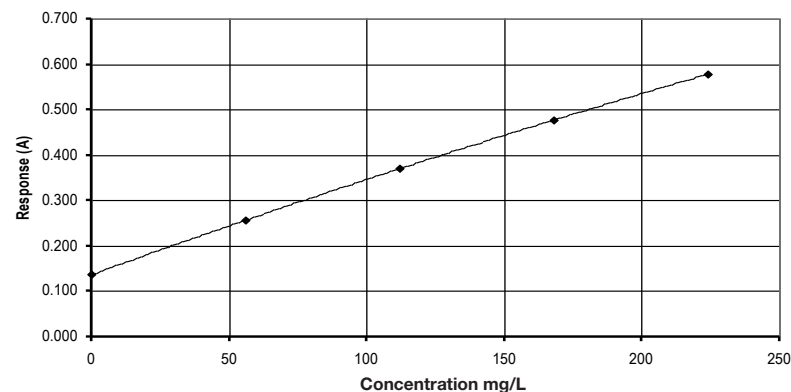


Figure 1. Calibration is second order using dilutions of a 224.0 mg/L standard.

Table 2. Precision

	Sample 1		Sample 2		Sample 3	
N	50		50		50	
Mean (mg/L)	31		77		172	
	SD	CV%	SD	CV%	SD	CV%
Within Run	0.791	2.6	0.884	1.1	5.049	2.9
Between Run	0.872	2.8	1.143	1.5	0.426	0.2
Total	1.177	3.8	1.445	1.9	5.067	2.9

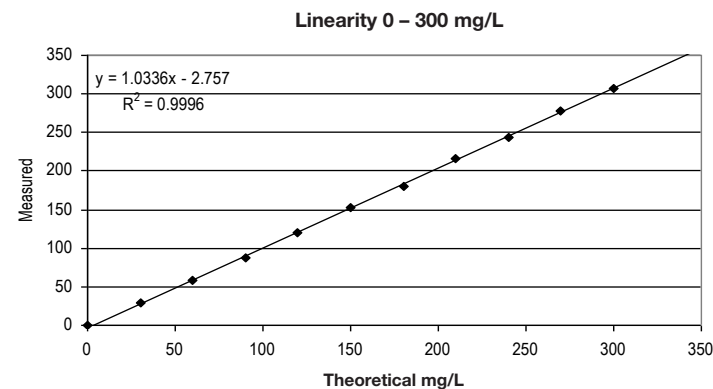


Figure 2. Method Performance Linearity







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L-Lactic acid is produced during the fermentation process by through malolactic fermentation (MLF). In this process the tart-tasting malic acid is converted to softer-tasting L-lactic acid via a decarboxylation reaction. In the wine industry, the course of malolactic fermentation is monitored by following the declining level of L-Malic acid and the increasing level of L-Lactic acid.

**Method:** Enzymatic test with L-Lactate-Dehydrogenase (L-LDH). Method is performed at 37 °C, using 340 nm filter.

**Chemistry:** L-Lactate-dehydrogenase

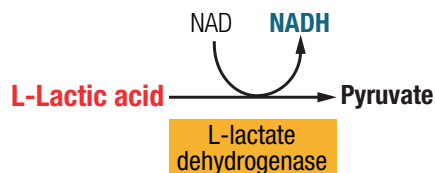


Table 1. Reagents

Reagent Part Number	Maximum Number of Tests	Measuring Range (mg/L)
984308	300	20 – 1600
984382 – Acid Combination Standard		

Table 2. Precision

	Sample 1		Sample 2		Sample 3	
N	50		50		50	
Mean (mg/L)	26		125		182	
	SD	CV%	SD	CV%	SD	CV%
Within Run	0.662	2.0	1.605	1.3	2.681	1.5
Between Run	0.789	3.0	1.570	1.3	1.631	0.9
Total	1.029	4.0	2.246	1.8	3.138	1.7

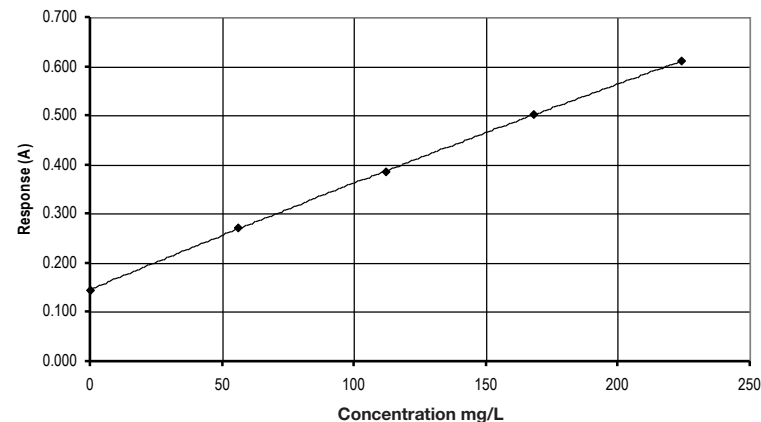


Figure 1. Calibration is second order using dilutions of a 224.0 mg/L standard.

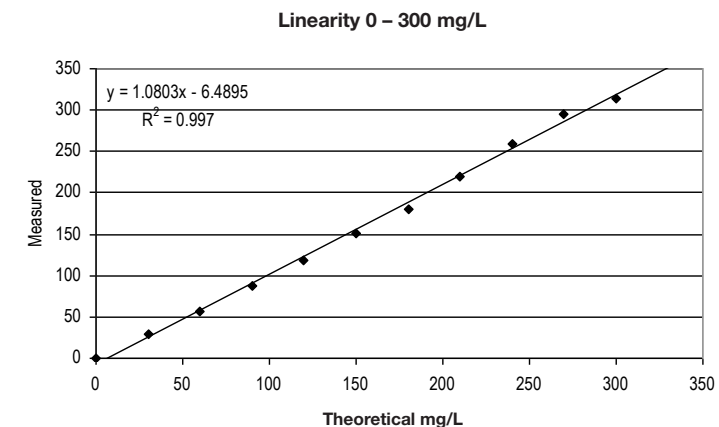


Figure 2. Method Performance Linearity





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In the wine industry, the level of L-Malic acid is monitored, along with L-Lactic acid, during malolactic fermentation. L-Malic acid is found in many applications as a food preservative (E296) and flavor-enhancing compound, such as in the manufacture of low-calorie drinks. Its quantitative determination is especially important in the manufacture of wine, beer, bread, fruit and vegetable products. It is one of the most important fruit acids and has the highest concentration of all acids in wine. L-Malic acid causes a tart flavor in wine and a sour taste in fruit juices.

**Chemistry:** L-Malate-dehydrogenase and glutamate-oxalacetate-transaminase

Enzymatic test with L-Malate-dehydrogenase (L-MDH) and Glutamate-Oxalacetate-Transaminase (GOT). Method is performed at 37 °C, using 340 nm filter.

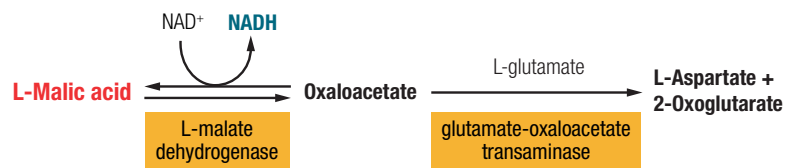


Table 1. Reagents

Reagent Part Number	Maximum Number of Tests	Measuring Range (g/L)
984310	300	0.05 – 20
984382 – Acid Combination Standard		

Table 2. Precision

	Sample 1		Sample 2		Sample 3	
N	50		50		50	
Mean (g/L)	1.2		2.4		3.8	
	SD	CV%	SD	CV%	SD	CV%
Within Run	0.011	0.9	0.023	0.9	0.024	0.6
Between Run	0.017	1.4	0.035	1.4	0.065	1.7
Total	0.020	1.7	0.042	1.7	0.069	1.8

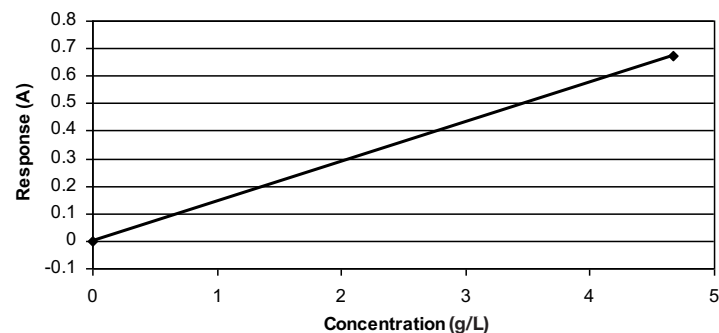


Figure 1. Calibration

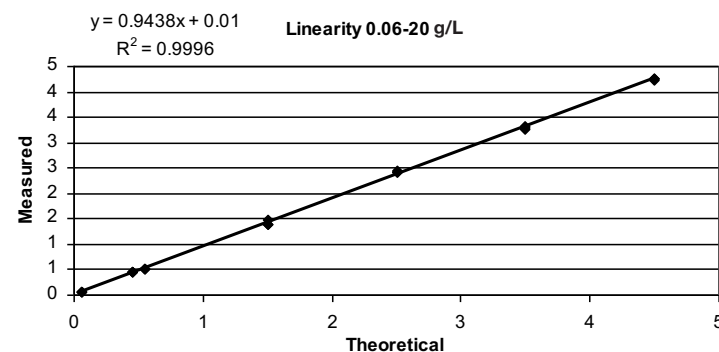


Figure 2. Method Performance Linearity





# Oxalic Acid

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## Oxalic Acid

- L-Ascorbic Acid

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## Reagent Ordering Table

The main acids of citrus fruits are citric and malic acids with trace amounts of tartaric, benzoic, oxalic and succinic acids. In the context of beer, precipitated oxalate leads to particulate and haze formation, gushing, and the white mineral deposit called “beer stone,” the latter being responsible for the blocking of beer piping.

**Chemistry:** Oxalate oxidase

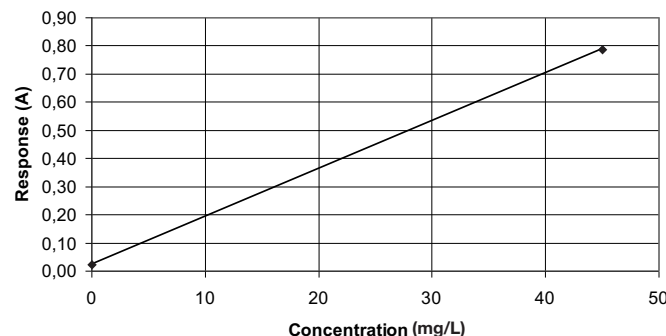
**Method:** Oxalate is oxidized to carbon dioxide and hydrogen peroxide by oxalate oxidase. Hydrogen peroxide reacts in the presence of POD (peroxidase) with MBTH (3-methyl-2-benzothiazolinone hydrazone) and DMAB (3-dimethylamino benzoic acid) forming a blue quinone compound. The intensity of color is proportional to the concentration of oxalic acid in the samples and it is read at 600 nm.

**Table 1. Reagents**

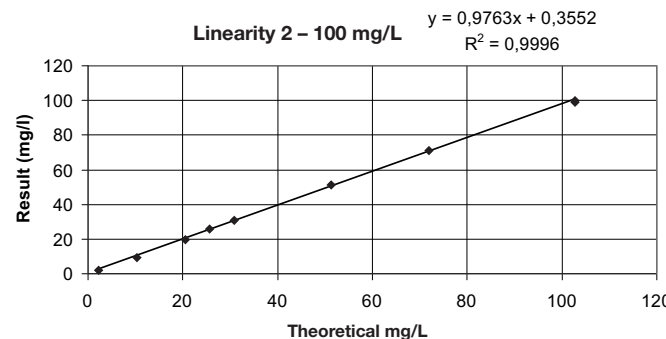
Reagent Part Number	Maximum Number of Tests	Measuring Range (mg/L)
984348	250	2 – 100
984393 – Oxalic Acid Standard		

**Table 2. Precision**

	Home Brew Beer		Lager Beer		Dark Beer	
	SD	CV%	SD	CV%	SD	CV%
N	40		40		40	
Mean (g/L)	2		3		13	
Within Run	0.056	3.4	0.023	0.7	0.159	1.3
Between Run	0.035	2.1	0.075	2.5	0.163	1.3
Total	0.066	4.0	0.078	2.6	0.228	1.8



**Figure 1. Calibration**



**Figure 2. Method Performance Linearity**







L-Ascorbic Acid

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Ascorbic acid acts as a preservative and is used to reduce the risk of oxidation in your bottled beer or wine. Oxidation can leave off aromas and off taste in beer and wine. Ascorbic Acid is an organic acid with antioxidant properties. It is also known as vitamin C. Ascorbic acid is an excellent ingredient for vitamin supplementation. Ascorbic acid is often added to fruit juices, dried fruit, cereal, milk powder, and other snack foods for this purpose.

