Agriculture and Food

AIMS Agriculture and Food, 8(1): 1–24.
DOI: 10.3934/agrfood.2023001
Received: 09 June 2022
Revised: 15 September 2022
Accepted: 13 November 2022
Published: 02 December 2022

http://www.aimspress.com/journal/agriculture

Research article

Validation of a rapid test to dose SO_2 in vinegar

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Abstract: Sulfur dioxide is generally used in wine and vinegar production. It is employed to decrease the bacteria' growth, improve the wines' aroma (since it supports the extraction of polyphenols during maceration), protect the wines from chemical oxidation and the musts from chemical and enzymatic oxidation (blocking free radicals and oxidase enzymes such as tyrosinase and laccase). The composition and storage conditions (i.e., pH, temperature, and alcohol levels) affect oenological results. In various countries, competent authorities have imposed legal limits since it can have toxic effects on humans. It is crucial to dose SO_2 levels to allow vinegar production and compliance with legal limits. The iodometric titration named "Ripper test" is the legal method used to dose it in vinegar. In this work, an automatized colorimetric test was validated using the international guidelines ISO/IEC (2017) to allow its use instead of the Ripper test. The test reliability was verified on white, red, and balsamic vinegar with low or high SO_2 content. The automatized test showed linearity, precision, and reproducibility similar to the Ripper test, but the accuracy parameter was not respected for the vinegar with a low concentration of SO_2. Therefore, the automatized colorimetric test can be helpful to dose SO_2 in vinegar with high concentrations of SO_2.

Keywords: sulfur dioxide; automatized test validation; food analysis; vinegar analysis; SO_2 dosage; validation of food analytical method

1. Introduction

Sulfur dioxide (SO_2) is a colorless gas, pungent, toxic, and suffocating [1]. It is obtained by burning sulfur and pyrites. It becomes liquid below 10 °C [2]. The use of sulfur dioxide in food processing is very complex [3]. It is used as an antiseptic to inhibit the development of microorganisms. It is more effective against bacteria than against yeasts. Its effect is directly proportional to the dose of use and inversely proportional to the level of contamination [4]. It is employed as an antioxidant compound. It can protect wines from chemical oxidation blocking oxygen and protect musts before fermentation, inhibiting the action of oxidase enzymes (tyrosinase and laccase). The SO_2 protects the aroma of wines and favors the extraction of intracellular components such as anthocyanins and polyphenols when added to grapes before maceration [5]. The dosages of SO_2 in wines and vinegar vary depending on the composition and storage conditions. A low pH, high temperature, and high alcohol content increase the active sulfur molecule fraction. A high salt concentration decreases the concentration of molecular sulfur dioxide [6]. The SO_2 in vinegar production is limited since sulfur dioxide can have toxic effects on humans and damage the wine, interfering with the aromatic baggage and causing the attenuation of the aromas. The World Health Organization has included sulfur dioxide among the preservatives (E220) and indicated the dose of 0.7 mg/kg of body weight as a dose allowable daily [7]. In addition to the toxic effect, sulfur dioxide also can have an allergenic action, so since November 2005, with the entry into force in Europe of the EC Directive no.89/2003 ("allergens directive"), it has become mandatory to report the presence of sulfites and sulfur dioxide in wine and in any other food, when the concentration exceeds 10 mg/L or 10 mg/kg, expressed as SO_2 [8]. Currently, national and community legislation sets legal limits on the presence of sulfites in wines and vinegar. European legislation sets maximum limits of 160 mg/L for reds and 210 mg/L for whites, with exceptions that allow the Member State to raise the maximum value of 40 mg /L in unfavorable years. The European limit (160 mg/L) must be observed for red wines in Italy, while the more restrictive national limit (200 mg/L) applies to whites. Higher values apply to sweet wines [9]. The Ripper test is the official method used to determine SO_2. It consists of directly titrating the vinegar with iodine using starch as an indicator [10]. This test is cheap, but its sensibility can be affected by iodine which can interfere with ascorbic acid, and it can be performed only by experienced personnel. Some new methods have been proposed to speed up the work in the laboratory [3,11], which require validation processes before they can be used in legal analyses. The development of analytical methods in food control, particularly in the case of legislative compliance valuation, needs the demonstration that they are "appropriate for purpose" through method validation [12]. The critical aspect is to confirm method applicability by providing test reliability and suitability in complex food matrices [13–19]. This work aims to validate an automated colorimetric test to dose sulfur dioxide in vinegar.

2. Materials and methods

2.1. Reagents

Enzytec^{TM} liquid SO_2 Cod. E28600 was purchased from R-Biopharm AG (Darmstadt, Germany). The distilled water was obtained from Sigma-Aldrich (Milan, Italy). The sulphuric acid solution (25%) was prepared from the concentrated acid (Sigma-Aldrich). EDTA, potassium iodide, potassium iodate, hydrogen peroxide, and starch were acquired from Merck. Co. (Darmstadt, Germany). Iodine was provided by Acros Organics (Geel, Belgium).

2.2. Samples preparation

Three commercial vinegar types were tested: white, red, and balsamic wine vinegar.

2.3. Apparatus

The analyzer iCubio iMagic M9, fully automatized, was used to detect the total SO_2 content in vinegar. The apparatus pipette reagents and samples into the cuvette, allow the incubation at a controlled temperature, read absorbance at the specific wavelength, and calculate the concentration of the SO_2 by a calibration curve. The parameter used in automated photometric systems were: temperature 20 to 37 °C; wavelengths 340 nm (±5 nm); optical path 1 cm; reaction 10 min (20–25 °C) or 5 min (37 °C).

2.4. Determination of total SO_2 content

The method reported in the kit instruction (Enzytec^{TM} liquid SO_2) was respected. The kit contained: Buffer: two vials $\geq$ 100 mL; Chromogen: two vials $\geq$ 25 mL and Calibrator (SO_2 = 150 mg/L): one vial $\geq$ 3.5 mL.

The first step consisted of preparing Reagent 1 by mixing 2000 μL of reagent (Buffer) together with 2000 μL calibrator solution and 2000 μL of the sample, after three minutes, the absorbance was read. Successively, Reagent 2 was obtained by mixing 500 μL of reagent (chromogen) together with 500 μL calibrator solution and 500 μL of sample. After 20 minutes (25 °C), the absorbance was read.

Enzytec fluid Acid combination Standard (ID-No 5460, 3 × 3 mL) was used to calibrate the automated photometric systems.

