J. Braz. Chem. Soc., Vol. 14, No. 1, 59-64, 2003.
Printed in Brazil - ©2003 Sociedade Brasileira de Química
0103 - 5053 $6.00+0.00

Article

Use of a Mixing Chamber for Sample Preparation and Multiple Collection in Sequential Injection Analysis: Determination of Sulfate in Wines

Helena R. Silva, Marcela A. Segundo and António O. S. S. Rangel*

Escola Superior de Biotecnologia, Universidade Católica Portuguesa, R. Dr. António Bernardino de Almeida, 4200-072 Porto, Portugal

Foi desenvolvido um sistema de análise por injeção sequencial para a determinação de sulfato em vinhos. A metodologia foi baseada na formação de $BaSO_4$, detectado turbidimetricamente a 420 nm. Visando a diluição e acidificação da amostra, esta e ácido foram enviados para uma câmara de mistura situada numa das portas laterais da válvula de seleção. Após mistura e homogeneização do conteúdo da câmara, várias alíquotas (no máximo 6) foram retiradas e analisadas. O sistema apresentou uma resposta linear entre 300 e 1500 mg L^{-1} de K_2SO_4, usando padrões com etanol a 10% v/v. Os resultados obtidos usando o sistema automático foram estatisticamente comparáveis com os obtidos pelo método de referência, apresentando desvios relativos inferiores a 3,5%. A velocidade de amostragem obtida foi de 5 amostras por hora com desvio padrão relativo (RSD) inferior a 10%.

A sequential injection system for determination of sulfate in wines was developed. It was based on the formation of $BaSO_4$, measured turbidimetrically at 420 nm. As sample dilution and acidification were required, both sample and acid were sent to a mixing chamber placed in a lateral port of the selection valve; after thorough mixing, up to six aliquots could be drawn and analyzed. A linear calibration curve was established using 10% v/v ethanol standards with concentrations between 300 and 1500 mg L^{-1} of K_2SO_4. The results obtained by the present methodology were statistically comparable with those furnished by the reference procedure, with relative deviations < 3.5%. The sample frequency was about 5 samples per h with relative standard deviations (RSD) < 10%.

Keywords*:* sequential injection, mixing chamber, turbidimetry, sulfate, wine

Introduction

First introduced in 1990,[1] sequential injection (SI) is a simple and convenient concept of flow analysis. In its simplest configuration, SI analysis consists in the sequential aspiration of well defined sample and reagent zones into a holding coil by means of a multi-position valve. The flow is then reversed and the stacked zones are mixed and propelled to the detector, where the reaction product formed is monitored. The main advantages pointed out to SI when compared to other flow methodologies are the possibility of accomplishing different analysis without system reconfiguration[2] and the considerable reagents saving, since its consumption is not continuous.[3] Moreover, many devices can be clustered around the selection valve, such as gas-diffusion/dialysis units, mixing chambers and pre-concentration columns, allowing sample pre-treatment in its different side channels.[4]

So far, several manifolds including a mixing chamber in a side channel of the selection valve have been reported. Sample dilution[5] and efficient mixing of sample and reagents[6] were achieved in different systems. Enzyme activity measurement[7] and kinetic determination of analytes[8] were also performed in sequential injection systems equipped with a mixing chamber. Finally, the mixing chamber was also used both as dilution chamber and detection cell when it was connected to a fiber optic detector for determination of total biomass.[9]

One of the disadvantages of placing the mixing chamber in a side port of the selection valve is the increased amount of time necessary for each analytical cycle, considering that the chamber must be properly washed after each determination. In the present work, a novel procedure to perform several determinations from the same pre-treated sample is proposed. In order to accomplish this, sample and reagent/carrier could be sent to the mixing chamber; after thorough mixing, several aliquots could be drawn and analyzed, allowing replicate measurements from

* e-mail: rangel@esb.ucp.pt

the diluted sample and avoiding successive washing of the mixing chamber.