**Chemistry:** Colorimetric test with the tetrazolium salt MTT

**Method:** Reaction principle is colorimetric. Method is performed at 37 °C, using 575 nm filter.

L-Ascorbic acid (L-Ascorbate) and other reducing substances in the sample are able to reduce the tetrazolium salt MTT [3-(4,5 dimethylthiazolyl-2)-2,5 diphenyltetrazolium bromide] by means of the electron carrier PMS (phenazinium methosulfate) to form a coloured formazan. This reaction determines the sum of L-Ascorbic acid and reducing substances in the sample.



Assay is designed specific for L-Ascorbate determination by using a sample blank, where L-Ascorbate is oxidatively removed by Ascorbate Oxidase (AO). The absorbance difference between the sample and the sample blank is equivalent to the amount of L-Ascorbate in the sample.



In optimized conditions designed in the application, the MTT-formazan gives a chromogenic complex with a maximum absorbance at 578 nm, proportional to the concentration of L-Ascorbic acid (L-Ascorbate) in the sample.

Table 1. Reagents

Reagent Part Number	Maximum Number of Tests	Measuring Range mg/L
984635	250	50 – 2500

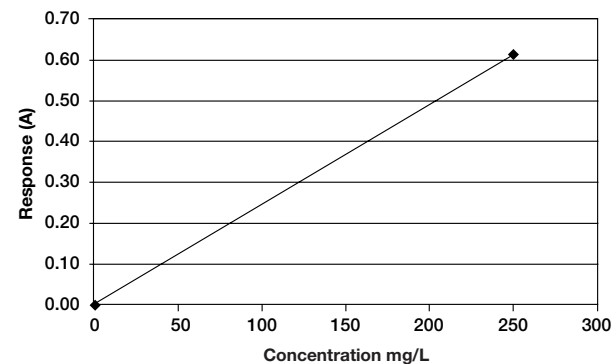


Figure 1. Calibration Curve

Table 2. Precision

	Orange Juice		Raspberry/Blueberry Juice	
N	30		30	
Mean (mg/L)	118		496	
	SD	CV%	SD	CV%
Within Run	1.014	0.9	4.080	0.8
Between Run	0.523	0.4	0.836	0.2
Total	1.141	1.0	4.164	0.8

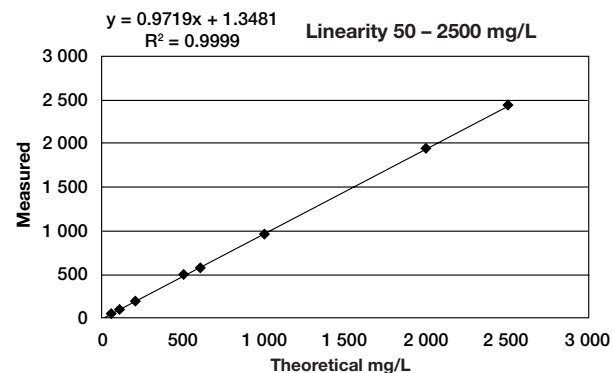


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**Cation testing in beverages**

Cations like calcium and magnesium are present in fruits as a natural source. These cations present in the source water used for wine-, beer- and cider-making affects the alcoholic beverage's color and taste. Potassium is the primary cation present in grape tissue. Potassium is a critical factor in acid salt formation, tartrate precipitation and buffer capacity. Ammonia can affect flavor and microbial stability of finished wine products. Determining cations, such as potassium, magnesium and calcium, in fruit juices is important due to the dietary significance of such cations.

**Analytical techniques for cation analysis**

Common cations can be determined by potentiometric titration in higher concentration and/or by Ion Selective Electrode (ISE) in ppm level concentration. [Ion Chromatography \(IC\)](#) is a highly selective method to determine simultaneous cation analysis in fermentation samples, wine, beer, water and other bioproduction processes. IC is an ideal technique for simultaneous cation analysis. The [Inductivity Coupled Plasma \(ICP\)](#) technique is a preferred fast analytical tool for cations (except ammonia), together with heavy metals. Common cations in beverages can be measured rapidly and accurately by colorimetric or enzymatic methods. The Gallery discrete analyzer can automate photometric measurements to improve productivity in wine, beer and cider testing labs.





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Ammonia is the most important inorganic source of yeast available nitrogen (YAN). During the fermentation process, testing for ammonia is an important way to monitor nitrogen levels. Ammonia's role is important in the fermentation process as it serves as nutrients for growth and metabolic activity of yeast during fermentation. Ammonia is one of the essential nitrogenous compounds. Nitrogenous compounds are a vital part of all living organisms and plays a vital role in the wine-, beer- and cider-making process.

Ammonia can affect flavor and microbial stability of finished wine products. Ammonia is mainly produced by microbial protein catabolism of organic material. Therefore, high concentrations of ammonia can indicate the decomposition of substances like milk, meat and seafood, ammonia being a major component of the off-flavor or odor.

**Method:** Enzymatic test with glutamate dehydrogenase (GLDH). Method is performed at 37 °C, using 340 nm filter.

**Chemistry:** Glutamate dehydrogenase

The amount of NADH consumed in the reaction by glutamate dehydrogenase (GLDH) is stoichiometric with the amount of ammonia in the sample. Method is performed at 37 °C using a 340 nm filter.



Table 1. Reagents

Reagent	Part Number	Maximum Number of Tests	Measuring Range (mg/L)
	984320	300	10 – 420
	984766*	1000	10 – 420

\* Arena discrete analyzer only.

**Samples:** The concentration of ammonia in white and red grape juice and wine can usually be determined without any sample treatment, except for filtration and dilution, if necessary. For red wine, it may be necessary to remove some of the color by the addition of 0.2 g of PVPP per 10 mL of sample.

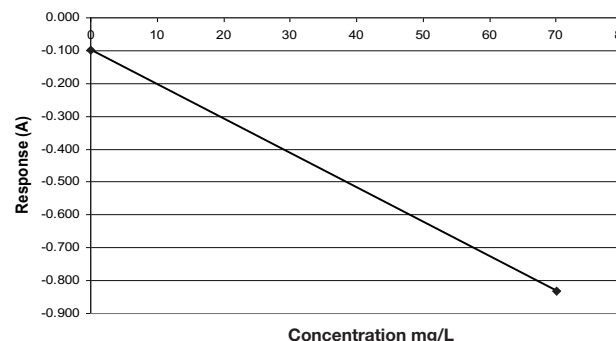


Figure 1. Calibration

$$y = 1.0281x - 3.1235$$

$$R^2 = 0.9996$$

Linearity 10 – 420 mg/L

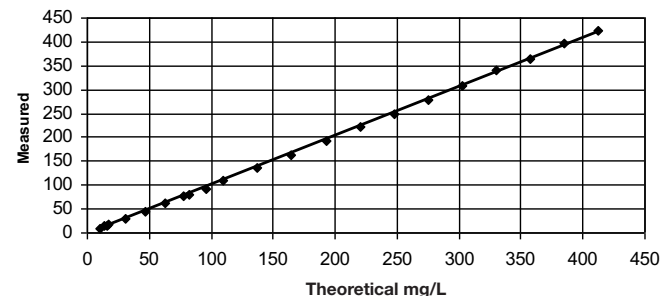


Figure 1. Method Performance Linearity

Table 2. Precision

	Sample 1 (White Wine)		Sample 2 (Red Wine)		Sample 3 (White Wine)	
	SD	CV%	SD	CV%	SD	CV%
N	50		50		50	
Mean (mg/L)	11		29		63	
Within Run	0.0197	0.8	0.211	0.7	0.217	0.3
Between Run	0.155	1.3	0.279	1.0	0.635	1.0
Total	0.183	1.6	0.350	1.2	0.671	1.1





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Calcium is a primary contributor to water hardness, along with magnesium, and is the most influential ion in the brewing process. Calcium is instrumental to many yeast, enzyme and protein reactions, both in the mash and in the boil. It promotes clarity, flavor and stability in the finished beer. In industrial water, scaling is mainly due to the presence of calcium and magnesium salts, which are less soluble in hot condition. Boiler scaling causes loss of boiler efficiency, plus heat transfer, and eventually leads to boiler tube rupture, resulting in expensive component replacements.

Calcium determination is critical to maintaining taste in the brewing industry, as well as, extending the life of expensive utility equipment. In the wine industry, to predict the formation of wine stones together with tartaric acid.

Method: Calcium ions form a highly coloured complex with Arsenazo III at neutral pH. The amount of complex is measured at 660 nm.

**Chemistry:** Arsenazo III

Calcium ions form a highly coloured complex with Arsenazo III at neutral pH. The amount of complex is measured at 660 nm.

Table 1. Reagents

Reagent Part Number	Maximum Number of Tests	Measuring Range (mg/L)
984361	350	10 – 200

Table 2. Precision

	Lager Beer		Red Wine		Dark Beer	
N	20		20		20	
Mean (mg/L)	29		65		89	
	SD	CV%	SD	CV%	SD	CV%
Within Run	0.059	0.2	0.238	0.4	0.202	0.2
Between Run	0.017	0.1	0.072	0.1	0.017	0.0
Total	0.062	0.2	0.248	0.4	0.202	0.2

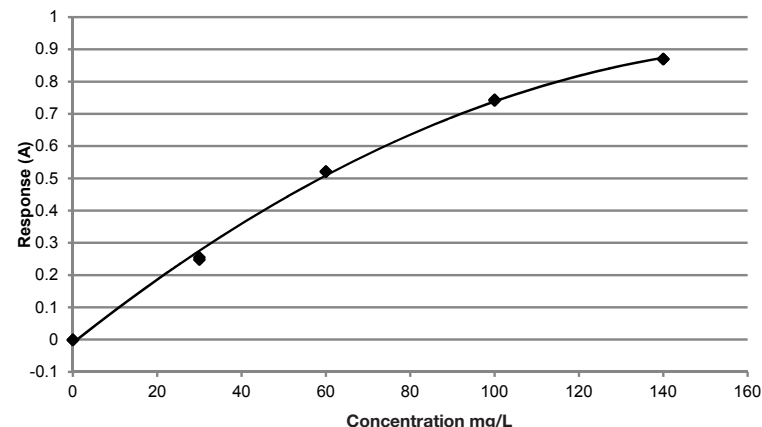


Figure 1. Calibration is second order using standard dilutions. Standards can be diluted automatically by the analyzer.

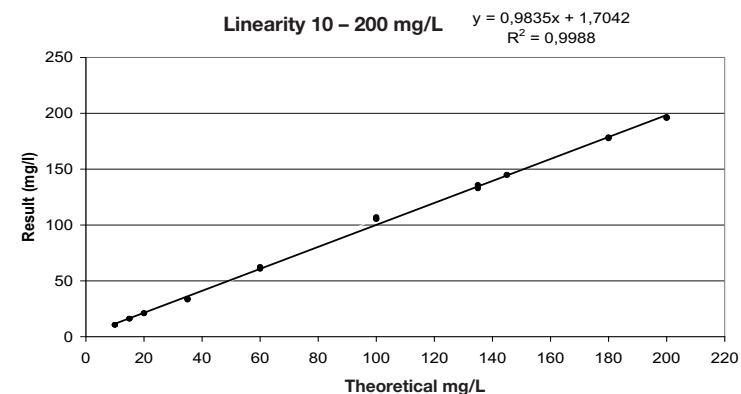


Figure 2. Method Performance Linearity





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Magnesium is used to protect yeasts from ethanol stress in the production of high alcohol-content fruit wines. It is used as cell division, enzyme activator and stress suppressor.

**Method:** Xylidyl blue forms a red coloured complex with magnesium in alkaline conditions. The resulting complex is measured at 510 nm.

**Chemistry:** Xylidyl Blue

Xylidyl blue forms a red-colored complex with magnesium in alkaline conditions. The resulting complex is measured at 510 nm.

