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Determination of Sulfites by UV-Vis Spectrophotometry in Red Wine and White Wine

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Abstract

Sulphites are natural or synthetic additives widely used by the food industries. They are used to improve and preserve the stability and quality of the organoleptic characteristics of food. Due to potential serious cases of hypersensitivity beyond a threshold, the presence and content of sulphites must be monitored in food, and more particularly in wine, in order to thus preserve the health of consumers. To do this, it is essential to develop reliable methods for the quantification of sulphites in wine and this is the subject of this study. UV-Visible spectrophotometry programmed at 565nm was used to determine the sulfite concentrations (µg/mL) in a sample of commercially purchased white and red wine. The results showed that the method used was linear (range of linearity ranging from 0.05 µg/mL to 5 µg/mL with a coefficient of determination of R^2 = 0.9969), accurate (CV ranging from 0.823% to 1.976% for intra-day precision and 1.229 to 1.487 for inter-day precision) and accurate (average recovery rate of 98.59%) in accordance with Agency guidelines French Standardization. The application of this method for the determination of sulphites revealed respective levels of 57.271 mg/L and 136.063 mg/L for red wine and white wine, levels in accordance with European regulations which set the maximum limit at 150 mg /L for red wine and 200 mg/L for white wine. This method could therefore make it possible to determine the quality of wines on the market and prevent the harmful effects of sulphites on the health of consumers.

Keywords*: sulphites, white wine, red wine, dosage, UV-visible spectrophotometry.*

Introduction

Food additives are substances added to preserve or improve the safety, freshness, taste, texture or appearance of food (Joint FAO/WHO Codex Alimentarius Commission, 2018). Among these substances, sulfur dioxide and its salts occupy a prominent place in the industry due to their broad application. They are added to a wide range of food products such as fruit juices, fruit and vegetables, pastries, seafood or wine (S. L. Taylor et al, 1986). However, since the 1980s, serious cases of hypersensitivity have been reported in individuals following consumption of sulfite-treated foods (R Carballo et al, 2003). The US Food and Drug Administration then required the presence of sulfites to be mentioned on labels for concentrations greater than or equal to 10 mg/kg or 10mg/L (Food and drug administration, 2018). This initiative is followed by the European Union, which even requires limit values for sulfites in different types of food (European Commission, 2002). So, in the particular case of the wine industry, where the taste, color and stability of wine depend on the presence of sulfites (S. L. Taylor et al, 1986), it is vital to develop sensitive and reliable methods for monitoring sulfites in wine.
The aim of the present work is to develop an effective method for the determination of sulfites in wine for the food industry.

Materials And Methods

Materials

Sampling
The wine samples (red and white) chosen for this study were 750mL bottles of wine with the words "CONTAINS SULFITES" written on their packaging. These wine samples came from the same manufacturer. The samples were coded as follows: VR for red wine and VB for white wine.

Reference substance and chemicals used
Sodium bisulfite with a purity of 92% and a dosage of 1000mg from the manufacturer Acros Organics, batch number RK-88229-25, was used as the reference substance.
Tous les produits chimiques utilisés étaient de qualité analytique : EDTA (100%, SIGMA) ; Chlorure de para rosaniline (90%, Alfa Aesar) ; Acide chlorhydrique (37%, Carlo Erba) ; Solution de formaldéhyde 0,1% (35%, officinale).
Distilled water was used to prepare the various reagents.

Equipment

We used a DENVER INSTRUMENT SI 403 analytical balance, accurate to 0.0001g, for weighing, a NEUATION TD1605AB1350 vortex mixer for mixing solutions, and a spectrophotometer (JENWAY 7315) combined with a desktop computer running the relevant software driver for the determination of sulfites in wine.

Methods

Preparation of the reference substance (RS)

A mass of 0.1 g of sodium bisulfite solution powder was taken into a 100 mL volumetric flask and dissolved with distilled water up to the mark.

