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Development of method for the speciation of inorganic iron in wine samples

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ARTICLE INFO

Article history:
Received 24 June 2007
Received in revised form
12 August 2007
Accepted 3 September 2007
Published on line 5 September 2007

Keywords:
Speciation analysis
Iron(II) determination
Total iron determination
Wine samples
Box Behnken design

ABSTRACT

In this paper, we proposed a procedure for the determination of iron(II) and total iron in wine samples employing molecular absorption spectrophotometry. The ligand used is 2-(5-bromo-2-pyridylazo)-5-(diethylamino)-phenol (Br-PADAP) and the chromogenic reaction in absence or presence of ascorbic acid (reducing agent) allows the determination of iron(II) or total iron, respectively. The optimization step was performed using a multivariate technique (Box Behnken design) involving the factors pH, acid ascorbic concentration and reaction time.

The method allows the determination of iron(II) and iron(III) in wine samples, with limits of detection and quantification 0.22 and 0.72 $\mu g\,L^{-1}$, respectively. The precision expressed as relative standard deviation (R.S.D.) was 1.43 and 0.56% (both, $n = 11$) for content of iron(II) in wine samples of 1.68 and 4.65 $mg\,L^{-1}$, and 1.66 and 0.87% (both, $n = 11$) for content of total iron in wine samples of 1.72 and 5.48 $mg\,L^{-1}$.

This method was applied for determination of iron(II) and total iron in six different wine samples. In these, the iron(II) content varied from 0.76 to 4.65 $mg\,L^{-1}$ and from 1.01 to 5.48 $mg\,L^{-1}$ for total iron. The results obtained in the determination of total iron by Br-PADAP method were compared with those that were performed after complete acid digestion in open system and determination of total iron employing FAAS. The method of regression linear was used for comparison of these results and demonstrated that there is no significant difference between the results obtained with these two procedures.

1. Introduction

Iron is a mineral element present in wine in the concentration range 0.5–25.0 $mg\,L^{-1}$ and is important to the wine technologist because it may cause cloudiness or a colour change when present in a large excess [1–3]. If wine is kept under airtight conditions, iron, being in a reducing medium, exists exclusively as iron(II) and is soluble even if present in large amounts [4]. However, when wine is aerated, the dissolved oxygen oxidizes the iron(II) to iron(III), which is responsible for the precipitation of colouring matter (blue casse) and for the cloudiness in white wines (white casse). Cacho et al. studied the influence of iron, copper, and manganese on wine oxidation, measuring the evolution of different compounds sensitive to this process, such as: anthocyanins, tannins, total phenol content and acetaldehyde. They concluded that the oxidation depends directly of the concentration of these cations in the wine [5]. Oszmianski et al. studied the oxidation process of (+)-catechin in wine-like model solutions in presence of iron(II) ions. They observed that the rate of cat-

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doi:10.1016/j.aca.2007.09.002

echin oxidation and the extent of browning increase with the iron level. Colourless compounds and yellow pigments are formed in larger amounts at higher iron concentrations [6].

This way, methods for iron speciation in wines are opportune and several procedures have been performed for the determination of iron species in wine samples [7–15]. Valcarcel and co-workers [7] performed an on-line system for the determination of iron in wine, based on the ferro(III)-thiocyanate complex. Wang and Mannino [8] established a method employing adsorptive stripping voltammetry for the determination of iron(III) and total iron in wines. Ajlec and Stupar [9] performed a method using ion exchange chromatography and flame atomic absorption spectrometry (FAAS) for the determination of iron(II) and iron(III) in wine. Weber [10] used a high-performance liquid chromatography (HPLC) system coupled with an electrochemical detector for the determination of iron(II) and another on-line system employing FAAS for the determination of total iron in wine. Perez-Conde and co-workers [11] proposed a flow-through fluorescent sensor for the determination of iron(III) and total inorganic iron in wine. Costa and Araujo [12] proposed a sequential method for the determination of iron(III) and total iron in Portuguese table wines by sequential injection analysis (SIA) employing FAAS. Paleologos et al. [13] performed a procedure for the determination of free and bound iron in wines using cloud point extraction and FAAS. Riganakos and Veltsistas [14] compared two spectrophotometric procedures proposed for the determination of the total iron wines. Stafilov and co-workers [15] proposed methods for the determination of iron(II), iron(III) and organically bounded iron in wines using for separation liquid–liquid and solid-phase extraction.