**Table 1. Reagents**

Reagent Part Number	Maximum Number of Tests	Measuring Range (mg/L)
984358	350	10 – 400

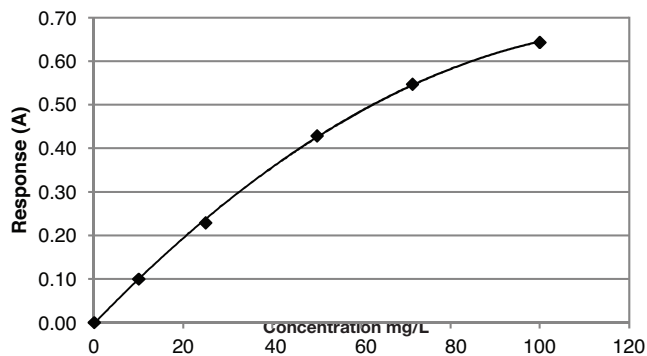


Figure 1. Calibration is second order using 1000 mg/L standard dilutions.

**Table 2. Precision**

	Beer		Red Wine		White Wine	
N	50		50		50	
Mean (mg/L)	76		96		72	
	SD	CV%	SD	CV%	SD	CV%
Within Run	0.699	0.9	0.934	1.0	0.716	1.0
Between Run	1.462	1.9	3.314	3.4	0.791	1.1
Total	1.620	2.1	3.443	3.6	1.067	1.5

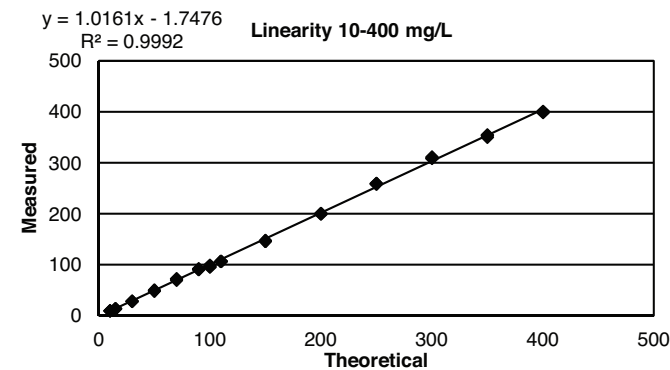


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Potassium is rich in orange juice, tomato juice. Determining of potassium in fruit juices is important due to the dietary significance. Potassium is the important cation in wine production process. High potassium concentrations can cause large increases in pH during primary and malolactic fermentations, which drive the finished wine into high pH range. Potassium is a critical factor in acid salt formation, tartrate precipitation and buffer capacity.

**Method:** Potassium ion reacts with tetraphenylborate (TPB) to give a stable precipitate, which is maintained as homogenous suspension due to pH conditions and the presence of stabilizers. The suspension is measured at 540 nm. The increase of the absorbance is directly proportional to the concentration of potassium in the sample.

**Chemistry:** Tetraphenylborate (TPB)

Table 1. Reagents

Reagent Part Number	Maximum Number of Tests	Measuring Range (mg/L)
984307	300	50 – 2500

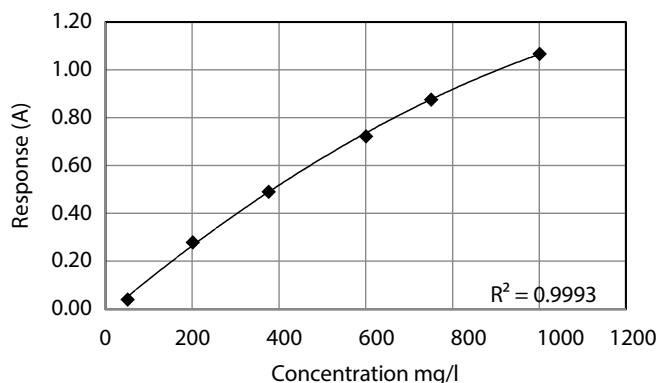


Figure 1. Calibration using 3000 mg/L potassium standard.

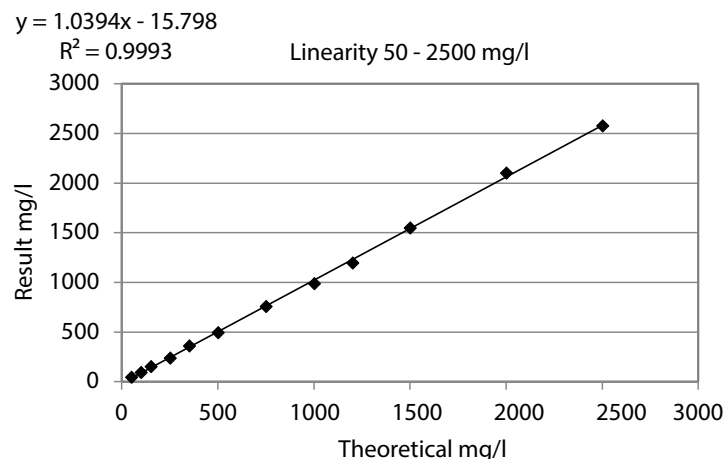


Figure 2. Method Performance Linearity

Table 2. Precision

	White Wine		Red Wine		Spiked Sparkling Wine	
	SD	CV%	SD	CV%	SD	CV%
N	50		50		50	
Mean (mg/L)	795		1473		1984	
Within Run	27	3.3	22	1.5	26	1.3
Between Run	11	1.4	39	2.6	70	3.5
Total	29	3.6	44	3.0	75	3.8







### Sugar analysis

Simple carbohydrates, sometimes called sugars, include a number of mono- and disaccharides. They are rapidly catabolized by the body to be used as energy. Simple carbohydrates are found naturally in foods, such as fruits, milk and milk products. They are also found in processed and refined sugars, such as table sugar, syrups, candy and sodas.

A chemical subgroup of carbohydrates, sugars are sweet-tasting, water-soluble and good sources of energy. Two six-carbon sugars, glucose and fructose monosaccharides are the most important sugars in grape juice. They provide sweetness to the juice and are consumed by yeast during the fermentation process. Sugar content in the juice of ripe grapes varies from 150 to 200 g/L. In unripe grapes, glucose is predominant. In ripe fruit, glucose and fructose are generally present in equal amounts with a slight variation depending upon the particular varietal. In overripe grapes, the level of fructose exceeds the concentration of glucose. During fermentation, yeast converts glucose and fructose into alcohol and carbon dioxide. The amount of alcohol produced is roughly related to the sugar content present in the original juice. Refined sugar, the disaccharide sucrose, is sometimes added during fermentation or secondary fermentation to provide additional sweetness and structure.

The ability to measure and manage levels of fructose and glucose in juice or wine production ensures a good final product, and is an indicator of quality. In wine, the content of D-Glucose and D-Fructose (total sugar) represents the amount of sugar available for fermentation by yeast. With too little glucose, fermentation will not happen properly. In addition, the authenticity of fruit juice is often checked using the ratio of fructose/glucose as specific natural sugar ratios exist for many fruits. Measuring sucrose can also be used to indicate total glucose, because during fermentation, yeast breaks down sucrose into glucose and fructose, then consumes the glucose followed by fructose. The amount of sucrose is calculated by subtracting the amount of free glucose from total glucose.

Grape juice is composed of 70% to 80% water, 20% carbohydrates, and 1% organic acids, phenolics, vitamins, minerals and nitrogenous compounds. Sugars, organic acids and phenolics provide flavor, while the vitamins, minerals, and nitrogenous compounds are essential participants in the successful growth of yeast and the process of fermentation. Finished wine has a similar composition to unfermented juice, but contains much lower levels of sugar, approximately 8% to 14% alcohol, and a wider range of minor components.

### Analytical techniques for sugar analysis

While total reducible sugar concentration can be determined by auto titration, selective and sensitive determination of individual sugar is possible by [HPLC with RI detector](#) or high-performance anion-exchange chromatography with [pulsed amperometric detection \(HPAE-PAD\)](#). Ion chromatography is best suitable for fast, high-resolution separation of mono-, di-, tri-, tetra- and penta-saccharides in food and beverage samples without the need for derivatization. For routine high-throughput sugar analysis, the Gallery discrete analyzer can be used to determine glucose, fructose and sucrose in beverages.

The Gallery discrete analyzer fully automates discrete analysis based on photometric and enzymatic measurements providing fast, reproducible results that allow laboratories to measure multiple analytes simultaneously, while reducing total analysis and operator time. For example, glucose levels in homogenous juice and wine samples can be determined using the automated discrete analyzer, which has the ability to analyze 100 samples for both glucose and fructose in 90 min. with first results completed in less than 15 min.

The analysis methods for use with discrete analyzers are well-known enzymatic and colorimetric tests that have been verified according to international reference methods. In addition, the system's ready-to-use reagents:

- Reduce hands-on time
- Require less reagent usage
- Produce less waste



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D-Glucose and D-Fructose are the predominant reducing sugars in grape and other fruit juices. The ratio of glucose to fructose in mature grapes is 1:1, but ranges from 0.74 – 1.12 according to variety, maturity and fermentation conditions. Fructose is one of the primary fermentable sugars in wine-making, and is used as a process indicator and quality indicator for the final product. In juice, fructose is used as a quality indicator and the fructose/glucose ratio is used for authenticity checks.

**Method:** Enzymatic test with hexokinase (HK), phosphoglucose isomerase (PGI) and glucose-6-phosphate dehydrogenase (G6P-DH). Method is performed at 37 °C, using 340 nm filter and 600 nm as a sidewavelength.

**Chemistry:** Hexokinase, phosphoglucose isomerase and glucose-6-phosphate dehydrogenase



Table 1. Reagents

Reagent Part Number	Maximum Number of Tests	Low Measuring Range (mg/L)	Measuring Range (g/L)
984302	300	5 – 500	0.7 – 200
984380 – Sugar Combination Standard			

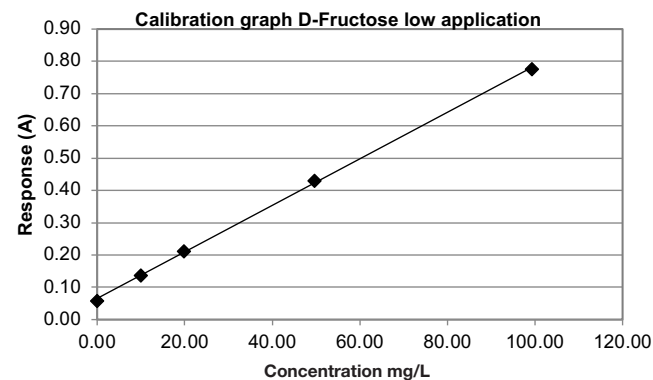


Figure 1. Calibration is linear using 1000 mg/L standard dilutions.

Table 2. Precision: low calibration range.

	Candy 1		Candy 2		Candy 3	
	SD	CV%	SD	CV%	SD	CV%
N	30		30		30	
Mean (mg/L)	16.10		59.02		25.00	
Within Run	0.169	1.0	0.133	0.2	0.097	0.4
Between Run	0.071	0.4	0.103	0.2	0.103	0.4
Total	0.183	1.1	0.168	0.3	0.142	0.6

Table 3. Precision: high calibration range.

	White Wine		Juice 1		Juice 2	
	SD	CV%	SD	CV%	SD	CV%
N	50		50		50	
Mean (g/L)	8.0		37.7		79.0	
Within Run	0.094	1.2	0.367	1.0	0.452	0.6
Between Run	0.055	0.7	0.101	0.3	0.631	0.8
Total	0.109	1.4	0.381	1.0	0.776	1.0



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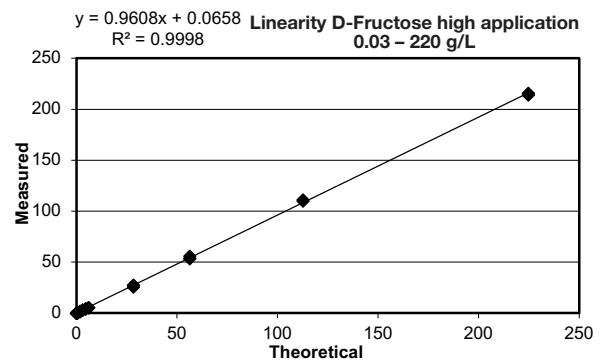
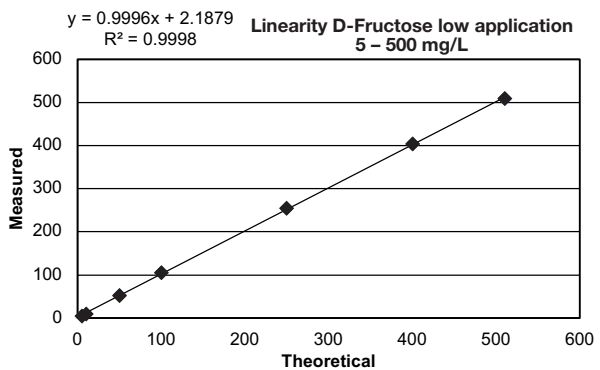


Figure 2. Method Performance Linearity





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Glucose is one of the primary sugars available for yeast fermentation and is naturally occurring in grape juice. Sugar can also be added from other sources such as cane/beet (sucrose) or corn sugar (glucose) to adjust the alcohol level of the finished product. In juice, glucose is used as a quality indicator and the fructose/glucose ratio for an authenticity check.