2.5. Reference procedure to determine SO_2 content

The "Ripper" method was performed according to the Portuguese regulation (IPQ 1987), based on a procedure from the Organisation Internationale de la Vigne et du Vin [20]. Vinegar (10.00 mL) was put into an Erlenmeyer flask (V = 500mL), an aliquot of 1% w/v starch indicator (5.00 mL), and a sodium hydrogen carbonate were added. After ten minutes, 5.00 mL of 33% (v/v) sulfuric acid was added, and the solution was immediately titrated with an 0.25 $\text{mmol}\cdot\text{L}^{-1}$ iodine solution to a blue endpoint (color stable for 20 seconds).

2.6. Automatized method validation process

Linearity was determined by performing three replicates of calibration curves of high-concentration red, white and balsamic wine vinegar (19, 38, 75, 150 mg/L) and low-concentration balsamic wine vinegar (1.88, 3.75,7.50, 15 mg/L).

Method precision was evaluated by conducting ten analyses on the same sample and verifying normality by Shapiro-Wilk [21] and the anomalous data from the Huber test [22].

The LLOQ (signal/noise ratio $\geqslant$ 10) and LLOD (lowest concentrations of SO_2 that were detectable in all replicates but not necessarily quantified and distinguished from zero) defined method sensitivity. The LLOQ dilution factor gives the lower end of the measuring range.

$$\text{Dilution factor} = \frac{read\ concentration*100}{\text{weight}*\text{ rate}} \quad (1)$$

The upper end of the measuring range was given by the last point of the calibration curve line. Reproducibility and repeatability were detected to validate method precision:

$$\text{Reproducibility} = \frac{Standard\ deviation\ of\ analyzed\ samples}{Standard\ deviation\ of\ reference\ samples} \quad (2)$$

Uncertainties (type A and B) were measured following as reported by Dini et al. [23,24] and the EURACHEM/CITAC guide [12].

Type A uncertainties were estimated from 10 repeated readings of the same sample.

$$U_{\text{Type A}} = \sqrt{\frac{variance}{\text{Degrees of freedom}}} \quad (3)$$

Type B uncertainties considered were:

The uncertainties related to standard preparation (U(mr)); uncertainties related to the calibration curve (U(ct)), uncertainties related to balances (U(bt)), uncertainties related to accuracy (due to burette use) (U(m)), uncertainties related to accuracy (due to 50 mL pipette use) (U(p))

U(mr) was found from each standards' analysis certificate. U(ct) was appraised for standard at three concentrations in triplicate. U(bt) was decided considering a certificate of repeatability (0.000029 g), calibration (0.00060 g), and stability (0.000032 g). U(m) was evaluated from a certificate of calibration (0.1 mL) and repeatability (0.0010 mL). The U(p) was found from a certificate of calibration (0.028 mL), variation in volume based on temperature (0.0003 mL), and repeatability (0.001 mL). The method accuracy was found.

$$\text{Accuracy} = \frac{|\bar{X}_{\text{Offic}} - \bar{X}|}{\sqrt{S_r^2 + U_{Official}^2}} \leq t_{\text{p}} \quad (4)$$

$|\bar{X}_{\text{Offic}}$=official method value

$\bar{X}_{\text{x}}$=media repeatability values

S_r^2=standard deviation^2

$U_{Official}^2$= reference material uncertainty ^{2}

2.7. Statistical Analysis

The statistical analyses were performed by Statistica software Version 7.0 (StatSoft, Inc. USA).

3. Results and discussion

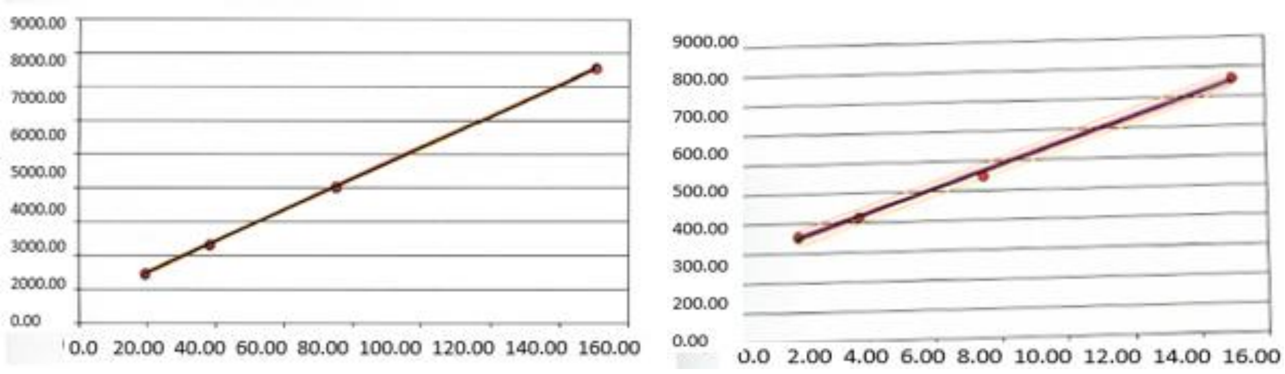
In commodity laboratories, automated equipment often substitutes the official methods. The automated analyzers do not require specialized personnel, improve safety, reduce the analysis time, and decrease the cost of analyses. This work validated a colorimetric method, performed by an automated analyzer, to determine NaCl levels in canned tomatoes using the international guidelines ISO/IEC (2017) [25]. According to international guidelines, the primary validation process explains a method's operative limits and performance not adequately characterized. In this case, the validation process was necessary to establish the commercial test's validity when applied to the assay of SO_2 in vinegar. The vinegar is a complex matrix, and the presence of interferents can negatively affect the results reliability. The objective was achieved by comparing the results obtained by the colorimetric method to those obtained by the Volhard test, considered a reference method (Ministerial Decree 03/02/1989–SO GU SG n 168 20/07/1989 Met 33). The parameters evaluated were working range (linearity range, LOQ, LOD, measuring range), recovery, precision, accuracy, and measurement uncertainty (associated with the analytical data). Statistical analyses were used to estimate validation qualities against fixed acceptance criteria.

3.1. The working range

The working range defines the impact of the sample preparation (i.e., dilutions) and the analytical procedure on the reliability of the results. The procedure's suitability for the intended use is confirmed by a linear relationship between analyte concentration and response.

3.2. Method linearity

The method linearity was evaluated by regression coefficient determination (Figure 1, Table S1). The ANOVA test estimated the distribution of residuals (procedure errors) across the calibration curve (Figure 2, Table S2).



