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

1. Scope

Megazyme's Total Sulfite Assay Kit (K-TSULPH) is a reliable and accurate method used for the rapid measurement and analysis of total sulfite (sulphite) in wine, beverages, foodstuffs and other materials. This method measures total sulfite in mg/L and is used widely in the wine industry. This method has been validated for red and white wines at the Bundesamt für Weinbau, Austria.

2. Planning

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

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

This assay is specific for total sulfite. Compounds containing free thiols (e.g. cysteine, β -mercaptoethanol and dithiothreitol) or thiol reactive compounds (e.g. aldehydes and maleimide compounds) can also react in the total sulfite assay. Aldehyde levels in wine do not cause interference of the total sulfite assay.

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 sulfite to the sample in the initial extraction steps.

3.2. Working Range

Assay follows the Total Sulfite Assay Kit (K-TSULPH) standard procedure. 0.05 mL of sulfite standard was used as sample, with a range of concentrations (5-400 mg/L sulfite) which corresponds to 0.25-20 μ g of sulfite per cuvette. Absorbance A₂ was read after 3 min, at 405 nm and at 25°C as recommended in the procedure.



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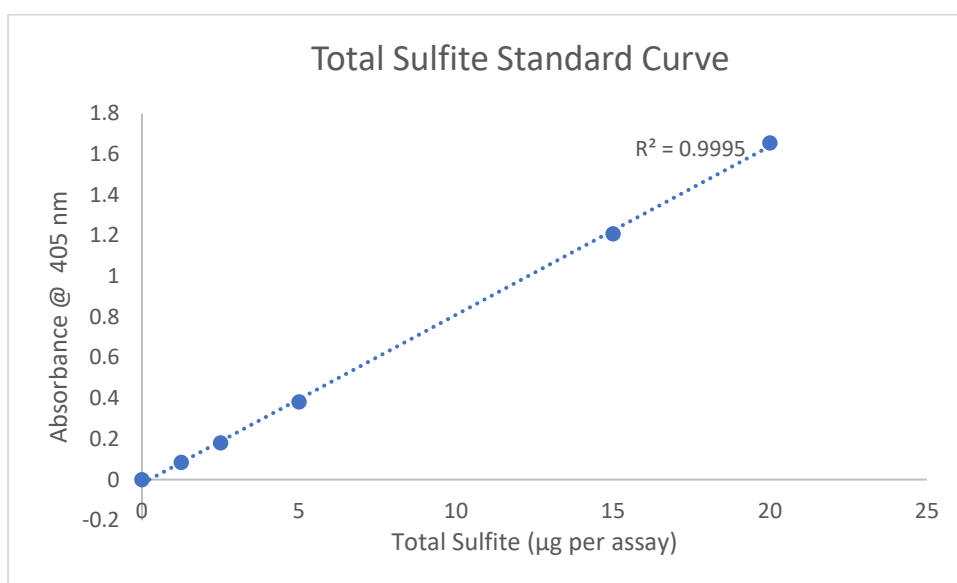
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Total Sulfite Concentration [$\mu\text{g}/\text{assay}$]	$\Delta A_{405\text{nm}}$
0	0.000
1.25	0.251
2.5	0.496
5.0	0.972
15.0	1.232
20.0	1.507





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3.3. LOD and LOQ

The **instrument limit of detection**, as per kit booklet, is 5.28 mg/L of sulfite, which is derived from an absorbance difference of 0.020 with the maximum sample volume of 0.05 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 (K-TSULPH) procedure.

- The LOD is the lowest concentration of the analyte that can be detected by the method. LOD is calculated as $3 \times s'0$; where $s'0$ is the standard deviation of a number of samples A1 reading.
- The LOQ is the lowest level at which the kit's performance is acceptably repeatable. LOQ is calculated as $kQ \times s'0$; where $s'0$ is the standard deviation of a number of samples A1 reading. The IUPAC default value for kQ is 10.
- For Total Sulfite Assay Kit (K-TSULPH)

LOD – For 0.05 mL of sample (maximum volume)

Total Sulfite = 2.9 mg/L

LOQ – For 0.05 mL of sample (maximum volume)

Total Sulfite = 10.018 mg/L

* **Note:** The above detection limits are for samples as used in the assay i.e. after any required sample preparation (e.g. deproteinisation). The dilution used in pre-treatment must be accounted for while establishing the detection limits for specific samples.

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3.4. Trueness (*Bias*)

Comparison of the mean of the results (x) achieved with Total Sulfite Assay Kit (K-TSULPH) 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 bisulphite supplied with the Total Sulfite Assay Kit (K-TSULPH) at 400 mg/L.

Relative Bias $b(\%)$

	n	Ref Material (mg/L)	Mean (mg/L)	$b(\%)$
Total Sulfite	16	400	428.86	7.22

3.5. Precision

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

For the purpose of this report different lot numbers of the kit standard is used as the reference material.

Reproducibility

	n	Ref Material (mg/L)	Mean (mg/L)	Standard Deviation	%CV
Total Sulfite	16	400	428.86	13.59	3.17



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Repeatability of this kit can be assessed using wine samples. This is a measure of the variability in results by a single analyst, using real samples, using the same equipment and over a short period of time. The use of wine samples shows one of the many applications of this kit.

Repeatability

	n	Mean (mg/L)	Standard Deviation	%CV
White Wine	11	81.4	2.3	2.83
Red Wine	12	83.77	1.13	1.35

4. Conclusion

The method outlined in this document is a robust, quick and easy method for the measurement of sulfite in various matrices. It has been used for many years and is fully automatable for high throughput analysis of samples. 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)	0.25-20
LOD (mg/L)	2.9
LOQ (mg/L)	10.02
Relative Bias <i>b</i> (%)	7.22
Reproducibility (%CV using sodium bisulfite)	3.17
Repeatability (%CV using white wine)	2.83
Repeatability (%CV using red wine)	1.35