Preparation of sample solutions

10 mL of wine was withdrawn and inverted into a beaker into which 30 mL of distilled water was added. The filtrate obtained from the mixture (wine and distilled water) was made up to 100 mL with distilled water and measured according to the modified para rosaniline-formaldehyde variant protocol (Yongjie Li and Meiping Zhao. 2006). According to this protocol, 2.5 mL of the para rosaniline-formaldehyde mixture was placed in a 10mL test tube, then 2.5 mL of SR or 2.5mL of wine sample was added. The resulting mixture was thoroughly homogenized by vortexing.

Preparation of working solvents

A 0.001mol/L EDTA solution was obtained by dissolving 0.19 g EDTA powder in a 500 mL volumetric flask of distilled water.

Para-rosaniline Chloride solution of concentration 0.001g/mL was prepared by dissolving 1g of para-rosaniline chloride powder in 10mL of absolute ethanol contained in a 100mL volumetric flask, then distilled water was added up to the mark.

Formaldehyde Solution 0.1% (1.2 mol/L) was obtained by diluting a 0.71mL volume of the 37% purity formaldehyde solution in a 250mL volumetric flask.

Storage of SR and working solvents

SR and the para rosaniline-formaldehyde mixture solution are stored at 4°C. The other solutions are stored at room temperature.

Determination of validation parameters

These parameters (linearity, precision, accuracy) were determined in accordance with the guidelines of the Agence Française de Normalisation (AFNOR) in standard NF V 03-110 (Norme NF V03-110, AFNOR, 2010), which covers assay methods in the food industry.

Linearity

Linearity was assessed by preparing a standard range from 0.05 µg. mL-1 to 5 µg. mL-1 by successive dilutions of the reference solution (SR) with respective intermediate concentrations of 0.05; 1.25; 1.67; 2.5; 3.33 and 5µg. mL-1 (ICH Harmonised Tripartite Guideline. 2005). The calibration curve was established from the absorbances obtained for each concentration, then the coefficient of determination (R2) and the equation of the line were deduced. However, before determining linearity, a spectral scan (from 400 to 700 nm) was performed to determine the wavelength of maximum absorption λmax (Figure 1) and plot the calibration curve (Figure 2).

Reliability

Intra-day precision or repeatability was determined by taking eighteen readings for each chosen concentration level (1.25 µg. mL-1 ; 1.67 µg. mL-1 and 2.5 µg. mL-1), i.e. three series of six successive readings, each concentration level having been prepared six times. Spectrophotometer readings were taken on the same day, with a four-hour interval between them.

Intermediate or inter-day precision was determined by performing eighteen readings (i.e. three series of six successive readings) of three respective concentration levels (1.25 µg. mL-1 ; 1.67 µg. mL-1 and 2.5 µg. mL-1), on the spectrophotometer over three successive days by the same operator.

The mean absorbance of the three series of readings and the coefficients of variation (CV) were calculated for each test, and the data reported in Tables I and II.

Accuracy

The accuracy test was carried out by adding a 1 mL volume of the reference solution to a 1 mL volume of the unfortified sample contained in a 10 volumetric flask. The mixture was made up to the mark with distilled water (1/10 dilution). The absorbances obtained after reading were converted to µg. mL-1 using the straight line equation. Recovery percentages (PR) were calculated according to the following formula (ICH Harmonised Tripartite Guideline. 2005) (Eq.1):

$$PR=(QF-QI)/QA\times100 \quad \text{(Eq.1)}$$

Where PR represents the Recovery Percentage; QF, QI and QA represent the Fortified, Initial and Added Quantities respectively.

Fortified Quantities (QF) are determined from the absorbances obtained and the equation line. By multiplying the concentration X (obtained from the equation line) by the dilution factor α = 10, we obtain QF.

Determining sulfite levels in wines

The average absorbances for each wine sample were used to determine the molar sulfite concentrations of each preparation (C1) from the equation of the straight line: y = ax +b C1=x=(y-b)/a (Eq.2).

Results And Discussion

Validation of the method for determining sulfites in wine

Before studying the validation parameters, we determined the absorption spectrum of sulfites by performing a spectral scan in the UV-visible range from 400 nm to 700 nm. This spectral scan enabled us to detect the maximum absorption wavelength λmax of sulfites, which is 565 nm as shown in Figure 1. This result is different from that obtained by Yongjie Li and Meiping Zhao (2006), who found a wavelength of 575 nm. This difference could be explained by the absence of a spectral range to detect maximum absorbance.