The iron(II) reacts with 2-(5-bromo-2-pyridylazo)-5-(diethylamino)-phenol (Br-PADAP) forming a complex, which has absorption peaks at 560 and 748 nm [16,17]. The absorption at 748 nm is lower than at 560 nm, however, at 748 nm the method is completely selective for iron(II). Br-PADAP reacts also with iron(III) forming a complex that has an absorption peak at 560 nm. Both complexes are stable and some procedures have been proposed involving separation and determination of iron(II) and iron(III) using this reagent [18–20]. Oszwaldowski et al. performed procedures for the separation of complexes of iron(II) and iron(III) with Br-PADAP employing micellar electrokinetic chromatography [18] and capillary electrophoresis [19]. A method using Br-PADAP was developed for the simultaneous determination of iron(III) and iron(II) in water samples employing reverse-phase liquid chromatography for the separation of the complexes [20]. Wang and Song [21] used Br-PADAP in method proposed for spectrophotometric determination of iron(III) in irrigation water.

Box Behnken design is a chemometric tool used for optimization of experimental conditions [22–24]. However, the use of this design is still few widespread in analytical chemistry. In recent years, several analytical methods have been optimized using this tool [25–27].

In the present paper, we proposed a procedure for the speciation analysis of inorganic iron in wine. It is based on the reactions of iron(II) with Br-PADAP in the absence and also presence of a reducing agent (ascorbic acid) for the determination of iron(II) and total iron, respectively. The optimization step was performed using a Box Behnken design. The method was applied for the determination of iron(II) and total iron in several wine samples.

2. Experimental

2.1. Instrumentation

Spectrophotometric measurements were made in a Cary 5E UV-visible Spectrophotometer (Varian) with 1.00-cm glass cells.

A Varian model SpectrAA 220 FS flame atomic absorption spectrometer (Mulgrave, Vistoria, Australia), equipped with a deuterium background corrector and automatic switching of hollow-cathode lamps was used. The iron hollow cathode lamp was operated with a current of 5.0 mA. The analytical wavelength at 248.3 nm was used with a spectral bandwidth of 0.1 nm. An air–acetylene flame was used with an acetylene flow rate of 2.0 L min^{-1}, an airflow rate of 13.5 L min^{-1} and a burner height of 13.5 mm.

2.2. Reagents

All reagents were of analytical grade unless otherwise stated.

Ultrapure water was obtained from an EASYpure RF purification system (Barnstedt, Dubuque, IA, USA). Nitric and hydrochloric acid were of Suprapur® quality (Merck, Darmstadt, Germany). Nitric and hydrochloric acid solution were prepared by direct dilution with water from the concentrated Suprapur® solutions.

Laboratory glassware was kept overnight in 10% (v/v) nitric acid solution. Before use, the glassware was rinsed with desmineralized water and dried in a dust-free environment.

Calibration solutions were prepared from 1000 mg L^{-1} iron stock solutions (Merck) by appropriate dilution with a 1% (v/v) nitric acid solution.

2.3. Reagents

2.3.1. Iron (II) solutions

Stock solution (0.50 g L^{-1}) of iron(II) was prepared by dissolving $Fe(NH_4)_2(SO_4)_2{\cdot}6H_2O$ (Merck) in 2% (v/v) hydrochloric acid solution (boiled recently). Working standard solutions were prepared daily by stepwise dilution of the stock solution with desmineralized water also boiled recently.

2.3.2. Br-PADAP solution

A 0.05% Br-PADAP solution was prepared by dissolving 0.05 g of Br-PADAP in 3.2 g of Triton X-100 in 10 mL of ethanol and diluting to volume with ethanol in a 100 mL volumetric flask.

Acetate buffer solution (pH 5.5) was prepared by dissolving 74.54 g of sodium acetate (Merck) and 5.2 mL concentrated acetic acid (Merck) with desmineralized water and diluting to 1 L.

Ascorbic acid solution (0.10%) was prepared daily by dissolution of this reagent in water.