High-concentration of red, white, and balsamic wine kinds of vinegar (mg/L)

Y = 46.6X + 567

R = 0.99995

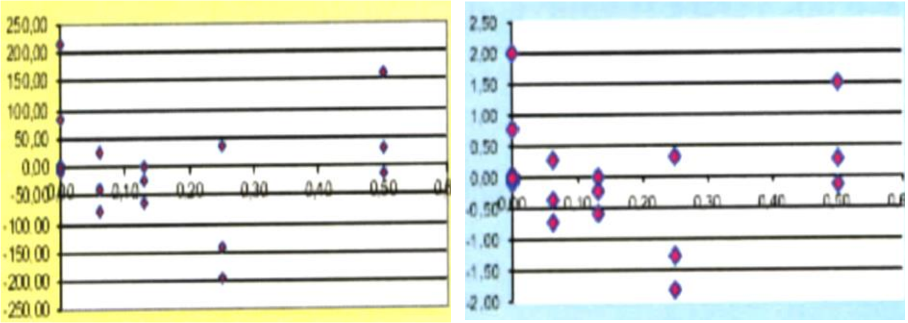
Low-concentration balsamic wine vinegar (mg/L)

Y = 77.4X + 552

R = 0.99873

Figure 1. Calibration curves.

Residual distributions in high and low concentration red and white and high concentration balsamic wine kinds of vinegar

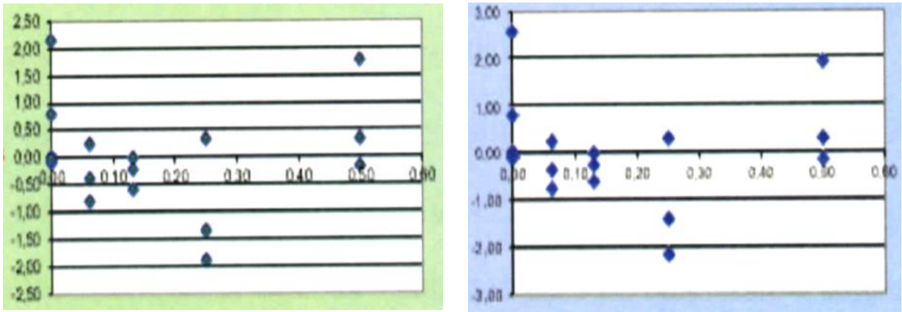


Absolute residuals

Normalized residuals

Residuals (+)	5
Residuals (−)	7
Sequences number	5
Reference parameter	6.83333
Correction factor	3.18182

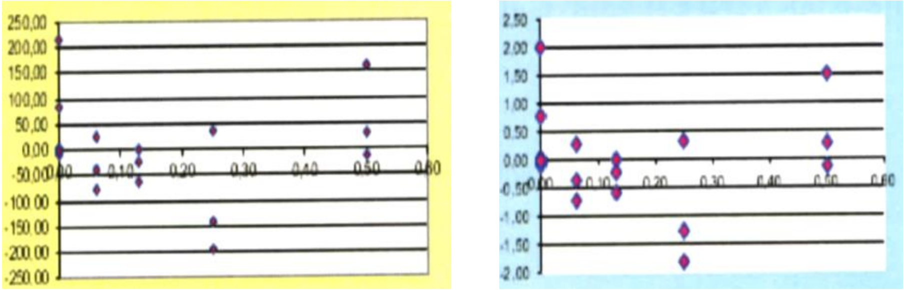
Normal residual distribution



Standardized residuals

Studentized residuals

Residual distributions in low-concentration balsamic wine vinegar

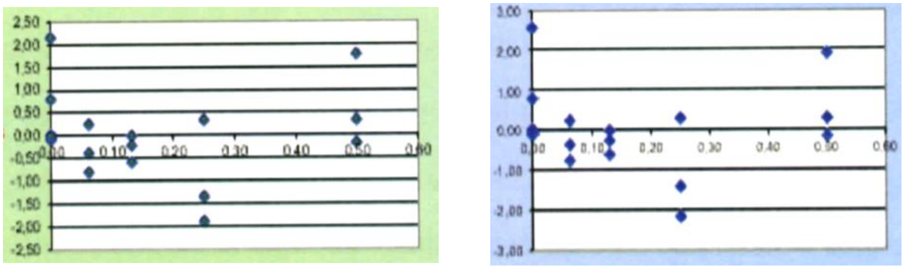


Absolute residuals

Normalized residuals

Residuals (+)	8
Residuals (−)	4
Sequences number	7
Reference parameter	6.33333
Correction factor	2.42424

Normal residual distribution



Standardized residuals

Studentized residuals

Figure 2. Residual distributions in wine vinegar.

The regression coefficient close to 1 of the calibration curve and the normal residual distribution evaluated by ANOVA confirmed the method's linearity.

3.3. Method sensitivity

The method detection limit was tested by repeated analysis of blank samples. LLOD and LLOQ were derived from the regression curve (Table 1).

Table 1. LLOD, LOOQ & measuring range of tested samples.

	LLOD (mg/L)	LLOQ (mg/L)	Measuring range (mg/L)
Low-concentration balsamic wine vinegar	1.38	4.03	4.03 ≤ Measuring range ≤ 15
Low-concentration red and white and high concentration balsamic wine kinds of vinegar	2.72	6.06	6.06 ≤ Measuring range ≤ 150

The test's measuring range, able to determine the concentrations of SO_2 admissible in vinegar by law, demonstrated the method's selectivity.

3.4. Method precision

Test precision serves to establish the effect of impurities on the dosage. Test precision was evaluated by estimating the repeatability and reproducibility of the test. The repeatability should be assessed by employing a minimum of 9 tests covering the range of the procedure. In this work, ten spectrophotometric analyses were carried out on the same sample of each type of vinegar to determine the repeatability of the two methods. The Shapiro-Wilk test was employed to prove the continuous variables' normal distribution, and the Huber test to evaluate the random errors (outliers) that deviate from a normal distribution.

The higher repeatability limit for the tested method than the Ripper test, the data normally distributed studied by Shapiro–Wilk, and the absence of outliers measured by the Huber test (Tables 2–6) demonstrated the compliance between the two methods.

The method reproducibility was reported in Table 7.

3.5. Accuracy test

Accuracy measures the agreement of a measurement with a reference value. It was obtained by comparing the measured results with an expected value. In this work, accuracy was determined by making ten analyses with both methods (official and colorimetric). The relative deviation % was calculated to evaluate the error (Tables 8–11).

Table 2. Precision of methods used for SO_2 concentration valuation when white wine vinegar with low SO_2 concentration was tested.