**Chemistry:** Hexokinase, and glucose-6-phosphate dehydrogenase

The D-Glucose test is an enzymatic test method that uses hexokinase (HK) with ATP (Adenosine 5-triphosphate) and glucose-6-phosphate dehydrogenase (G6P-DH) with NAD+ (Nicotinamide adenosine dinucleotide) and follows this reaction:



Table 1. Reagents

Reagent Part Number	Maximum Number of Tests	Low Measuring Range (mg/L)	Measuring Range (g/L)
984304	300	5 – 500	0.1 – 160
984380 – Sugar Combination Standard			

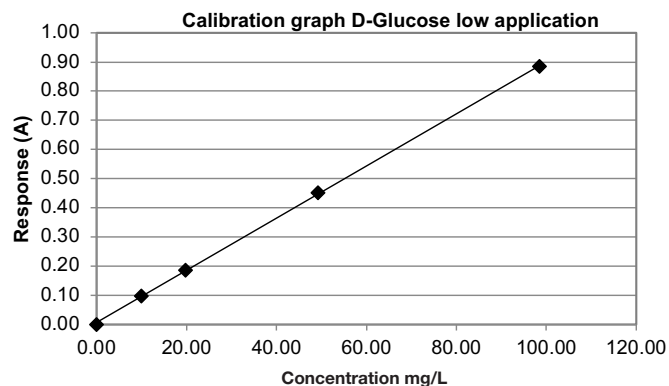
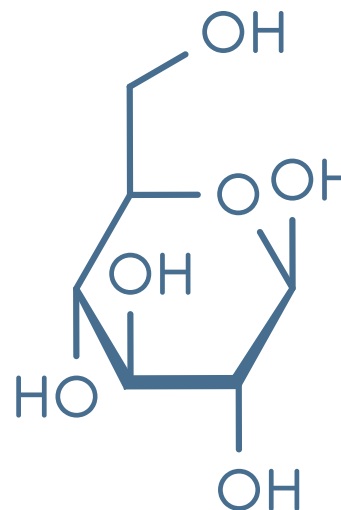


Figure 1. Calibration is linear using 1000 mg/L standard dilutions.



D-Glucose

Table 2. Precision: low calibration range.

	Candy 1		Candy 2		Candy 3	
N	30		30		30	
Mean (mg/L)	62.34		97.84		52.13	
	SD	CV%	SD	CV%	SD	CV%
Within Run	0.150	0.2	0.610	0.6	0.094	0.2
Between Run	0.223	0.4	0.346	0.4	0.116	0.2
Total	0.269	0.4	0.701	0.7	0.149	0.3

Table 3. Precision: high calibration range.

	White Wine		Juice 1		Juice 2	
N	50		50		50	
Mean (g/L)	12.5		34.6		69.4	
	SD	CV%	SD	CV%	SD	CV%
Within Run	0.101	0.8	0.253	0.7	0.371	0.5
Between Run	0.166	1.3	0.508	1.5	1.104	1.6
Total	0.194	1.6	0.567	1.6	1.165	1.7

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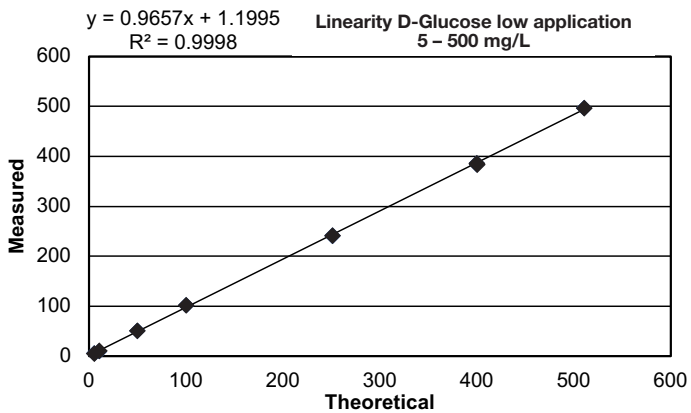
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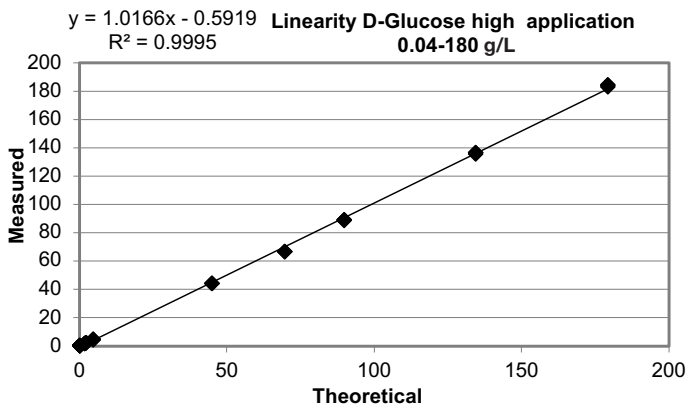


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Wine manufacturers are interested in the total concentration of glucose and fructose with test results reported as the sum of these two parameters.

**Method:** Enzymatic method for the determination of D-Fructose plus D-Glucose (total sugars). Based on the spectrophotometric measurement of INT-formazan formed through the combined action of hexokinase (HK), phosphoglucose isomerase (PGI), Glucose-6-phosphate dehydrogenase (G6PDH) and diaphorase.

**Chemistry:** Hexokinase, phosphoglucose isomerase and glucose-6-phosphate dehydrogenase

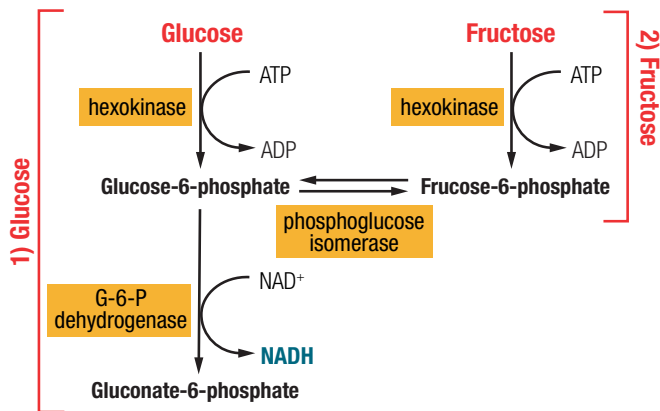


Table 1. Reagents

Reagent Part Number	Maximum Number of Tests	Measuring Range (g/L)
984314	300	0.04 – 200
984380 – Sugar combination standard		

Table 2. Precision

	Sample 1		Sample 2		Sample 3	
N	50		50		50	
Mean (g/L)	5.46		44.17		72.57	
	SD	CV%	SD	CV%	SD	CV%
Within Run	0.042	0.8	0.417	0.9	0.419	0.6
Between Run	0.038	0.7	0.396	0.9	0.924	1.3
Total	0.057	1.0	0.575	1.3	1.015	1.4

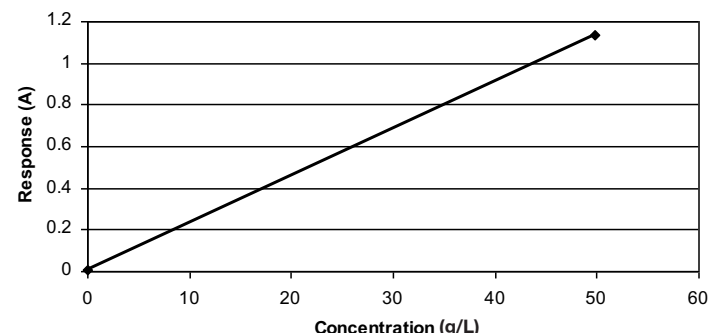


Figure 1. Calibration

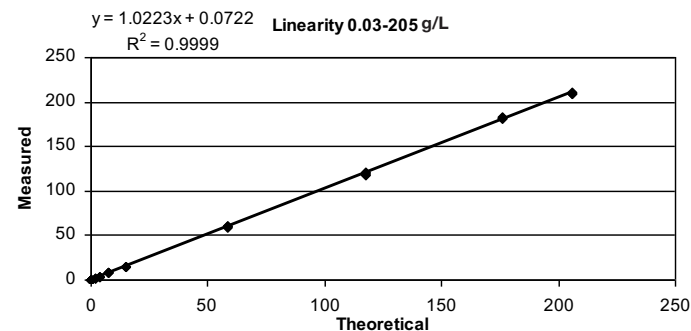


Figure 2. Method Performance Linearity







# D-Glucose & D-Fructose & Sucrose

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Sucrose

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Glucose and fructose are required in the fermentation process. The yeast enzyme (invertase) breaks down sucrose into glucose and fructose, consuming the glucose followed by fructose.

**Chemistry:** Enzymatic test with Beta-Fructosidase (Invertase), Hexokinase (HK), Phosphoglucose Isomerase (PGI) and Glucose-6-Phosphate Dehydrogenase (G6P-DH). Method is performed at 37 °C, using 340 nm filter.

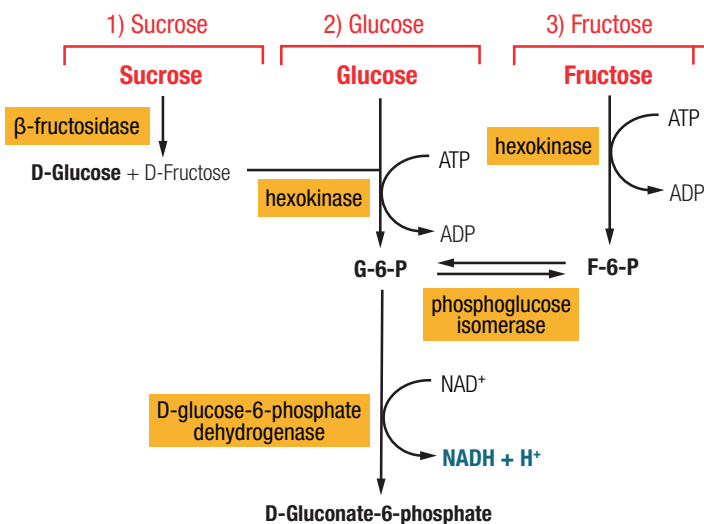


Table 1. Reagents

Reagent Part Number	Maximum Number of Tests	Measuring Range (g/L)
984317	300	0.24 – 200
984380 – Sugar Combination Standard		

Table 2. Precision

	Sample 1		Sample 2		Sample 3	
N	50		50		50	
Mean (g/L)	5.61		44.56		88.12	
	SD	CV%	SD	CV%	SD	CV%
Within Run	0.062	1.1	0.552	1.2	0.715	0.8
Between Run	0.067	1.2	0.473	1.1	2.287	2.6
Total	0.091	1.6	0.727	1.6	2.396	2.7

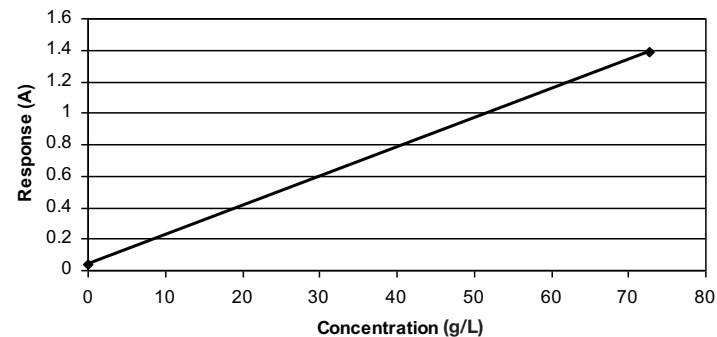


Figure 1. Calibration

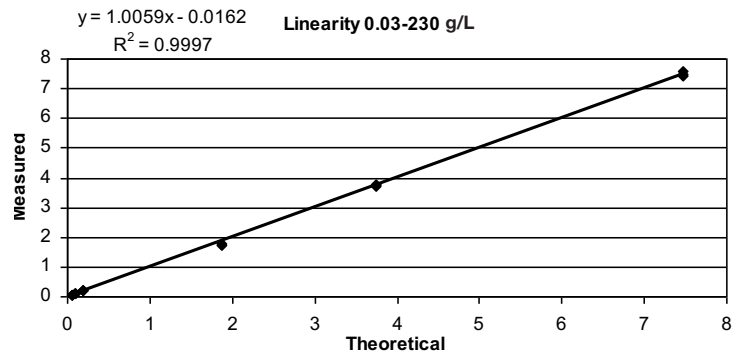


Figure 2. Method Performance Linearity



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- D-Glucose & D-Fructose & Sucrose

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The yeast enzyme (invertase) breaks down sucrose into glucose and fructose. Then it consumes glucose followed by fructose. Sucrose is calculated by subtraction of free glucose content from total glucose content.