Table 1 – Box Behnken design with real and coded values

Experiment	pH	AA concentration (mg mL^{-1})	Reaction time (min)	ABS
1	4 (−1)	20 (−1)	10 (0)	0.17526
2	6 (+1)	20 (−1)	10 (0)	0.18016
3	4 (−1)	60 (+1)	10 (0)	0.17154
4	6 (+1)	60 (+1)	10 (0)	0.17347
5	4 (−1)	40 (0)	5 (−1)	0.17582
6	6 (+1)	40 (0)	5 (−1)	0.18135
7	4 (−1)	40 (0)	15 (+1)	0.17570
8	6 (+1)	40 (0)	15 (+1)	0.17875
9	5 (0)	20 (−1)	5 (−1)	0.17358
10	5 (0)	60 (+1)	5 (−1)	0.17618
11	5 (0)	20 (−1)	15 (+1)	0.17332
12	5 (0)	60 (+1)	15 (+1)	0.17661
13	5 (0)	40 (0)	10 (0)	0.18227
14	5 (0)	40 (0)	10 (0)	0.18374
15	5 (0)	40 (0)	10 (0)	0.17924

Optimization of factors pH, ascorbic acid concentration and reaction time in the reaction of iron(II) with Br-PADAP.
AA, ascorbic acid.

2.4. *Procedure for determination of iron(II) in wine*

Transfer a sample volume containing iron(II) in the range of 1–25 μg to a 25 mL standard flask, add 2.0 mL of a 0.05% solution of Br-PADAP and 2 mL of acetate buffer solution (pH 5.5). Dilute to the mark with water, mix, and after 10 min, measure the absorbance at 748 nm, versus a blank solution. Determine the iron content considering the analytical curve established using standard solutions of iron using ascorbic acid for reduction of iron.

2.5. *Procedure for determination of total iron in wine by Br-PADAP method*

Transfer a sample volume containing iron in the range of 1–25 μg to a 25 mL standard flask, add 1.0 mL of a 0.10% solution of ascorbic acid, 2.0 mL of a 0.05% solution of Br-PADAP and 2 mL of acetate buffer solution (pH 5.5). Dilute to the mark with water, mix, and after 10 min, measure the absorbance at 748 nm, versus a blank solution.

2.6. *Procedure for determination of total iron in wine by FAAS method*

Transfer a sample volume of 10.0 mL to a 125-mL Erlenmeyer flask and add 3.0 mL of concentrated nitric acid and 5 mL of 30% (v/v) hydrogen peroxide. Heat and evaporate to dryness on a hot-plate and after dissolve the residue using 0.5 mol L^{-1} nitric acid solution. Transfer to a 25-mL volumetric flask and dilute to volume with 0.5 mol L^{-1} nitric acid solution. Determine the iron in the diluted solutions by FAAS.

3. Results and discussion

3.1. *Optimization of the experimental conditions of the method*

The reaction of iron(II) with Br-PADAP in aqueous media have been studied by several authors, and the experimental conditions are very established [16,17]. However, in this work, the experimental factors: pH, ascorbic acid concentration and reaction time were re-evaluated considering that wine is an organic matrix very complex [28–31]. Then, a re-optimization of these parameters was performed employing a Box Behnken design [22–27]. All the experiments were performed using a volume of 2 mL of a red wine sample containing total iron with a concentration of 4.10 mg L^{-1} and final dilution for 25 mL. The matrix with coded and real values and the responses as analytical signal (absorbance at 748 nm) are shown in Table 1. Replicates of the central point were performed for evaluation of the experimental error. All the experiments were carried out in random order. Experimental data were processed using a statistical program [32].

The Eq. (1) illustrates the relation between pH, ascorbic acid concentration (AAC), reaction time (time) and the analytical signal (AS), considering the real values:

$$\begin{aligned}\text{Analytical signal} = {} & 0.087891 + 0.022951(\text{pH}) - 0.001830(\text{pH})^2 \\ & +0.001103(\text{AAC}) - 0.000012(\text{AAC})^2 \\ & +0.002099(\text{time}) - 0.000081(\text{time})^2 \\ & -0.000037(\text{pH})(\text{AAC}) - 0.000124(\text{pH})(\text{time}) \\ & +0.000002(\text{AAC})(\text{time}) \qquad (1)\end{aligned}$$

This equation shows a critical point in the surface response, which is a maximum for the pH of (5.57), ascor-

Table 2 – Linear regression equations obtained for the determination of iron(II) in aqueous media and in the presence of four different wine samples

System	Linear regression equations
Aqueous media	$Abs = 0.5431.C_{Fe} + 0.0002$ $r = 0.9999$
Dry red wine	$Abs = 0.5446.C_{Fe} + 0.1008$ $r = 0.9986$
Sweet red wine	$Abs = 0.5398.C_{Fe} + 0.0897$ $r = 0.9989$
Sweet white wine	$Abs = 0.5435.C_{Fe} + 0.0611$ $r = 0.9991$
Dry white wine	$Abs = 0.5359.C_{Fe} + 0.0251$ $r = 0.9989$