Spectrophotometric method

	Sample
1	15.440
2	14.570
3	15.260
4	14.990
5	15.270
6	15.140
7	13.930
8	16.750
9	14.680
10	16.550

Data number (n)	10
Media (Xm)	15.258
Variance (s_r^2)	0.7317066667
Standard deviation (s_r)	0.855398543
t Student ($\nu = n - 1$; $p = 0.95$)	2.262157163
Coefficient of variation ratio (CV_r%)	5.606229798
Minimum (min)	13.93
Maximum (max)	16.75
Range	2.82
Median	15.2
Media-upper confidence limit ($p = 0.95$)	15.86991525
Media-lower confidence limit ($p = 0.95$)	14.64608475
Media-confidence interval ($p = 0.95$)	0.611915255
Degrees of freedom ($\nu = n - 1$)	9
Method repeatability limit	6.262157163
Normal Distribution (Shapiro-Wilk test 5%)	Yes
Outlier (Huber test 5%)	No

Ripper Schmitt method

	Sample
1	14.080
2	15.360
3	16.640
4	15.360
5	14.080
6	16.640
7	15.00
8	12.00
9	15.360
10	19.00

Data number (n)	10
Media (Xm)	15.352
Variance (s_r^2)	3.469084444
Standard deviation (s_r)	1.862547837
t Student ($\nu = n - 1$; $p = 0.95$)	2.262157163
Coefficient of variation ratio (CV_r%)	12.13228138
Minimum (min)	12
Maximum (max)	19
Range	7
Median	15.36
Media-upper confidence limit ($p = 0.95$)	16.68438646
Media-lower confidence limit ($p = 0.95$)	14.01961354
Media-confidence interval ($p = 0.95$)	1.332386458
Degrees of freedom ($\nu = n - 1$)	9
Method repeatability limit	5.958613384
Normal Distribution (Shapiro-Wilk test 5%)	Yes
Outlier (Huber test 5%)	No

Table 3. Precision of methods used for SO_2 concentration valuation when white wine vinegar with high SO_2 concentration was tested.

Spectrophotometric method

	Sample		
1	57.02	Data number (n)	10
2	54.32	Media (Xm)	53.311
3	50.42	Variance (s_r^2)	6.264498889
4	51.98	Standard deviation (s_r)	2.502898098
5	54.25	*t* Student ν = n − 1; p = 0.95)	2.262157158
6	49.46	Coefficient of variation ratio (CV_r%)	4.694899923
7	53.17	Minimum (min)	49.46
8	51.99	Maximum (max)	57.05
9	57.05	Range	7.59
10	53.45	Median	53.31
		Media-upper confidence limit (p = 0.95)	55.10146544
		Media-lower confidence limit (p = 0.95)	51.52053456
		Media-confidence interval (p = 0.95)	1.790465436
		Degrees of freedom (ν = n − 1)	9
		Method repeatability limit	8.00720404851
		Normal Distribution (Shapiro-Wilk test 5%)	Yes
		Outlier (Huber test 5%)	No

Ripper Schmitt method

	Sample		
1	48.00	Data number (n)	10
2	46.08	Media (Xm)	44.352
3	44.16	Variance (s_r^2)	4.919751111
4	42.88	Standard deviation (s_r)	2.218051197
5	42.24	*t* Student (ν = n − 1; p = 0.95)	2.262157158
6	44.80	Coefficient of variation ratio (CV_r%)	5.001017309
7	41.60	Minimum (min)	41.60
8	44.16	Maximum (max)	48.00
9	42.24	Range	6.40
10	47.36	Median	44.16
		Media-upper confidence limit (p = 0.95)	45.93869824
		Media-lower confidence limit (p = 0.95)	42.76530176
		Media-confidence interval (p = 0.95)	1.586698238
		Degrees of freedom (ν = n − 1)	9
		Method repeatability limit	4.095593024
		Normal Distribution (Shapiro-Wilk test 5%)	Yes
		Outlier (Huber test 5%)	No

Table 4. Precision of methods used for SO_2 concentration valuation when red wine vinegar with low SO_2 concentration was tested.

Spectrophotometric method

	Sample
1	21.02
2	20.89
3	22.07
4	21.45
5	22.25
6	21.31
7	22.14
8	22.98
9	23.05
10	22.87

Data number (n)	10
Media (Xm)	22.004
Variance (s_r^2)	0.65156
Standard deviation (s_r)	0.807192666
t Student ($\nu = n - 1$; $p = 0.95$)	2.262157158
Coefficient of variation ratio (CV_r%)	3.668390592
Minimum (min)	20.89
Maximum (max)	23.05
Range	2.16
Median	22.105
Media-upper confidence limit ($p = 0.95$)	22.58143085
Media-lower confidence limit ($p = 0.95$)	21.42656915
Media-confidence interval ($p = 0.95$)	0.577430847
Degrees of freedom ($\nu = n - 1$)	9
Method repeatability limit	2.582349252
Normal Distribution (Shapiro-Wilk test 5%)	Yes
Outlier (Huber test 5%)	No

Ripper Schmitt method

	Sample
1	29.18
2	28.67
3	27.98
4	28.03
5	28.67
6	28.67
7	28.67
8	29.70
9	28.67
10	29.55

Data number (n)	10
Media (Xm)	28.779
Variance (s_r^2)	0.318032222
Standard deviation (s_r)	0.563943457
t Student ($\nu = n - 1$; $p = 0.95$)	2.262157158
Coefficient of variation ratio (CV_r%)	1.959565852
Minimum (min)	27.98
Maximum (max)	29.70
Range	1.72
Median	28.67
Media-upper confidence limit ($p = 0.95$)	29.18242085
Media-lower confidence limit ($p = 0.95$)	28.37557915
Media-confidence interval ($p = 0.95$)	0.403420845
Degrees of freedom ($\nu = n - 1$)	9
Method repeatability limit	1.804152868
Normal Distribution (Shapiro-Wilk test 5%)	Yes
Outlier (Huber test 5%)	No

Table 5. Precision of methods used for SO_2 concentration valuation when red wine vinegar with high SO_2 concentration was tested.