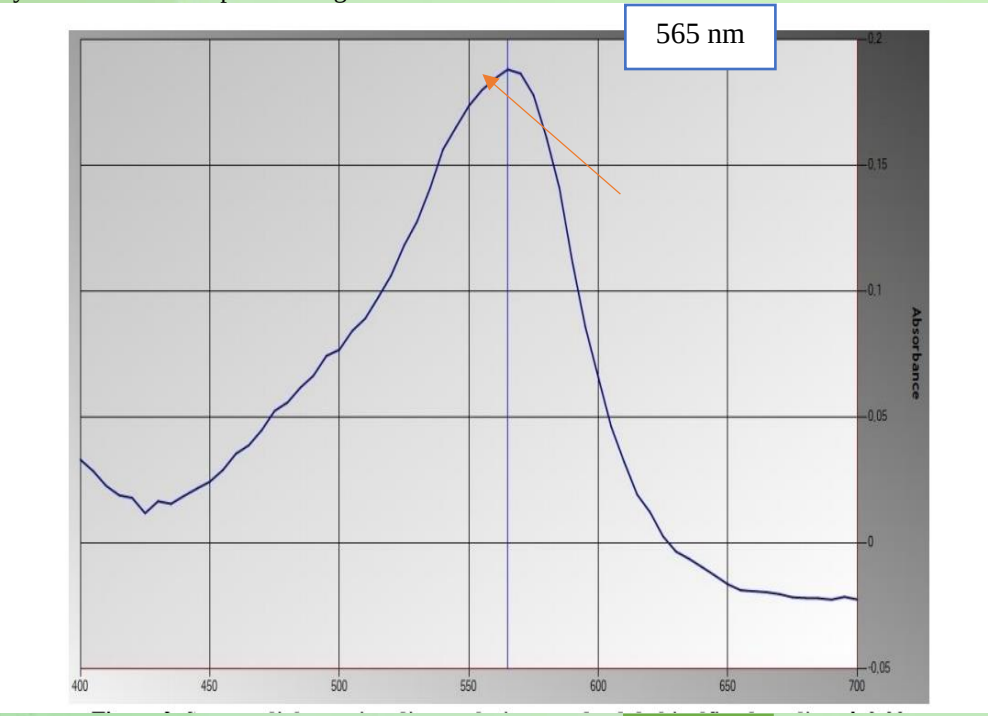

Figure 1. Absorption spectrum of sulfite n

Spectral data from the standard solution were used to plot the sulfi curve at 565 nm (Figure 2).