**Chemistry:** Enzymatic test with Beta-Fructosidase (Invertase), Hexokinase (HK) and Glucose-6-Phosphate Dehydrogenase (G6P-DH). Method is performed at 37 °C, using 340 nm filter and 600 nm as a side wavelength.



Table 1. Reagents

Reagent Part Number	Number of Tests	Low Measuring Range (mg/L)	Measuring Range (g/L)
984312	300	15 – 500	0.1 – 100
984380 – Sugar Combination Standard			

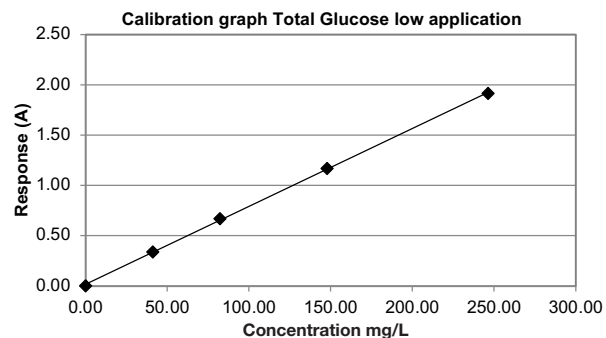


Figure 1. Calibration is linear and uses serial dilution from 760 mg/L standard.

Table 2. Precision: low calibration range

	Candy 1		Candy 2		Candy 3	
N	30		30		30	
Mean (mg/L)	62.34		97.84		52.13	
	SD	CV%	SD	CV%	SD	CV%
Within Run	0.150	0.2	0.610	0.6	0.094	0.2
Between Run	0.223	0.4	0.346	0.4	0.116	0.2
Total	0.269	0.4	0.701	0.7	0.149	0.3

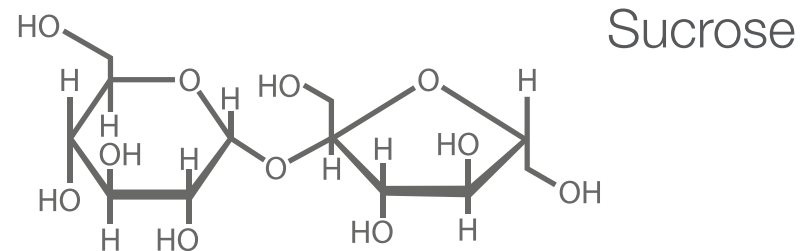


Table 3. Precision: high calibration range

	White Wine		Juice 1		Juice 2	
N	50		50		50	
Mean (g/L)	25.1		1.9		45.1	
	SD	CV%	SD	CV%	SD	CV%
Within Run	0.345	1.4	0.031	1.6	0.301	0.7
Between Run	0.216	0.9	0.007	0.3	0.646	1.4
Total	0.406	1.6	0.031	1.6	0.712	1.6

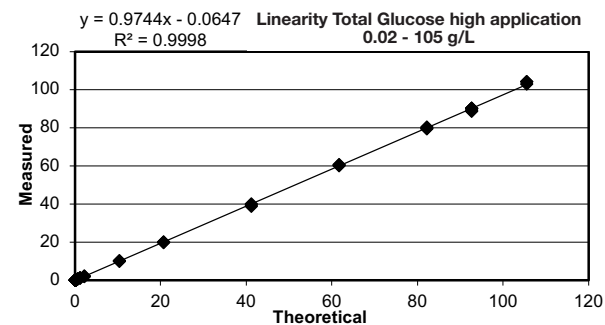
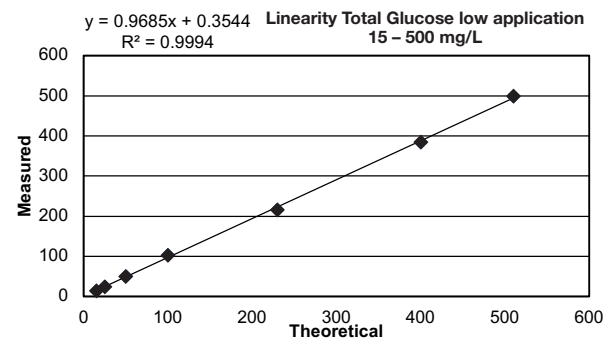


Figure 2. Method Performance Linearity

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## Metals

**Trace metal analysis**

Trace elemental screening and speciation analysis of food is receiving global attention. Some elements are an essential part of a healthy diet. Heavy metals occur naturally in the ecosystem through anthropogenic sources such as pollution. Living organisms require varying amounts of “heavy metals” such as iron, cobalt, copper, manganese, molybdenum and zinc. However, exposure to excessive levels can be damaging to organisms. This exposure can easily occur through dietary intake, including through the consumption of wine. There are many contributing factors that determine the metal content in wine, including soil, type of vineyard, various steps of the wine production cycle (from grape to the finished wine) and from wine processing equipment, conservation and bottling.

It is well known that the presence of these elements in water and fruit wine can influence the winemaking process or can change the taste and quality of the final product. Trace metals commonly present in these alcoholic drinks usually come from two main sources: environmental (e.g., soil) and contamination originating from cars, factories, fertilizers, pesticides and oenological practices (e.g., machinery, piping, use of fining agents, additives).

**Analytical technique for metal analysis**

[Atomic absorption spectrometry \(AAS\)](#) is still a popular choice for uncomplicated trace element analysis in food and beverages. [Inductively coupled plasma atomic emission spectroscopy \(ICP-OES\)](#) is a fast, multi-element technique with the capability to analyze 70+ elements in one analytical run and provides better sensitivity and a higher linear dynamic range than AAS. Due to its robust design, it can handle complex food sample matrices without degrading detection limits for trace contaminants. The speciation analyzer includes a metal-free [IC system](#) with high resolution ion exchange columns and simple online connectivity, together with high sensitivity ICP or [inductively coupled plasma mass spectrometry \(ICP-MS\)](#).

An integrated wet chemistry analyzer, based on discrete analysis, is an alternative routine analytical tool for selective metal analysis in beverage matrices. One major advantage is the analysis speed. Another added benefit is that the discrete analyzer requires no sophisticated skillset or additional hands-on analysis time.





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Some metals can affect the quality of the wine, especially iron, which can lead to haze formation in bottled wine. Therefore, it is recommended that winemakers screen for these metals prior to bottling. Iron is typically low in grape juice, but contamination during harvesting and processing can occur to boost its levels. Excess iron primarily impacts white wine (reducing clarity), but there is also the potential to cause a blue black deposit in red wine.

**Method:** Colorimetric test with Ferene S.

Method is performed at 37 °C, using 600 nm filter. Bound iron is liberated from proteins by guanidine buffer. Ascorbic acid is used to reduce the ferric iron to its ferrous state, which forms a coloured product with Ferene S. The intensity of the colour is measured at 600 nm.

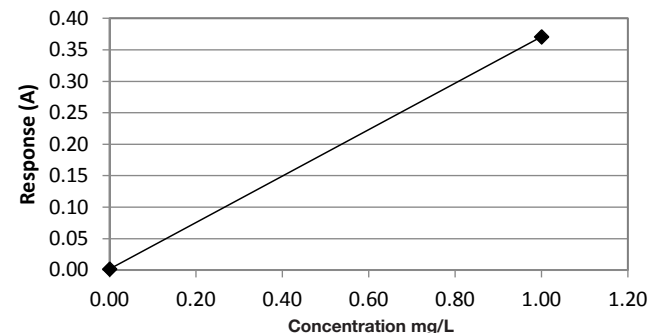
**Chemistry:** Ferene S

**Table 1. Reagents**

Reagent Part Number	Maximum Number of Tests	Measuring Range (mg/L)
984326	850	0.03 – 5.5

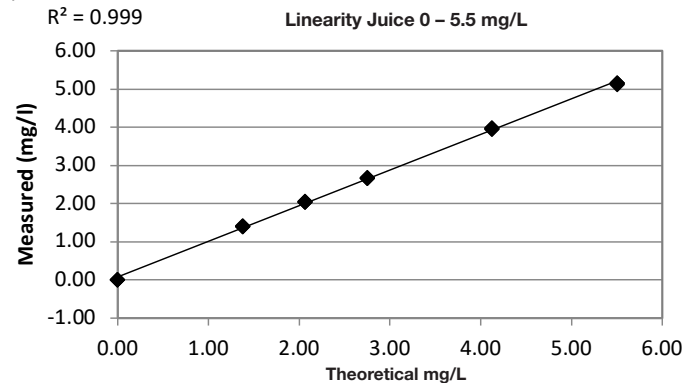
**Table 2. Precision**

	Dark Beer		Juice		Red Wine	
N	20		20		20	
Mean (mg/L)	0.05		1.70		2.60	
	SD	CV%	SD	CV%	SD	CV%
Within Run	0.001	1.8	0.022	1.3	0.011	0.4
Between Run	0.001	1.9	0.025	1.5	0.017	0.6
Total	0.001	2.6	0.033	2.0	0.020	0.8



**Figure 1. Calibration**

$y = 0.933x + 0.080$   
 $R^2 = 0.999$



**Figure 2. Method Performance Linearity**



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Total Acids (TA)

## Reagent Ordering Table

Titration is a common wet chemistry method of quantitative analysis used in beverage analysis to determine the unknown concentration of a known reactant, such as titratable acidity. It is commonly used in the wine industry for several process-critical parameters, such as free and total sulfur dioxide and citric acid analysis. Titrations are used to determine the total amount of hardness, calcium and alkalinity in brewing water, and total acidity in beer. Quality control analysis in the beverage industry is often a demanding process requiring high throughput, flexibility and accurate instrumentation. Laboratories choose automated methods for ease-of-use and optimized application, resulting in smaller volumes of reagents required and lower costs per test. An integrated wet chemistry analyzer, based on discrete analysis, is able to perform multiple facets of beverage analysis.





pH (colorimetric)

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- Fress SO<sub>2</sub>
- Total SO<sub>2</sub>
- Total Acids (TA)

Reagent Ordering Table

pH is a measure of free hydrogen ions in a solution (which corresponds to the chemical definition of acidity) and is used to gauge acidity. pH is critical in relationship to microbial stability, interactions of phenolic compounds, and color expression. Wine color stability, potassium bitartrate stability (cold stability), calcium stability and molecular SO<sub>2</sub> levels are directly related to wine pH.

Colorimetric pH measurement is recommended only where screening type of results is sufficient, like beer/wort samples. For accurate pH measurement in point of entry water, wine and fruit juices, electrochemistry method using pH electrode is recommended. pH by colorimetric is especially useful with Gallery plus Beermaster when, electrochemical pH measurement is not possible.

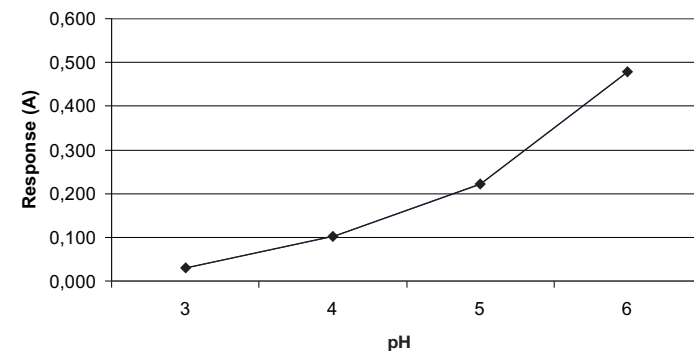
**Method:** Method is performed at 37 °C, using 575 nm filter and 700 nm as side wavelength.

Alternatively, pH can be measured electrochemically using a pH sensor. The Gallery discrete analyzer with an Electro Chemical Measurement (ECM) unit is capable of simultaneous pH, conductivity and photometric measurements.