Spectrophotometric method			
	Sample		
1	90.79	Data number (n)	10
2	97.33	Media (Xm)	94.87
3	98.96	Variance (s_r^2)	19.69057778
4	97.95	Standard deviation (s_r)	4.43740665
5	99.15	t Student ($\nu = n - 1$; $p = 0.95$)	2.262157158
6	99.16	Coefficient of variation ratio (CV_r%)	4.677354959
7	96.36	Minimum (min)	87.39
8	87.39	Maximum (max)	99.16
9	90.38	Range	11.77
10	91.23	Median	96.845
		Media-upper confidence limit ($p = 0.95$)	98.04432949
		Media-lower confidence limit ($p = 0.95$)	91.69567051
		Media-confidence interval ($p = 0.95$)	3.174329485
		Degrees of freedom ($\nu = n - 1$)	9
		Method repeatability limit	18.19603302
		Normal Distribution (Shapiro-Wilk test 5%)	Yes
		Outlier (Huber test 5%)	No
Ripper Schmitt method			
	Sample		
1	96.00	Data number (n)	10
2	99.20	Media (Xm)	92.177
3	95.36	Variance (s_r^2)	31.20502333
4	97.92	Standard deviation (s_r)	5.58614566
5	86.40	t Student ($\nu = n - 1$; $p = 0.95$)	2.262157158
6	86.40	Coefficient of variation ratio (CV_r%)	6.060238085
7	86.40	Minimum (min)	84.48
8	84.48	Maximum (max)	99.20
9	98.36	Range	14.72
10	94.25	Median	94.805
		Media-upper confidence limit ($p = 0.95$)	96.17308787
		Media-lower confidence limit ($p = 0.95$)	88.18091213
		Media-confidence interval ($p = 0.95$)	3.996087867
		Degrees of freedom ($\nu = n - 1$)	9
		Method repeatability limit	17.87104823
		Normal Distribution (Shapiro-Wilk test 5%)	Yes
		Outlier (Huber test 5%)	No

Table 6. Precision of methods used for SO_2 concentration valuation when balsamic wine vinegar with low SO_2 concentration was tested.

Spectrophotometric method

	Sample
1	14.00
2	11.52
3	16.44
4	12.80
5	16.60
6	12.44
7	14.20
8	13.12
9	11.55
10	11.68

Data number (n)	10
Media (Xm)	13.435
Variance (s_r^2)	3.527183333
Standard deviation (s_r)	1.878079693
t Student ($\nu = n - 1$; $p = 0.95$)	2.262157158
Coefficient of variation ratio (CV_r%)	13.97900776
Minimum (min)	11.52
Maximum (max)	16.60
Range	5.08
Median	12.96
Media-upper confidence limit ($p = 0.95$)	14.77849728
Media-lower confidence limit ($p = 0.95$)	12.09150272
Media-confidence interval ($p = 0.95$)	1.343497276
Degrees of freedom ($\nu = n - 1$)	9
Method repeatability limit	6.0083302472
Normal Distribution (Shapiro-Wilk test 5%)	Yes
Outlier (Huber test 5%)	No

Ripper Schmitt method

	Sample
1	10.88
2	9.60
3	12.16
4	10.24
5	10.24
6	8.96
7	7.68
8	9.65
9	8.32
10	8.32

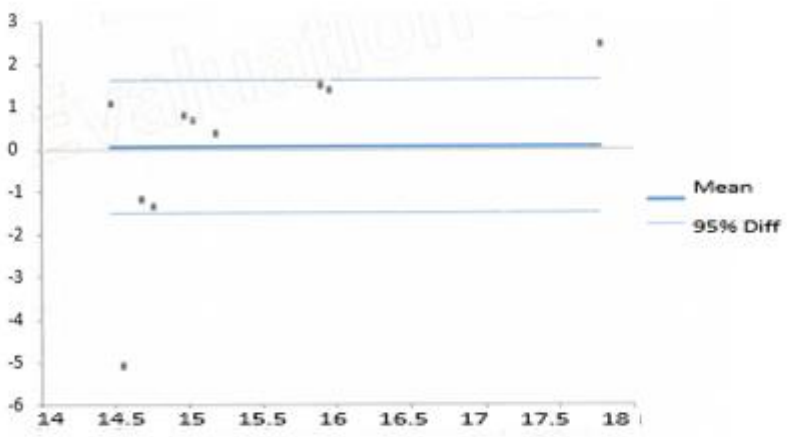
Data number (n)	10
Media (Xm)	9.605
Variance (s_r^2)	1.820694444
Standard deviation (s_r)	1.34933111
t Student ($\nu = n - 1$; $p = 0.95$)	2.262157158
Coefficient of variation ratio (CV_r%)	14.04821562
Minimum (min)	7.68
Maximum (max)	12.16
Range	4.48
Median	9.25
Media-upper confidence limit ($p = 0.95$)	10.57025333
Media-lower confidence limit ($p = 0.95$)	8.639746674
Media-confidence interval ($p = 0.95$)	0.965253326
Degrees of freedom ($\nu = n - 1$)	9
Method repeatability limit	4.316744105
Normal Distribution (Shapiro-Wilk test 5%)	Yes
Outlier (Huber test 5%)	No

Table 7. Method's reproducibility.

Sample	Reproducibility
White wine vinegar with a low SO_2 concentration	$\frac{0.855}{1.86} = 0.460$
White wine vinegar with a high SO_2 concentration	$\frac{2.50}{2.22} = 1.13$
Red wine vinegar with a low SO_2 concentration	$\frac{0.807}{0.560} = 1.43$
Red wine vinegar with a high SO_2 concentration	$\frac{4.44}{5.59} = 0.794$
Balsamic wine vinegar with a low SO_2 concentration	$\frac{1.88}{1.35} = 1.39$
Depending on the degree of freedom (n=9)	
Upper limit of reproducibility = 0.548; Lower limit of reproducibility = 1.480	

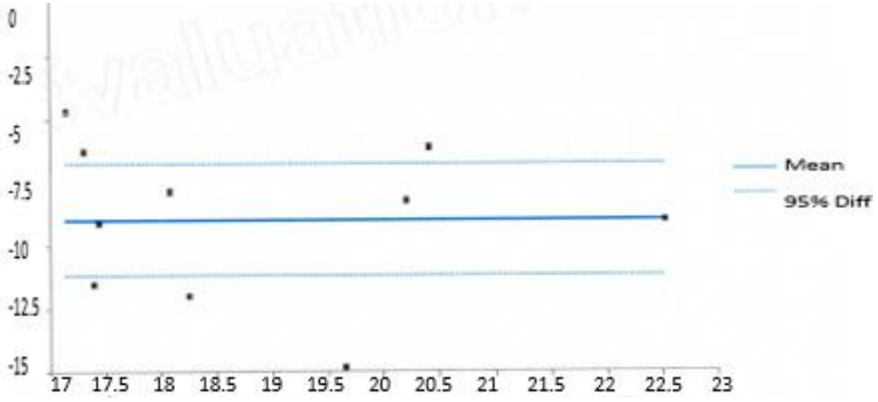
Table 8. Accuracy—white wine vinegar.