Table 3 – Determination of iron(II) and total iron in commercial wine samples

Wines	Iron(II) found Br-PADAP method ($mg\,L^{-1}$)	Total iron found Br-PADAP method ($mg\,L^{-1}$)	Total iron found FAAS method ($mg\,L^{-1}$)
Dry red	2.10 ± 0.16	2.40 ± 0.10	2.33 ± 0.06
Sweet red	4.65 ± 0.012	5.48 ± 0.002	5.62 ± 0.29
Sweet red	4.60 ± 0.004	4.75 ± 0.06	4.78 ± 0.04
Dry white	0.56 ± 0.01	1.01 ± 0.04	0.97 ± 0.16
Dry white	1.68 ± 0.013	1.72 ± 0.03	1.76 ± 0.09
Sweet white	2.76 ± 0.04	3.01 ± 0.25	2.84 ± 0.04

bic acid concentration ($37.87\,mg\,mL^{-1}$) and reaction time (9.14 min). The way of calculating these critical points has been published in previous papers [33,34]. This way, the experimental conditions established for the method are: pH 5.5, acid ascorbic concentration $40.0\,mg\,mL^{-1}$ and reaction time of 10 min.

3.2. *Analytical characteristics*

Analytical curves employing the procedure proposed for total iron were established by spiking four wine samples with increasing concentration of iron. All the results obtained in this experiment are summarized in Table 2. An evaluation of these results demonstrated that the slope of the analytical curve for iron obtained by the standard calibration technique is quite comparable with the slopes of the analytical curves obtained in the presence of the four different wines. This experiment demonstrate that iron(II) and total iron using the reducing agent (ascorbic acid) can be determinate in wines employing the standard calibration technique.

Metal ions as copper(II), zinc(II), manganese(II) and others, which also react with Br-PADAP and that could be present in wine samples do not interfere because the analytical measure is done at 748 nm and the Br-PADAP complexes of these metal ions have maximum absorption at 560 nm.

3.3. *Validation studies*

The limits of detection ($3\sigma/S$) and quantification ($10\sigma/S$), where (3σ) is standard deviation of the blank and (S) slope of analytical curve, were calculated as IUPAC recommendation [35]. These are 0.22 and $0.72\,\mu g\,L^{-1}$, respectively. The precision expressed as relative standard deviation (R.S.D.) was 1.43 and 0.56% (both, $n = 11$) for content of iron(II) in wine samples of 1.68 and $4.65\,mg\,L^{-1}$, and 1.66 and 0.87% for content of total iron in wine samples of 1.72 and $5.48\,mg\,L^{-1}$.

3.4. *Application of the method*

The proposed method has been applied for the determination of iron(II) and total iron in six different Brazilian wine samples bought in supermarkets in Salvador City, Brazil. In these, the iron(II) content varied from 0.76 to $4.65\,mg\,L^{-1}$ and from 1.01 to $5.48\,mg\,L^{-1}$ for total iron. Considering the absence of a certified reference material of wine for the evaluation of the accuracy of the method proposed for the determination of total iron, the same wine samples were also analyzed by FAAS method after complete acid digestion in open system. All the results are summarized in Table 3. The regression linear method was used for comparison of the results obtained [36]. This analysis demonstrated that the relationship established between the results obtained employing these methods is:

$$[\mathrm{FAAS}] = 1.0312 \pm 0.0730\ [\mathrm{Br-PADAP}] - 0.1072 \pm 0.2519\ (R^2 = 0.9974)$$

This equation demonstrates that the calculated slope and intercept do not differ significantly from the "ideal" values of 1 and 0, respectively, and that there is no evidence for systematic difference between these two methods [36].

4. Conclusions

The method proposed for inorganic iron speciation has precision and accuracy satisfactory and can be applied for the iron quantification in white and red wines. It is very simple compared with other published, which require separation procedures involving ion exchange chromatography [9], HPLC [10], liquid–liquid extraction [12,14], cloud point extraction [13] and solid phase extraction [15].

The iron contents obtained for the Brazilian wines analyzed are agreeing with data reported by literature. The iron(II) concentrations obtained are always higher than the iron(III) contents. It can be explains considering the reductive character of the wines.

Acknowledgements

The authors are grateful to Fundação de Amparo a Pesquisa do Estado da Bahia (FAPESB), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for providing grants and fellowships and for financial support.

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