**Chemistry:** Colorimetric test with pH indicator dyes in an aqueous solution.

**Table 1. Reagents**

Reagent Part Number	Maximum Number of Tests	Measuring Range (pH)
984349	330	3 – 7.2



**Figure 1. Point to point calibration**

**Table 2. Precision**

	Lager Beer		Home Brew Beer		Dark Beer	
	SD	CV%	SD	CV%	SD	CV%
N	20		20		20	
Mean (pH)	4.12		4.22		4.59	
Within Run	0.002	0.05	0.002	0.05	0.007	0.15
Between Run	0.006	0.14	0.017	0.41	0.005	0.11
Total	0.006	0.15	0.017	0.41	0.009	0.19







# pH and Conductivity by Electro chemistry

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Total Acids (TA)

## Reagent Ordering Table

### Automated pH and conductivity measurements – electrochemical method

pH and conductivity measurements are often a critical early step in water testing workflow. pH & Conductivity are the most common parameters tested for all the water samples, from point of entry water to the wastewater, to indicate the overall utility performance. pH measurement of beverages are important for many reasons including flavor and taste.

**Method:** Electrochemical measurement of pH and electrical conductivity from beverage and water samples is accurate using the automated Gallery and Gallery Plus discrete analyzers. Electro Chemical Measurement (ECM) is an integrated unit into the benchtop discrete photometric analyzer. It is capable to simultaneously measure both pH and conductivity alongside the photometric testing. Sample types can vary from water samples, like natural water, waste water and drinking water to beverage samples, like wine, juice, sparkling and still water.

Both conductivity and pH are measured at 37 °C in the Gallery discrete analyzer. However, results can be reported in different temperatures, e.g., at 25 °C, because the discrete analyzer software has a robust system with which the sample result may be correlated to a reference analyzer result automatically.

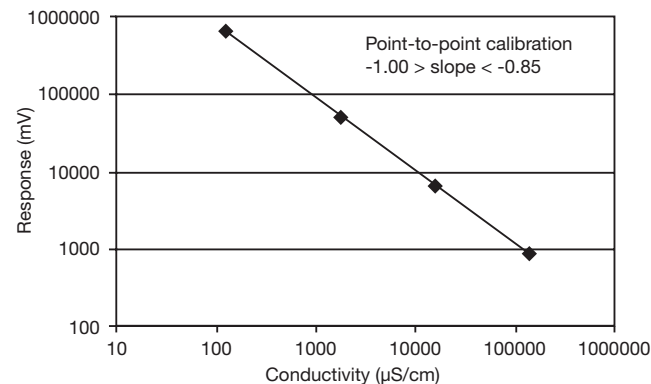
**Table 1. Reagents**

	Reagent Part Number	Description
	984340	ECM Prime
	984998	ECM Rinse Liquid
	984030	Washing solution 4.5 %
	980659	Cleansing solution
pH	984330	ECM pH 2 Standard
	984331	ECM pH 4 Standard
	984332	ECM pH 7 Standard
	984333	ECM pH 10 Standard
	984334	ECM pH 12 Standard
Conductivity	984339	ECM Conductivity 0.08 Standard
	984336	ECM Conductivity 1.4 Standard
	984337	ECM Conductivity 13 Standard
	984338	ECM Conductivity 112 Standard

### Measuring range

**pH:** The test has been developed to measure pH within a measuring range from pH 2-12.

**Conductivity:** The test has been developed to measure conductivity within a measuring range from 20 µS/cm to 112 mS/cm.



**Figure 1. Conductivity calibration.**

**Table 2. Conductivity Precision – beverages.**

	Wine		Juice		Sparkling Water	
N	30		30		20	
Mean	2.53 mS/cm		3.06 mS/cm		1357 µS/cm	
	SD	CV%	SD	CV%	SD	CV%
Within Run	0.005	0.2	0.002	0.1	6.772	0.5
Between Run	0.045	1.8	0.037	1.2	21.346	1.6
Total	0.045	1.8	0.037	1.2	22.395	1.7

**Table 3. Conductivity precision – water samples.**

	Drinking Water		Natural Water		Waste Water	
N	20		20		20	
Mean	59 µS/cm		194 µS/cm		8.19 mS/cm	
	SD	CV%	SD	CV%	SD	CV%
Within Run	0.294	0.5	0.709	0.4	0.035	0.4
Between Run	0.112	0.2	1.410	0.7	0.027	0.3
Total	0.315	0.5	1.578	0.8	0.045	0.5





# pH and Conductivity by Electro chemistry

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Fress SO<sub>2</sub>

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Reagent Ordering Table

**Accuracy / Method comparison.** The method comparison was done by comparing results from a Gallery discrete analyzer ECM and a benchtop conductivity meter. Slope and coefficient of determination (R<sup>2</sup>) are obtained from the linear relationship of the two methods.

Table 4.

Sample Matrix	N	Conductivity Range (µS/cm)	Slope	R <sup>2</sup>
Drinking Water	23	45 – 2572	1.06	1.000
Natural Water	20	26 – 8343	1.05	1.000
Waste Water	3	551 – 6858	1.15	1.000

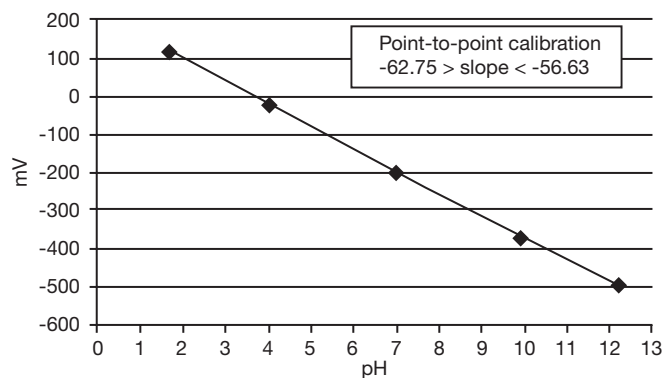


Figure 2. pH Calibration

Table 5. Precision – Beverage pH.

	Wine pH 3.3		Juice pH 3.4		Sparkling Water pH 5.3	
	SD	CV%	SD	CV%	SD	CV%
Within Run	0.001	0.02	0.001	0.03	0.021	0.39
Between Run	0.005	0.15	0.007	0.20	0.015	0.27
Total	0.005	0.16	0.007	0.20	0.025	0.48

Table 6. Precision – Water pH.

	Drinking Water pH 6.8		Natural Water pH 7.8		Waste Water pH 6.6	
	SD	CV%	SD	CV%	SD	CV%
Within Run	0.020	0.29	0.061	0.78	0.002	0.03
Between Run	0.018	0.27	0.007	0.09	0.005	0.08
Total	0.027	0.39	0.062	0.79	0.005	0.08

**Accuracy / Method comparison.** The method comparison was done by comparing results from a Gallery discrete analyzer ECM and a benchtop pH meter. Slope and coefficient of determination (R<sup>2</sup>) are obtained from the linear relationship of the two methods.

Table 7.

Sample Matrix	N	pH Range	Slope	R <sup>2</sup>
Wine	15	2.7 – 4.2	0.995	0.998
Juice	12	3.0 – 3.7	1.018	0.999
Drinking Water	23	4.6 – 7.9	1.011	0.910
Natural Water	20	3.7 – 8.9	0.873	0.981
Waste Water	3	6.7 – 7.5	0.919	0.999



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## Reagent Ordering Table

Sulphur dioxide (SO<sub>2</sub>) is used widely as an additive in various forms, most commonly as sulphites (or sulfites), in the wine, beverage and food industries where it acts primarily as an antimicrobial and antioxidant preservative.

Sulfur dioxide (SO<sub>2</sub>) is added to control the process of winemaking and serves many useful functions. For example, it acts as an enzyme inhibitor in musts to prevent juice from browning. As a microbiological control agent, SO<sub>2</sub> is added to the winemaking process to prevent oxidation in the finished product. In beer, it contributes to flavor stability and improved shelf life. In addition, due to the increased awareness of the adverse effects of sulphites and the prevalence of sulphite intolerance in some individuals, sulphite levels in foods and drinks are strictly regulated by various governing bodies; and therefore, there is a requirement for accurate determination of sulphite levels in foods, beverages and wines. The presence of total SO<sub>2</sub> (both free and bound) is regulated and, as a result, a warning statement is required on wine labels because sulfite is considered an allergen. The European Union established a maximum permitted level of total SO<sub>2</sub> in wine varying from 150 to 500 mg/L, which is dependent upon the sugar level of the product. In the U.S., the maximum level of total SO<sub>2</sub> permitted is 350 mg/L. In EU levels above 10 ppm (total) must be labeled. That is why it is important to measure also the low level in e.g. beers.

Free forms, SO<sub>2</sub> (gas) and bisulfate ion (HSO<sub>3</sub><sup>-</sup>), are bound to compounds that incorporate a carbonyl group, such as acetaldehyde. Free forms of SO<sub>2</sub> are pH and temperature dependent; and because of the acidic nature of wines, SO<sub>2</sub> is usually present and measured as bisulfate ion (HSO<sub>3</sub><sup>-</sup>). Results are reported as SO<sub>2</sub>.

Total SO <sub>2</sub>	Free SO <sub>2</sub>	Sulfur dioxide (SO <sub>2</sub> )
		Bisulfite (HSO <sub>3</sub> <sup>-</sup> )
		Sulfite (SO <sub>3</sub> <sup>2-</sup> )
	Combined SO <sub>2</sub>	Unstable bonds: Sugars, Polyphenols, Acids
		Stable bonds: Acetaldehyde

## Methods of Analysis

[Auto titration](#) is a common method of analysis. There are two common titration methods are used for free and total SO<sub>2</sub>. Ripper method based on iodometric titration and Aeration/oxidation method (AO) based on acid base titration. The determination of both free and total SO<sub>2</sub> by the AO method, also known as the Monier/Williams or Rankine/Pocock method, and is the basis of the OIV approved method OIVMA-AS323-04A (OIV methods). The free SO<sub>2</sub> AO method involves acidification of a sample of wine to liberate the SO<sub>2</sub> in the molecular form. A stream of air is then used to carry the liberated SO<sub>2</sub> to a reservoir of hydrogen peroxide where it oxidizes to form sulfuric acid. The generated sulfuric acid is then titrated against dilute sodium hydroxide in the presence of an indicator to determine the amount of acid formed and hence the original quantity of SO<sub>2</sub> in the wine. Total SO<sub>2</sub> is determined in the same manner with the original sample being heated to release the bound forms of SO<sub>2</sub>. However, the required equipment and reagents are relatively specialized and need to be carefully used and maintained. Each analysis takes about 20 minutes and requires the constant attention of a trained technician.

Iodometric titration (more commonly known as the Ripper method) allows a rapid determination of free and total SO<sub>2</sub> with limited equipment and can be automated for use on autotitrators; however, it suffers from significant interferences from other wine components and often gives artificially high results.

An automated discrete analysis method, which compares well with the reference AO method, is fast, simple to use, and carried out on equipment used for other wine analysis—proving itself to be a significant benefit for medium-to-large wineries. Compared to the FIA method, the photometric method requires only small volumes of reagents, making it the more economical and environmentally friendly choice. The automated SO<sub>2</sub> Free and total methods are very quick and easy to use. Analysis of 60 samples takes only 35 min. and allows simultaneous analysis of various sugars and acids, plus color and total SO<sub>2</sub>.







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Reagent Ordering Table

**Method:** The method is based on the reaction between sulfur dioxide, pararosaniline and formaldehyde. Method is performed at 37 °C, using 575 nm filter and for side wavelength 700 or 750 nm filter.

**Chemistry:** pararosaniline and formaldehyde

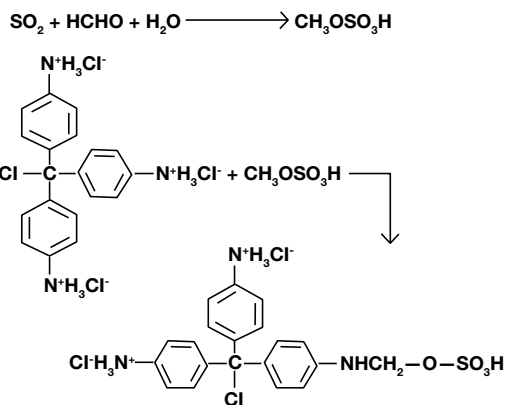


Table 1. Reagents

Reagent Part Number	Maximum Number of Tests	Measuring Range (mg/L)
984634	430	2 – 150

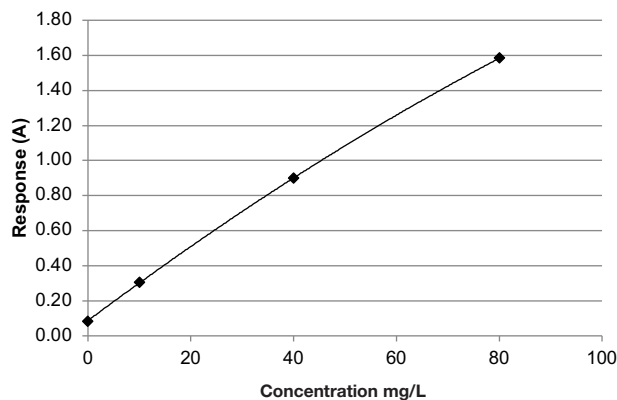


Figure 1. Example calibration curve.