With low SO_2 concentration



Student's test	
Hypothesized difference	0
t statistic	0.09
DF	9
p-value	0.9329

With high SO_2 concentration



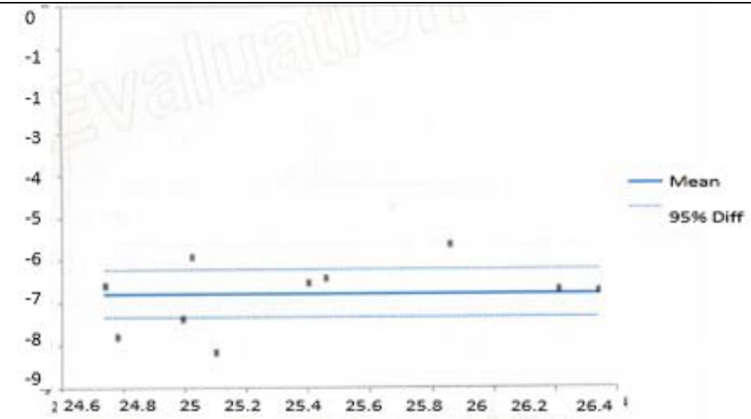
Student's test	
Hypothesized difference	0
t statistic	−9.10
DF	9
p-value	<0.0001

y = concentration official meth/colorimetric method

$x = \frac{\text{concentration official meth/colorimetric meth}}{2}$

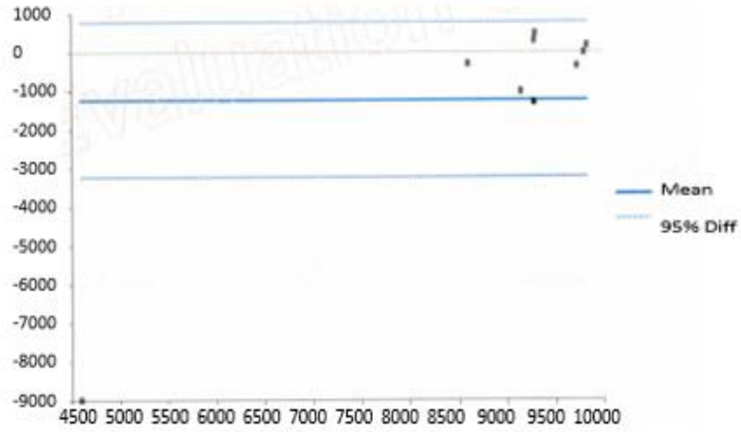
Table 9. Accuracy—red wine vinegar.

With low SO_2 concentration



Student's test	
Hypothesized difference	0
t statistic	−27.22
DF	9
p-value	<0.0001

With high SO_2 concentration



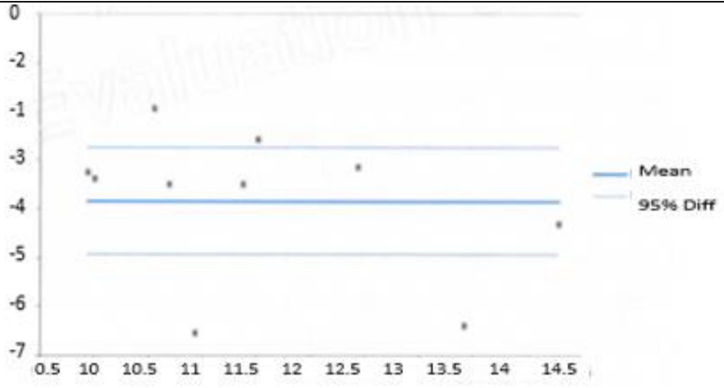
Student's test	
Hypothesized difference	0
t statistic	−1.38
DF	9
p-value	0.2019

y = concentration official method/colorimetric method

$$x = \frac{\text{concentration official method/colorimetric method}}{2}$$

Table 10. Accuracy—balsamic wine vinegar.

With low SO_2 concentration



Student's test	
Hypothesized difference	0
t statistic	−8.04
DF	9
p-value	<0.0001

y = concentration official method/colorimetric method

$$x = \frac{\text{concentration official method/colorimetric method}}{2}$$

Table 11. *t* Student data processing.

Vinegar samples	Average deviation	Average of the averages	Relative deviation %
White wine vinegar with a low SO_2 concentration	0.059	15.32	0.38
White wine vinegar with a high SO_2 concentration	−8.959	48.83	18.34
Redwine vinegar with low SO_2 concentration	6.775	25.39	26.68
Red wine vinegar with a high SO_2 concentration	−2.693	93.52	2.88
Balsamic wine vinegar with a low SO_2 concentration	−4.283	11.29	37.93

Our results showed that the average deviation was very high in samples with low SO_2 concentrations and decreased with high SO_2 concentrations (Table 11), demonstrating the matrix independence and measurement dependence of the systematic errors.

3.6. *Measurement uncertainty*

The measurement uncertainties evaluate the errors associated with a measurement. They affect the accuracy and precision of the measurements. The uncertainties measure is recommended by the international standard ISO/IEC 17025:2017 [25–27]. It gives the analytical procedure quality and supports the interpretation of results [26]. The uncertainties are categorized as Type A if they are measured by the statistical analysis of reiterated measurements (linked to the spread of experimental data) and Type B if they are evaluated by other available information (i.e., instrument specifications, apparatus calibration, etc.) Standard deviation measurements confirmed the type A results' reliability for the number of degrees of freedom considered (Tables 12–16). Also, type B uncertainties were considered irrelevant since they were lower than those from the Ripper test.

4. Conclusions

An automated colorimetric method was validated to determine the SO_2 concentration in vinegar samples, as it could be helpful in the laboratory routine to reduce the analysis time, use of specialized personnel, and analysis costs. The validation was obtained by comparing the colorimetric test with the "Ripper test" (reference test for European legislation).

The test measuring range, sensitivity, and precision complied with those obtained using the Ripper method. The accuracy parameter was not respected in samples containing low dosages of SO_2. The type A and B uncertainties of the rapid analytical method tested were lower than the Ripper method uncertainties. Therefore, this method can be considered reliable for determining SO_2 only in vinegar with a high concentration of SO_2. New studies must be performed to optimize method performance if it is to be used to determine low SO_2 levels in vinegar.

Table 12. Uncertainties-white wine vinegar with low SO_2 concentration.

