

Free Sulfite/Total Sulfite (DTNB Method)

FIAlab DTNB method for the free and total sulfite contained in wines and using the FIAlab-2500/2600/2700 system. May work with other types of samples.

Assay	Typical Throughput	Concentration Range	Notes
Free Sulfite	120 samples/hour	0 to 200 mg (N)/L	1 cm flow cell
Free Sulfite	90 samples/hour	5.0 to 200 mg (N)/L	10 cm flow cell

Principle:

The determination of free sulfite and total sulfite concentration is frequently needed in the wine making industry. Free sulfite concentration needs to be determined for the preserving of wine. Total sulfite concentration needs to be determined because it is government regulated. For both methods samples are mixed with acid to produce sulfur dioxide from the sulfite and a gas diffusion unit (see Ammonia method) is then used to transfer the sulfur dioxide to the acceptor solution of a reagent. This is all done so that the red coloration of wine doesn't cause interference in measurements. For total sulfite the samples are pretreated with sodium hydroxide to liberate all of the bound sulfite.

Comments:

For total sulfite the samples must be pretreated with sodium hydroxide as outlined below. Recommended Wavelengths: 412 nm primary, no reference wavelength. Make the sample loop from three inches of tubing (.03" ID) and use a 1 cm flow cell.

Interferences:

The gas diffusion unit eliminates the interferences from the colored wine sample.

Reagents:

Carrier:

DI Water

Reagent 1: Hydrochloric acid

For free sulfite use 4M HCl. For total sulfite use 6 M HCl

Reagent 2: DTNB Solution

0.06 g DTNB (**5,5'-Dithio-Bis** (2-Nitrobenzoic Acid))

10mL isopropanol.

1 L TOTAL: Balance with PBS buffer solution (Phosphate buffered Saline Sigma: P-3813).

Dissolve 0.06g of DTNB in the 10 mL of isopropanol. Prepare the PBS solution by adding one packet of PBS (Phosphate buffered Saline Sigma: P-3813) to 1 L of H₂O, and mix well. Add ~900 mL of PBS buffer solution to a 1 L volumetric flask. Add the dissolved DTNB solution to the flask. Fill flask to mark with PBS buffer solution, mix well.

FOR TOTAL SULFITE:

The samples must be pretreated with sodium hydroxide.

0.7g Sodium Hydroxide

10 mL of Sample

Dissolve the sodium hydroxide in the sample, and allow to sit for 5 minutes.

Standards:

1000ppm Sulfite Standard:

0.7415g Sodium Meta-bisulfite

500mL TOTAL: Balanced with Stabilizing buffer

Stabilizing buffer:

10.7g Sodium Phosphate, Dibasic

3.6g D-Mannitol

300mL Ethyl Alcohol

2 L TOTAL: Balanced with Deionized Water

Dissolve 10.7 g of sodium phosphate, dibasic and 3.6 g of D-Mannitol in about 500 mL of deionized water contained in a 2 liter flask. Add 300 mL of ethyl alcohol. Dilute to the mark with deionized water and mix well. Prepare as needed. Cover when not in use.