Table 2. Precision

	White Wine		Rosé Wine		Red Wine	
N	40		40		40	
Mean (mg/L)	31		5		24	
	SD	CV%	SD	CV%	SD	CV%
Within Run	0.480	1.5	0.168	3.4	0.442	1.8
Between Run	0.752	2.4	0.156	3.2	0.502	2.1
Total	0.892	2.9	0.230	4.6	0.669	2.8

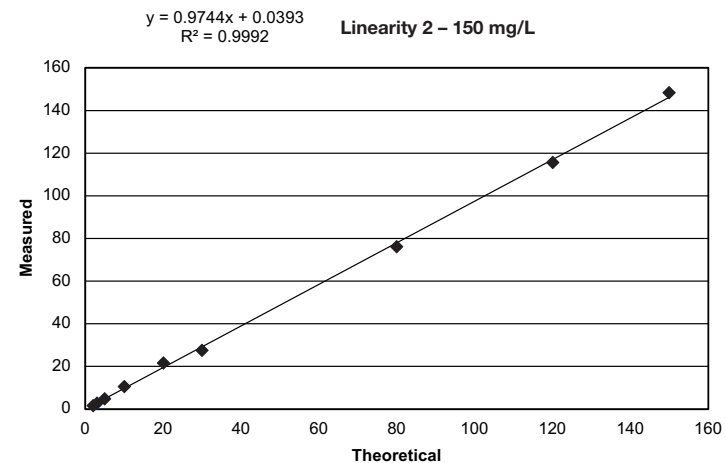


Figure 2. Method Performance Linearity

Download Application Note:

[Correlation of an Automated Discrete Analysis Sulfur Dioxide Method to Standardized Para-roaniline Methods in the Analysis of Beer](#)

Download:

[Fast and Accurate Automated Method for Free Sulfite Analysis in Wine](#)

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Total SO<sub>2</sub>

In foods and beverages, reversibly bound sulfite consists primarily of adducts with carbonyl compounds and hydroxysulfonates. These adducts are stable at low-to-intermediate pH and completely dissociate into free sulfite above pH 8.5. The combined free and reversibly bound sulfite is referred to as total SO<sub>2</sub>. Both free and total sulfite are of interest to the food and beverage industries. For instance, sulfite in wine combines with acetaldehyde to form the hydroxyl/sulfonate adduct with only a small proportion of the sulfite present as free sulfite. Because the concentration of free sulfite is an important parameter in the wine industry, an analysis method is needed that accurately differentiates free from bound sulfite. Total sulfur dioxide (free and bound) in wines is regulated by legislation since it is an allergen.

**Method:** The method is based on the reaction between sulfur dioxide and 5,5'-dithiobis nitrobenzoic acid (DTNB) in basic conditions. Method is performed at 37 °C using a 405 nm filter and for side wavelength 700 or 750 nm filter.

**Chemistry:** 5,5'-dithiobis nitrobenzoic acid.



Table 1. Reagents

Reagent Part Number	Maximum Number of Tests	Low Measuring Range (mg/L)	Measuring Range (mg/L)
984345	300	2 – 50	20 – 300



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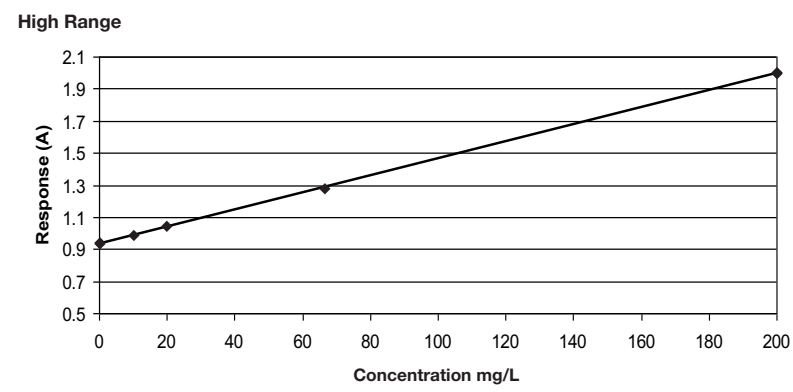
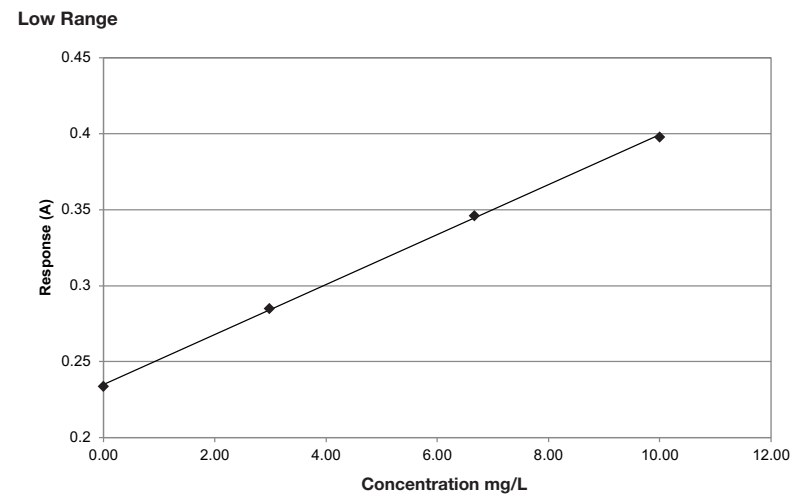


Figure 1. Linear calibration in low and high range.

Table 2. Precision

	Beer		Wine 1		Wine 2	
N	30		50		50	
Mean (mg/L)	9.5		48.3		66.0	
	SD	CV%	SD	CV%	SD	CV%
Within Run	0.671	7.0	1.181	2.4	1.253	1.9
Between Run	0.222	2.3	1.166	2.4	2.370	3.6
Total	0.707	7.4	1.659	3.4	2.681	4.1

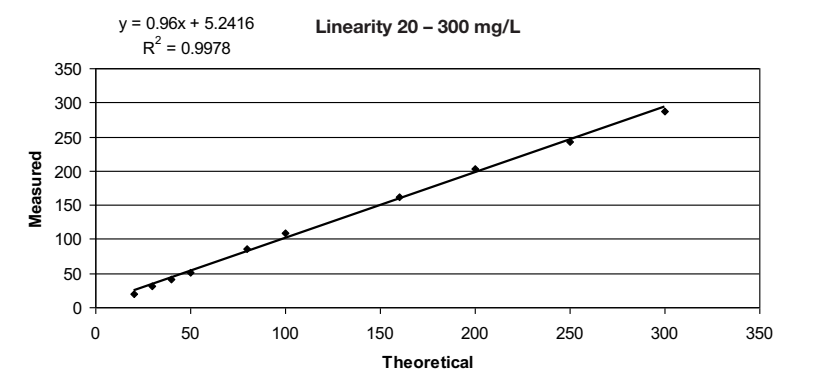
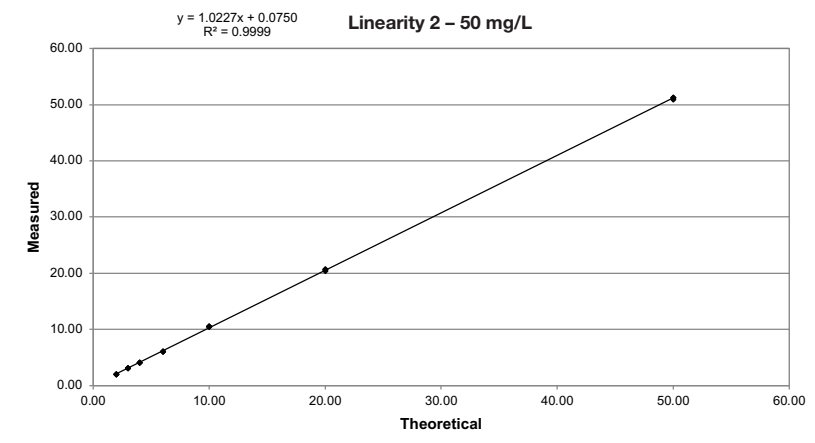


Figure 2. Method Performance for low and high range





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chemistryFree and Total SO<sub>2</sub>Fress SO<sub>2</sub>Total SO<sub>2</sub>

Total Acids (TA)

## Reagent Ordering Table

Acids play a significant role in the taste, color and microbial stability of juice. Analysis is essential to ensure that it fulfills the required quality and authenticity characteristics. In various phases of the production process, from raw materials to finished products, the health standards and uniform quality of juices are constantly confirmed.

**Total Acids (TA)** measures total available hydrogen ions in solution. This measurement includes both the free hydrogen ions and the undissociated hydrogen ions from acids that can be neutralized by sodium hydroxide. Results obtained from the analysis of wine, liqueur and cider during the production process are important for alcoholic beverage making. There will be differences in reported results depending upon the analytical procedure used. In Europe, total acidity in musts and wines is defined by the Office International de la Vigne et du Vin (OIV) as the sum of titratable acids up to pH 7.0 using a NaOH solution. Neither carbonic acid nor sulfur dioxide are included in the expression of total acidity. In the U.S., the Association of Official Analytical Chemists (AOAC) has established a pH of 8.2 using a titration indicator with phenolphthalein as the end point. Total acidity is usually expressed as grams of tartaric acid per liter.

Colorimetric determination of total acids provides a rapid, user-friendly way to analyze total acidity in alcoholic beverages, such as white wine, red wine, cider and liqueur. One major advantage of the automated total acids method is speed. It takes less than 18 minutes to analyze 26 samples. An added benefit is that the method requires no sophisticated skillset or additional hands-on analysis time.

## Titration Method

- Large sample volume
- Electrode care and maintenance
- Slow
- Requires expertise in technique

## Gallery Discrete Analysis Method

- Very small sample and reagent volumes
- Easy to use and maintain
- Rapid analysis
- No sophisticated skill set

Table 1. Reagents

Reagent Part Number	Maximum Number of Tests	Measuring Range (g/L)
984632 – Total Acids (Wine pH 7)	400	1 – 18 as Tartaric acid, 0.5 – 12 as Sulfuric acid
984633 – Total Acids (Juice pH 8)	400	0.5 – 15 as Citric acid

**Method – Total Acids (Wine, pH 7):** This method is adapted from OIV/ Total Acidity for wine samples (OIVMA-AS313-01). Sample is added to buffer solution and measured together with bromothymol blue indicator. Change in blue color is measured at wavelength 620 nm (side wavelength 700 or 750 nm).

**Chemistry:** Bromothymol blue

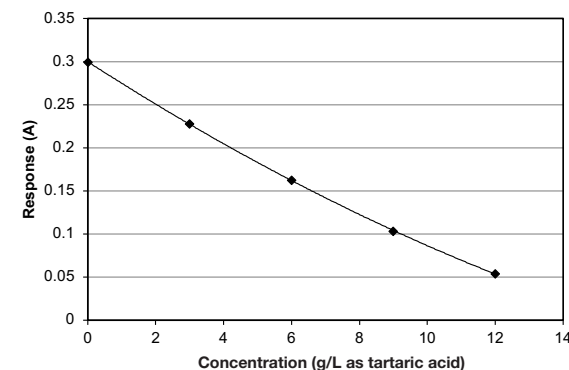


Figure 1. Calibration uses 36 g/L tartaric acid standard; the test has been developed to determine total acidity within a measuring range from 1.0 to 18.0 g/L expressed as tartaric acid.

Table 2. Precision: method performance is tested with wine samples.