Type A standard uncertainties					
X	Spectrophotometric method	Ripper-Schmitt method			
1	15.44	14.08			
2	14.57	15.36			
3	15.26	16.64			
4	14.99	15.36			
5	15.27	14.08			
6	15.14	16.64			
7	13.93	15.00			
8	17.10	12.00			
9	14.68	15.36			
10	16.55	19.00			
X_m	15.2930	15.3520			
Y = 47x + 547					
Standard deviation	0.9274	1.8625			
Relative deviation (s_r)	0.0606	0.1213			
Type A $_{\text{uncertainity}} = \sqrt{\frac{variance}{\text{Degrees of freedom}}}$	0.309	0.621			
Type B systematic uncertainties					
Spectrophotometric method					
	U(p)	U(mr)	U(ct)	U(bt)	U(m)
Type B uncertainty Xm		7.500000	1.244771		
Type B uncertainty Xm/radq	0.000000	2.165064	0.359334	0.000000	0.000000
Uncertainty u $(Xm)_{B/Xm}$	0.0000000	0.014434	0.054693	0.0000000	0.0000000
Resulting relative uncertainty u(y)/y	0.08293				
Resulting uncertainty u(y)	1.268				
Coverage factor k (2 < k < 3)	2				
Extended uncertainty U(y)	2.536				
Ripper -Schmitt method					
	U(p)	U(mr)	U(ct)	U(bt)	U(m)
Type B uncertainty Xm	0.050000	0.050000			0.030000
Type B uncertainty Xm/radq	0.014434	0.014434	0.000000	0.000000	0.008660
Uncertainty u $(Xm)_{B/Xm}$	0.002887	0.000940		0.0000000	0.000087
Resulting relative uncertainty u(y)/y	0.12136				
Resulting uncertainty u(y)	1.863				
Coverage factor k (2 < k < 3)	2				
Extended uncertainty U(y)	3.726				

U(p) uncertainties related to accuracy (due to 50 mL pipette use); U(mr) uncertainties related to standard preparation; U(ct) uncertainties related to the calibration curve; U(bt) uncertainties related to balances; U(m) uncertainties related to accuracy (due to burette use).

Table 13. Uncertainties-white wine vinegar with high SO_2 concentration.

Type A standard uncertainties					
X	Spectrophotometric method		Ripper-Schmitt method		
1	57.02		48.00		
2	54.32		46.08		
3	50.42		44.16		
4	51.98		42.88		
5	54.25		42.24		
6	49.46		44.80		
7	53.17		41.60		
8	51.99		44.16		
9	57.05		42.24		
10	53.45		47.36		
X_m	53.3110		44.3520		
Y = 46.6x + 567					
Standard deviation					
Relative deviation (s_r)					
Type B systematic uncertainties					
Spectrophotometric method					
	U(p)	U(mr)	U(ct)	U(bt)	U(m)
Type B uncertainty Xm		0.050000	0.417939		
Type B uncertainty Xm/radq	0.000000	0.014434	0.120649	0.000000	0.000000
Uncertainty u $(Xm)_{B/Xm}$	0.0000000	0.000096	0.120649	0.0000000	0.0000000
Resulting relative uncertainty u(y)/y	0.12946				
Resulting uncertainty u(y)	6.902				
Coverage factor k (2 < k < 3)	2				
Extended uncertainty U(y)	13.803				
Ripper -Schmitt method					
	U(p)	U(mr)	U(ct)	U(bt)	U(m)
Type B uncertainty Xm	0.050000	0.050000			0.030000
Type B uncertainty Xm/radq	0.014434	0.014434	0.000000	0.000000	0.008660
Uncertainty u $(Xm)_{B/Xm}$	0.002887	0.000325		0.0000000	0.000087
Resulting relative uncertainty u(y)/y	0.05009				
Resulting uncertainty u(y)	2.222				
Coverage factor k (2 < k < 3)	2				
Extended uncertainty U(y)	4.444				

U(p) uncertainties related to accuracy (due to 50 mL pipette use); U(mr) uncertainties related to standard preparation; U(ct) uncertainties related to the calibration curve; U(bt) uncertainties related to balances; U(m) uncertainties related to accuracy (due to burette use).

Table 14. Uncertainties-red wine vinegar with low SO_2 concentration.

Type A standard uncertainties					
X	Spectrophotometric method			Ripper-Schmitt method	
1	21.02			29.18	
2	20.89			28.67	
3	22.07			27.98	
4	21.45			28.03	
5	22.25			28.67	
6	21.31			28.67	
7	22.14			28.67	
8	22.98			29.70	
9	23.05			28.67	
10	22.87			29.55	
X_m	22.0040			28.7790	
Y = 47x + 547					
Standard deviation	0.8072			0.5639	
Relative deviation (s_r)	0.367			0.0196	
$\text{Type A}_{\text{uncertainty y}} = \sqrt{\frac{variance}{\text{Degrees of freedom}}}$	0.269			0.188	
Type B systematic uncertainties					
Spectrophotometric method					
	U(p)	U(mr)	U(ct)	U(bt)	U(m)
Type B uncertainty Xm		0.050000	0.417939		
Type B uncertainty Xm/radq	0.000000	0.014434	0.120649	0.000000	0.000000
Uncertainty u $(Xm)_{B/Xm}$	0.0000000	0.000096	0.120649	0.0000000	0.0000000
Resulting relative uncertainty u(y)/y	0.12610				
Resulting uncertainty u(y)	2.775				
Coverage factor k (2 < k < 3)	2				
Extended uncertainty U(y)	5.550				
Ripper -Schmitt method					
	U(p)	U(mr)	U(ct)	U(bt)	U(m)
Type B uncertainty Xm	0.050000	0.050000			0.030000
Type B uncertainty Xm/radq	0.014434	0.014434	0.000000	0.000000	0.008660
Uncertainty u $(Xm)_{B/Xm}$	0.002887	0.000502		0.0000000	0.000087
Resulting relative uncertainty u(y)/y	0.01981				
Resulting uncertainty u(y)	0.570				
Coverage factor k (2 < k < 3)	2				
Extended uncertainty U(y)	1.140				

U(p) uncertainties related to accuracy (due to 50 mL pipette use); U(mr) uncertainties related to standard preparation; U(ct) uncertainties related to the calibration curve; U(bt) uncertainties related to balances; U(m) uncertainties related to accuracy (due to burette use).

Table 15. Uncertainties-red wine vinegar with high SO2 concentration.

