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Validation Report: Total Sulfite Assay Kit (Enzymatic) (cat. no. K-ETSULPH)

1. Scope

Megazyme's Total Sulfite Assay Kit (Enzymatic), (K-ETSULPH) is a simple, highly specific and reliable, enzymatic method for the measurement and analysis of total sulfite (as SO₂) in wines, beverages, foodstuffs and other materials. This method measures total sulfite in g/L. Methods based on this enzymatic Total Sulphite Assay Kit principle, have been accepted by DIN, EN, MEBAK and NMKL.

2. Planning

The purpose of this report is to verify and validate the current method as detailed by the enzymatic Total Sulfite Assay Kit (Enzymatic) (K-ETSULPH).

3. Performance characteristics

The selectivity, working range, limit of detection, limit of quantification, trueness (*bias*) and precision of this kit is detailed in this report.

3.1. Selectivity

Sulfite oxidase will react with sulfite, isothiocyanates and isothiocyanate glycosides while sulfates, thiosulfates, sulfinic acid compounds and sulfides do not react. Sulfonic acid compounds can cause a creep reaction under these assay conditions.

The presence of L-ascorbic acid will slow the sulfite oxidase reaction and L-ascorbic acid levels higher than 50 µg per manual assay should be removed during sample preparation.

Interfering substances in the sample being analysed can be identified by including an internal standard. Quantitative recovery of this standard would be expected. Losses in sample handling and extraction are identified by performing recovery experiments, i.e. by adding sodium sulfite to the sample in the initial extraction steps.

Due to the characteristic instability of aqueous sulfite samples, recoveries of less than 100% should be expected. This is consistent with values obtained with other commercially available enzymatic sulphite test kits.



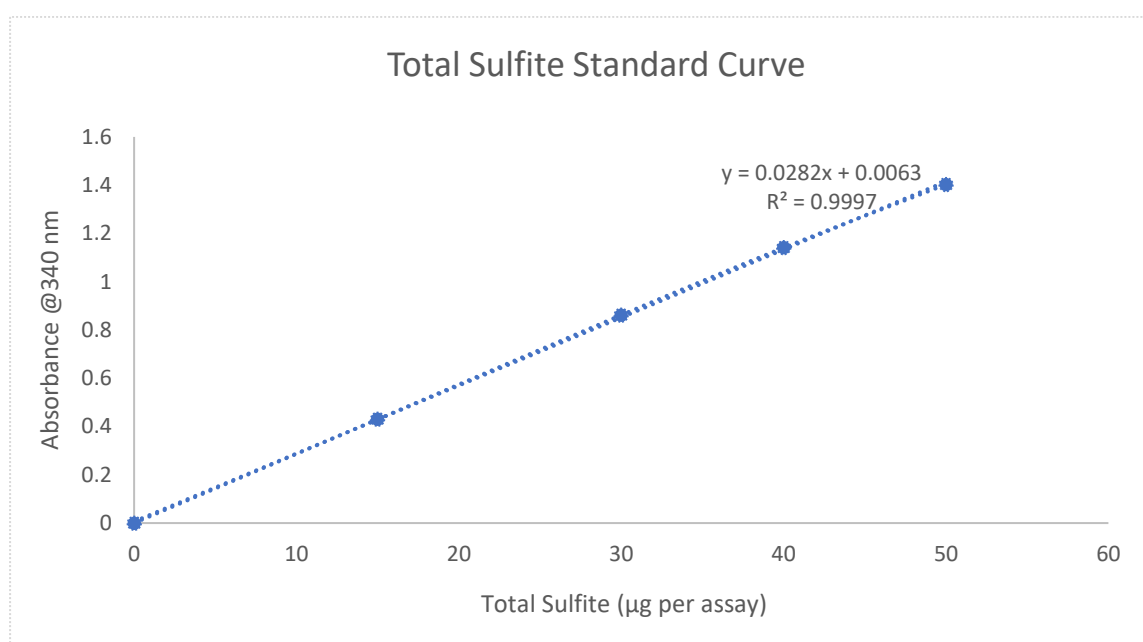
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3.2. Working Range

Assay follows the Total Sulfite Assay Kit ((Enzymatic) (K-ETSULPH) standard procedure. Sodium sulfite standard was used as a sample across a range of concentrations (0.01-0.5 g/L) which corresponds to 1-50 µg of sulfite (as SO₂) per cuvette.

The working range is linear between 1-50 µg of sulfite per assay.



3.3. LOD and LOQ

The **instrument limit of detection** for the standard manual assay procedure is 0.34 mg/L, which is derived from an absorbance difference of 0.020 with the maximum sample volume of 2.00 mL.

The **calculated limit of detection (LOD)** and the **calculated limit of quantification (LOQ)** for this report purpose is based on the analysis of samples that have been taken through the whole Total Sulfite Assay kit (Enzymatic) (K-ETSULPH) measurement procedure.

- The Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated as $3 \times \sigma$ of the blank sample solution absorbance and $10 \times \sigma$ of the blank sample solution absorbance, respectively, where σ is the standard deviation of the absorbance values from 10 replicates.



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- For Total Sulfite Assay Kit (Enzymatic) (K-ETSULPH)

LOD – For 2.0 mL of sample (maximum volume)

Total Sulfite = 0.240 mg/L

LOQ – For 2.0 mL of sample (maximum volume)

Total Sulfite = 0.765 mg/L

* **Note:** The above detection limits are for samples as used in the assay, after any sample preparation, if required. The dilution used in pre-treatment must be accounted for while establishing the detection limits for specific samples.

3.4. Trueness (*Bias*)

Comparison of the mean of the results (x) achieved with the Total Sulfite Assay Kit (Enzymatic) (K-ETSULPH) method with a suitable reference value (x ref). For this report, Relative Bias is calculated in per cent as: **$b(\%) = \frac{x - x_{ref}}{x_{ref}} \times 100$** . The reference material for this purpose is sodium sulfite supplied with the Total Sulfite Assay Kit (Enzymatic) (K-ETSULPH) and made to a concentration of 0.3 g/L, as instructed. **Due to the characteristic instability of aqueous sulphite samples, recoveries of less than 100% should be expected.**

Relative Bias *b*(%)

	n	Ref Material (g/L)	Mean (g/L)	<i>b</i> (%)
Total Sulfite	22	0.3	0.2728	-9.08

3.5. Precision

This report details the reproducibility of the Total Sulfite Assay Kit (Enzymatic) (K-ETSULPH), it is a measure of the variability in results, on different occasions and by different analysts over an extended period of time.

Reproducibility

	n	Ref Material (g/L)	Mean (g/L)	Standard Deviation	%CV
Total Sulfite	22	0.3	0.2728	0.0038	1.39



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4. Conclusion

The method outlined in this document is a robust, quick and easy method for the measurement of total sulfite in various matrices. Data presented in this report verifies and validates that this method is fit for the purpose intended, which is summarised below.

Validation Summary	Total Sulfite
Working range (μg in cuvette)	1-50
LOD (mg/L)	0.240
LOQ (mg/L)	0.765
Relative Bias <i>b</i> (%)	-9.08
Reproducibility (%CV using kit standard)	1.39