	Red Wine		Red Wine		White Wine	
	SD	CV%	SD	CV%	SD	CV%
N	50		50		50	
Mean	4.15		5.15		6.81	
Within run	0.036	0.9	0.047	0.9	0.052	0.8
Between run	0.087	2.1	0.032	0.6	0.032	0.5
Total	0.094	2.3	0.057	1.1	0.061	0.9

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Free and Total SO<sub>2</sub>

Fress SO<sub>2</sub>

Total SO<sub>2</sub>

**Total Acids (TA)**

Reagent Ordering Table



$y = 0.969x + 0.1868$   
 $R^2 = 0.9989$  **Linearity 1 – 18 g/L as tartaric acid**

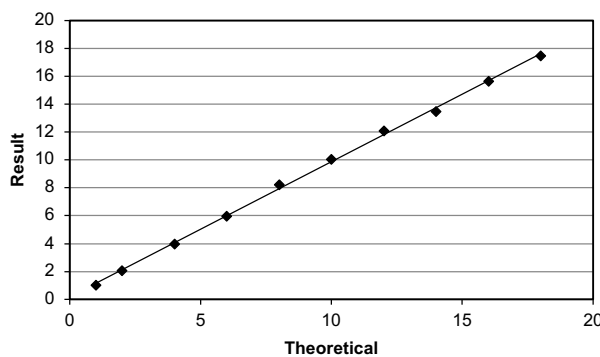


Figure 2. Method Performance Linearity

**Method – Total Acids (juice, pH 8):** Sample is added to buffer solution and measured together with bromothymol blue indicator. Change in blue color is measured a wavelength 620 nm

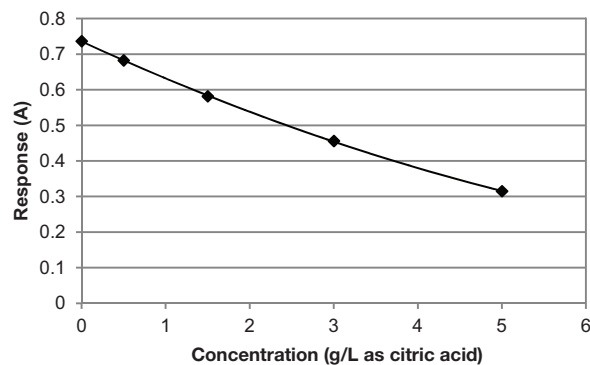


Figure 3. Calibration using dilutions of 15 g/L citric acid. The test has been developed to determine total acidity within a measuring range from 0.5 to 15.0 g/l expressed as citric acid.

$y = 1.0049x - 0.0286$   
 $R^2 = 0.9998$  **Linearity 0.5 – 15 g/L as citric acid**

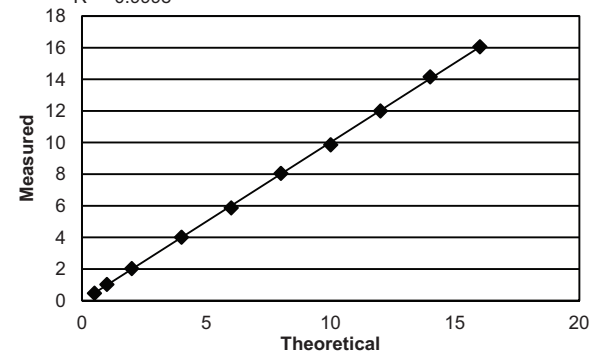


Figure 4. Method Performance Linearity

Table 3. Precision

	Juice		Juice		Juice	
N	50		50		50	
Mean	1.70		2.51		4.28	
	SD	CV%	SD	CV%	SD	CV%
Within run	0.017	1.0	0.015	0.6	0.027	0.6
Between run	0.036	2.1	0.048	1.9	0.026	0.6
Total	0.040	2.3	0.050	2.0	0.037	0.9



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Catalog Number	Description	Analyte	Interference Filter (nm) main / side (alternative)	Test Range mg/L	Method
984347	Acetaldehyde system reagents (250 tests)	Acetaldehyde	340	2 – 500 mg/L	Enzymatic test with acetaldehyde dehydrogenase
984318	Acetic Acid system reagents (300 tests)	Acetic Acid	340	0.04 – 3.00 g/L	Enzymatic test with acetate kinase
984303	Acetic Acid system reagents (1000 tests)	Acetic Acid	340	0.04 – 3.00 g/L	Enzymatic test with acetate kinase
984342	Alpha-Amino Nitrogen by NOPA system reagents (300 tests)	Alpha-Amino Nitrogen	340 / 700 (750)	20 – 300 mg/L	Colorimetric test with OPA (o-Phthaldialdehyde) and NAC (N-acetylcysteine)
984320	Ammonia system reagents (300 tests)	Ammonia	340	10 – 420 mg/L	Enzymatic test with glutamate dehydrogenase
984766	Ammonia system reagents (1000 tests)	Ammonia	340	10 – 420 mg/L	Enzymatic test with glutamate dehydrogenase
984353	BC System liquid system reagents (360 tests)	Bitterness	275	5 – 100 BU	Column extraction of bittering components from beer and wort and subsequent photometric determination
984354	BC Diluent system reagents (360 tests)	Bitterness	275	5 – 100 BU	Column extraction of bittering components from beer and wort and subsequent photometric determination
984355	BC Eluent system reagents (360 tests)	Bitterness	275	5 – 100 BU	Column extraction of bittering components from beer and wort and subsequent photometric determination
984356	Acetic Acid system reagents (300 tests)	Acetic Acid	340	0.04 – 3.00 g/L	Enzymatic test with acetylcoenzyme A synthetase (ACS)
984361	Calcium system reagents (350 tests)	Calcium	660	10 – 200 mg/L	Colorimetric test with Arsanazo III
984327	Citric Acid system reagents (250 tests)	Citric Acid	340	15 – 3000 mg/L	Enzymatic test with citrate lyase
984300	Ethanol system reagents (300 tests)	Ethanol	340	0.01 – 10 g/L	Enzymatic test with alcoholdehydrogenase
984302	D-Fructose system reagents (300 tests)	D-Fructose	340 / 600	0.7 – 200 g/L 5 – 500 mg/L	Enzymatic test with hexokinase, phosphoglucose isomerase and glucose-6-phosphate dehydrogenase
984305	Beta-Glucan – High MW system reagents (350 tests)	Beta-Glucan	405 / 600	15 – 500 mg/L	Novel colorimetric test
984304	D-Glucose system reagents (300 tests)	D-Glucose	340 / 600	0.1 – 160 g/L 5 – 500 mg/L	Enzymatic test with hexokinase and glucose-6-phosphate dehydrogenase
984764	D-Glucose system reagents (1000 tests)	D-Glucose	340 / 600	0.1 – 160 g/L 5 – 500 mg/L	Enzymatic test with hexokinase and glucose-6-phosphate dehydrogenase
984314	D-Glucose + D-Fructose system reagents (300)	D-Glucose D-Fructose	340 / 600	0.04 – 200 g/L	Enzymatic test with hexokinase, phosphoglucose isomerase and glucose-6-phosphate dehydrogenase
984313	D-Glucose + D-Fructose system reagents (1000)	D-Glucose D-Fructose	340 / 600	0.04 – 200 g/L	Enzymatic test with hexokinase, phosphoglucose isomerase and glucose-6-phosphate dehydrogenase
984317	D-Glucose + D-Fructose + Sucrose system reagents (300 tests)	D-Glucose D-Fructose Sucrose	340 / 600	0.24 – 200 g/L	Enzymatic test with betafructosidase (Invertase), hexokinase, phosphoglucose isomerase and glucose-6-phosphate dehydrogenase
984710	D-Gluconic Acid (250 tests)	D-Gluconic Acid	340 / 750 (700)	50-5000 mg/L	Enzymatic test with 6-P-gluconate-dehydrogenase and gluconate kinase

Number of tests/ kit is test flow dependent number.





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984301	D-Glucose + D-Fructose + Sucrose system reagents (1000 tests)	D-Glucose D-Fructose Sucrose	340 / 600	0.24 – 200 g/L	Enzymatic test with betafructosidase (Invertase), hexokinase, phosphoglucose isomerase and glucose-6-phosphate dehydrogenase
984316	Glycerol system reagents (300 tests)	Glycerol	340	0.07 – 30 g/L	Enzymatic test with glycerokinase, ADP dependent hexokinase and glucose-6-phosphatedehydrogenase
984765	Glycerol system reagents (1000 tests)	Glycerol	340	0.07 – 30 g/L	Enzymatic test with glycerokinase, ADP dependent hexokinase and glucose-6-phosphatedehydrogenase
984326	Total Iron (Fe) system reagents (850 tests)	Iron	600	0.03 – 5.5 mg/L	Colorimetric test with Ferene S
984322	D-Isocitric Acid system reagents (300 tests)	D-Isocitric Acid	340	10-600 mg/L	Enzymatic test with isocitrate-dehydrogenase
984306	D-Lactic Acid system reagents (300 tests)	D-Lactic Acid	340	25 – 1600 mg/L	Enzymatic test with D-lactatedehydrogenase
984308	L-Lactic Acid system reagents (300 tests)	L-Lactic Acid	340	20 – 1600 mg/L	Enzymatic test with L-lactatedehydrogenase
984358	Magnesium system reagents (350 tests)	Magnesium	510	10 – 400 mg/L	Colorimetric test with Xylidyl Blue I
984310	L-Malic Acid system reagents (300 tests)	L-Malic Acid	340 / 700 (750)	0.05 – 20 g/L	Enzymatic test with L-malatedehydrogenase and glutamate-oxalacetate-transaminase
984311	L-Malic Acid system reagents (1000 tests)	L-Malic Acid	340 / 700 (750)	0.05 – 20 g/L	Enzymatic test with L-malatedehydrogenase and glutamate-oxalacetate-transaminase
984348	Oxalic Acid system reagents (250 tests)	Oxalic Acid	600 / 700	2 – 100 mg/L	Enzymatic test with oxalate oxidase
984349	pH (colorimetric) (330 tests)	pH	575 / 700	3 – 7.2 pH at 37 °C	Colorimetric test with pH indicator dyes
984634	Sulfur Dioxide system reagents (430 tests)	Free SO <sub>2</sub>	575 / 750 (700)	2 – 150 mg/L	Colorimetric test with paraosanine
984345	Sulfur Dioxide system reagents (300 tests)	Total SO <sub>2</sub>	405 / 750 (700)	2 – 50 mg/L 20 – 300 mg/L	Colorimetric test with DTNB
984312	Sucrose (Total Glucose) system reagents (300 tests)	Sucrose	340 / 600	0.1 – 100 g/L 15 – 500 mg/L	Enzymatic test with betafructosidase, hexokinase and glucose-6-phosphate dehydrogenase
984328	Proteins system reagents (450 tests)	Total Protein (Biuret)	540 / 700	0.5 – 15 g/L	Colorimetric test with Biuret
984346	Total Polyphenol system reagents (280 tests)	Polyphenols	700	50-3000 mg/L	Colorimetric test with Folin-Ciocalteau reagent
984309	Tartaric acid (480 tests)	Tartaric acid	540	0.5 – 12 g/L	Colorimetric test with ammonium monovanadate
984632	Total Acids (Wine pH 7) system reagents (400 tests)	Total Acids (Wine pH 7)	620 / 700 (750)	1-18 g/L as tartaric acid	Colorimetric tests with bromothymol blue
984633	Total Acids (Juice pH 8) system reagents (400 tests)	Total Acids (Juice pH 7)	620 / 700 (750)	0.5-15 g/L as Citric acid 0.5-12 g/L as Sulfuric acid	Colorimetric tests with bromothymol blue

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984396	Acetaldehyde Standard (100 mg/L)
984394	NOPA Standard for Alpha-Amino Nitrogen test (150 mg/L)
984384	Alcohol Standard (0.5 g/L)
984380	Sugar Combination Standard for D-Fructose (0.500 g/L), D-Glucose (0.500 g/L), Sucrose (0.500 g/L), or total Glucose (0.760 g/L) tests
984383	Beta-Glucan Standard (500 mg/L)
984386	Glycerol Standard (0.2 g/L)
984382	Acid Combination Standard for D-Lactic Acid (0.200 g/L), L-Malic Acid (0.500 g/L), L-Lactic Acid (220 mg/L), and D-Lactic Acid (220 mg/L)
984393	Oxalic Acid Standard (45 mg/L)
984331	ECM pH 4 Standard
984332	ECM pH 7 Standard
984330	ECM pH 2 Standard
984331	ECM pH 4 Standard
984332	ECM pH 7 Standard
984333	ECM pH 10 Standard
984334	ECM pH 12 Standard
984339	ECM Conductivity 0.08 Standard
984336	ECM Conductivity 1.4 Standard
984337	ECM Conductivity 13 Standard
984338	ECM Conductivity 112 Standard

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