Type A standard uncertainties					
X	Spectrophotometric method			Ripper-Schmitt method	
1	90.79			96.00	
2	97.33			99.20	
3	98.96			95.36	
4	97.95			97.92	
5	99.15			86.40	
6	99.16			86.40	
7	96.36			86.40	
8	87.39			84.48	
9	90.38			95.36	
10	91.23			94.25	
X_m	94.8700			92.1770	
Y = 46.6x + 567					
Standard deviation	4.4374			5.5861	
Relative deviation (s_r)	0.0468			0..0606	
Type A $_{\text{uncertainty y}}$ = $\sqrt{\frac{variance}{\text{Degrees of freedom}}}$	1.479			1.862	
Type B systematic uncertainties					
Spectrophotometric method					
	U(p)	U(mr)	U(ct)	U(bt)	U(m)
Type B uncertainty Xm		0.050000	0.417939		
Type B uncertainty Xm/radq	0.000000	0.014434	0.120649	0.000000	0.000000
Uncertainty u (Xm)$_{B/Xm}$	0.0000000	0.00096	0.120649	0.0000000	0.0000000
Resulting relative uncertainty u(y)/y	0.12940				
Resulting uncertainty u(y)	12.276				
Coverage factor k (2 < k < 3)	2				
Extended uncertainty U(y)	24.552				
Ripper -Schmitt method					
	U(p)	U(mr)	U(ct)	U(bt)	U(m)
Type B uncertainty Xm	0.050000	0.050000			0.030000
Type B uncertainty Xm/radq	0.014434	0.014434	0.000000	0.000000	0.008660
Uncertainty u (Xm)$_{B/Xm}$	0.002887	0.000157		0.0000000	0.000087
Resulting relative uncertainty u(y)/y	0.06067				
Resulting uncertainty u(y)	5.593				
Coverage factor k (2 < k < 3)	2				
Extended uncertainty U(y)	11.185				

U(p) uncertainties related to accuracy (due to 50 mL pipette use); U(mr) uncertainties related to standard preparation; U(ct) uncertainties related to the calibration curve; U(bt) uncertainties related to balances; U(m) uncertainties related to accuracy (due to burette use).

Table 16. Uncertainties-balsamic wine vinegar with low SO_2 concentration.

Type A standard uncertainties		
X	Spectrophotometric method	Ripper-Schmitt method
1	14.00	10.88
2	11.52	9.60
3	16.44	12.16
4	12.80	10.24
5	16.60	10.24
6	12.44	8.96
7	14.20	7.68
8	13.12	5.12
9	11.55	8.32
10	11.68	8.32
X_m	13.4350	9.1520
$Y = 77.4x + 552$		
Standard deviation	1.8781	1.9564
Relative deviation (s_r)	0.1398	0.2138
Type A $_{\text{uncertainty y}} = \sqrt{\frac{variance}{\text{Degrees of freedom}}}$	0.626	0.652

Type B systematic uncertainties					
Spectrophotometric method					
	U(p)	U(mr)	U(ct)	U(bt)	U(m)
Type B uncertainty Xm		0.050000	0.000000		
Type B uncertainty Xm/radq	0.000000	0.014434	0.000000	0.000000	0.000000
Uncertainty u $(Xm)_{B/Xm}$	0.0000000	0.000096	0.0000000	0.0000000	0.0000000
Resulting relative uncertainty u(y)/y	0.13979				
Resulting uncertainty u(y)	1.878				
Coverage factor k ($2 < k < 3$)	2				
Extended uncertainty U(y)	3.756				
Ripper -Schmitt method					
	U(p)	U(mr)	U(ct)	U(bt)	U(m)
Type B uncertainty Xm	0.050000	0.050000			0.030000
Type B uncertainty Xm/radq	0.014434	0.014434	0.000000	0.000000	0.008660
Uncertainty u $(Xm)_{B/Xm}$	0.002887	0.001577		0.0000000	0.000087
Resulting relative uncertainty u(y)/y	0.21379				
Resulting uncertainty u(y)	1.957				
Coverage factor k ($2 < k < 3$)	2				

U(p) uncertainties related to accuracy (due to 50 mL pipette use); U(mr) uncertainties related to standard preparation; U(ct) uncertainties related to the calibration curve; U(bt) uncertainties related to balances; U(m) uncertainties related to accuracy (due to burette use).

Conflict of interest

The authors declare no conflict of interest.

Supplementary

Table S1. Concentrations used to develop the calibration curve.

	Standard	X (g/L)	Y_{found}	$Y_{calculated}$
High and low concentration red and white and high concentration balsamic wine vinegars (mg/L)	1	19	1451.6008	1450
	2	38	2319.5044	2340
	3	75	4022.3055	4060
	4	150	7598.7301	7560
	1	19	1483.0711	1450
	2	38	2323.9193	2340
	3	75	4057.2913	4060
	4	150	7559.4303	7560
	1	19	1474.3658	1450
	2	38	2361.0670	2340
	3	75	4037.2093	4060
	4	150	7549.0236	7560
Low concentration balsamic wine vinegar (mg/L)	1	1.88	708.8872	698
	2	3.75	845.173	843
	3	7.5	1100.3899	1130
	4	15	1726.0638	1710
	1	1.88	717.8597	698
	2	3.75	846.4987	843
	3	7.5	1100.8734	1130
	4	15	1726.8831	1710
	1	1.88	717.468	698
	2	3.75	841.4904	843
	3	7.5	1100.2545	1130
	4	15	1728.1739	1710

Table S2. Residual probability.

High and low concentration red and white and high concentration balsamic wine vinegars					
x	h_i leverages coefficient	e_i absolute residuals	e_{Ni} normalized residuals	e_{Si} studentized residuals	e_{ji} standardized residuals
19.0	0.171	−1.31	−0.051	−0.056	−0.054
38.0	0.118	−18.9	−0.742	−0.790	−0.774
75.0	0.084	40.5	−1.590	−1.661	−1.852
150	0.293	40.4	1.584	1.884	2.226
19.0	0.171	30.2	1.182	1.299	1.351
38.0	0.118	−14.5	−0.569	−0.606	−0.585
75.0	0.084	−5.56	−0.218	−0.228	−0.217
150	0.293	1.12	0.044	0.052	0.049
19.0	0.171	21.5	0.841	0.924	0.917
38.0	0.118	22.6	0.888	0.945	0.90
75.0	0.084	−25.6	−1.005	−1.050	−1.057
150	0.293	−9.29	−0.364	−0.433	−0.415

Low concentration balsamic wine vinegar					
x	h_i leverages coefficient	e_i absolute residuals	e_{Ni} normalized residuals	e_{Si} studentized residuals	e_{ji} standardized residuals
1.88	0.171	11.1	0.515	0.565	0.545
3.75	0.119	2.61	0.121	0.129	0.123
750	0.084	−32.5	−1.509	−1.576	−1.725
15.0	0.293	12.6	0.5186	0.697	0.678
1.88	0.171	20.1	0.932	1.023	1.026
3.75	0.119	3.93	0.183	0.195	0.185
7.50	0.84	−32.0	−1.486	−1.553	−1.691
15.0	0.293	13.4	0.625	0.743	0.725
1.88	0.171	19.7	0.914	1.003	1.004
3.75	0.119	−1.08	−0.050	−0.053	−0.050
7.50	0.084	−32.6	−1.515	−1.583	−1.735
15.0	0.293	14.7	0.685	0.814	0.799

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