565nm

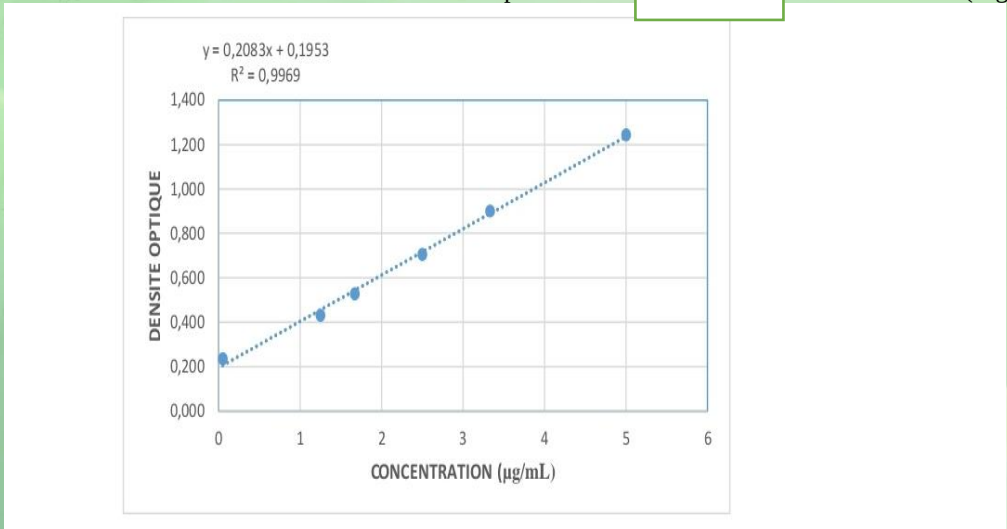

Figure 2. Calibration curve for sulfites at 565 nm

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The linearity of the method was determined by assessing the coefficient of determination R2 obtained from the calibration curve. Our result complies with Pharmacopoeia USP 38 NF 33 version 2015 (USP 38 - NF 33, Version 2015), which states that the coefficient of determination must be greater than 0.9950. The coefficient of determination of our study, which was 0.9969, is significantly close to 0.9993 obtained by Yongjie Li and Meiping Zhao (2006).
The fidelity of our method gave CVs of 0.823%; 0.953% and 1.976% at 8, 12 and 16 hours respectively for intra-day fidelity and 1.430%; 1.229% and 1.487% for inter-day fidelity. Our method complies with the CVs given by Pharmacopoeia USP 38 NF 33 version 2015 (USP 38 - NF 33, Version 2015), which stipulates that the CVs for intra-day and inter-day fidelity must be less than 2% respectively. Our method is therefore accurate.

Table 1. Repeatability (intra-day precision) of analysis by the modified method

Concentrations (µg/mL)	Optical density	Mean	Standard deviation	Coefficient of variation (%)
2,5	0,696	0,699	0,00471	0,823
	0,705			
	0,703			
	0,692			
	0,694			
	0,702			
1,67	0,535	0,530	0,00406	0,953
	0,537			
	0,528			
	0,527			
	0,530			
	0,524			
1,25	0,439	0,4242	0,00417	1,976
	0,415			
	0,425			
	0,419			
	0,427			
	0,420			

Table 2. Intermediate repeatability (inter-day reliability) of analysis using the modified method

Concentration (µg/mL)	Day 1	Day 2	Day 3	Coefficient of variation (%)
2,5	0,696	0,687	0,691	1,430
	0,705	0,685	0,688	
	0,703	0,688	0,668	
	0,692	0,689	0,689	
	0,694	0,698	0,670	
	0,702	0,687	0,668	
1,67	0,535	0,522	0,534	1,229
	0,537	0,518	0,520	
	0,528	0,511	0,521	
	0,527	0,517	0,520	
	0,530	0,521	0,515	
	0,524	0,516	0,523	
1,25	0,439	0,436	0,468	1,487
	0,415	0,432	0,429	
	0,425	0,427	0,412	
	0,419	0,430	0,442	
	0,427	0,436	0,442	
	0,420	0,436	0,427	

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Accuracy gave an average recovery rate of 98.59%, in line with the specifications given by Pharmacopoeia USP 38 NF 33 version 2015 (USP 38 - NF 33, Version 2015), which recommends that the average recovery rate be contained within the acceptability range of 98% to 102%. Our method is therefore accurate. The 98.59% rate obtained in our study is also close to the 98% rate obtained by Yongjie Li and Meiping Zhao (2006).
The results of the validation criteria obtained justify the application of this method to the quantitative determination of sulfites in wine.

Determination of sulfite levels in wines

Sulfite levels in 750 mL bottles of red and white wine were 57.271 mg. L-1 and 136.063 mg. L-1. These concentrations were in line with standards, as European regulations (Council Regulation (EC) No. 1493/1999) set the maximum limit for sulfite concentration at 150 mg. L-1 for red wines and 200 mg. L-1 for white wines.
However, a concentration of 12.9 mg. L-1, lower than those found in our study, was determined by Yongjie Li and Meiping Zhao (2006). This discrepancy could be explained by the fact that different food samples were used. Indeed, Yongjie Li and Meiping Zhao, (2006) used preserved almond (solid food) whereas we used red and white wine (liquid food).

Conclusion

At the end of this study, the aim of which was to develop an analytical method for the determination of sulfites in wine, we concluded that the modified para rosaniline-formaldehyde method of determination by UV-VISIBLE spectrophotometry is reliable, with satisfactory validation criteria. It can be validly used for routine control of sulfites in wine. The sulfite concentration obtained complies with European regulations for wine products. However, short- and long-term studies of sulfite stability in wine will be required.

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