The feasibility of this procedure was tested using the determination of sulfate in wines. Sulfate is usually found in musts; additional sulfate in wine may arise from the oxidation of sulfur dioxide or from the addition of calcium sulfate in the treatment known as plastering.[10] Moreover, the fraudulent addition of H_2SO_4 to wine leads to a clearly detectable increase in sulfate content. Automation of this determination could be interesting considering that the reference method proposed by OIV[11] involves a time-consuming gravimetric procedure.

The methodology chosen was based on the precipitation of sulfate in the presence of barium chloride in acid media. The precipitate formed was turbidimetrically monitored at 420 nm. As the application of this methodology to wine samples can bring problems related to matrix color and acidity, sample dilution and pH adjustment were required. The proposed procedure was then applied to this particular analytical problem.

Experimental

Reagents and solutions

All chemicals used were of analytical reagent grade with no further purification, and deionized water with a specific conductance less than 0.1 μS cm^{-1} was used throughout.

A stock standard sulfate solution containing 5000 mg L^{-1} of K_2SO_4 was prepared by dissolving 1.000 g of solid in distilled water and diluting it to 200 mL. Working solutions in the range 200-2000 mg L^{-1} were prepared by suitable dilution of the stock solution and ethanol addition up to 10% (v/v). For initial studies, 1% ethanol (v/v) working standard solutions were prepared in the range 50-500 mg L^{-1} using the same stock solution. These solutions also contained 0.1 mol L^{-1} HCl.

For interference studies, a series of 500 mg L^{-1} K_2SO_4 standard with 10% (v/v) ethanol were prepared, each one with just one potential interfering species. The following salts were weighed and dissolved in each standard: KH_2PO_4, KNO_3, Na_2SO_3 and $KHCO_3$ for assessing PO_4^{3-}, NO_3^-, SO_3^{2-} and HCO_3^- interference; KCl, NaCl, $CaCl_2 \cdot 2H_2O$ and $MgCl_2 \cdot 6H_2O$ for assessing K^+, Na^+, Ca^{2+} and Mg^{2+} interference.

A washing buffer solution containing 40 g of EDTA (dissodium salt), 7 g of ammonium chloride and 57 mL of 25% (m/m) ammonia, was prepared by dissolving the different chemicals in succession in 500 mL of distilled water and diluting to 1 L.

A 1.0 g L^{-1} polyvinyl alcohol (PVA) solution was prepared by dissolving 0.5 g of solid in 200 mL of boiling water with continuous stirring; after cooling, the volume was made up to 500 mL with water. The reagent solution was prepared by dissolving 4 g of $BaCl_2 \cdot 2H_2O$ in 20 mL of the PVA solution.

A 0.6 mol L^{-1} hydrochloric acid solution was prepared by diluting 5 mL of 36% (m/m) hydrochloric acid in 95 mL of water.

Apparatus

A Gilson Minipuls 3 peristaltic pump, equipped with PVC pumping tubes, was connected to the central channel of a ten port electrically actuated selection valve (Valco VICI C25-31180E).

A Novaspec II visible spectrophotometer equipped with a Hellma 178.712QS flow-through cell (internal volume 18 μL) was used as detection system and the wavelength was set at 420 nm.

A mixing chamber (MC), made of acrylic and furnished with a magnetic bar, was placed over a magnetic stirrer; the schematic representation is depicted elsewhere.[12] The internal volume was *ca*. 900 μL.

Omnifit PTFE tubing (0.5 and 0.8 mm id) and Gilson end-fittings and connectors were used to connect the different parts of the manifold.

Data acquisition and device control were achieved using a 386 personal computer (Samsung SD700) equipped with an Advantec PCL-818HG interface card and a PCLD-8115 wiring terminal board, running a homemade software written in QuickBasic 4.5.

Manifold and procedure

System components were arranged as shown schematically in Figure 1. The holding coil (HC) length was 300 cm while the reaction coil (RC) length was 50 cm. The tubing connecting the selection valve and the MC was 5 cm long; the waste tubing in the MC was 6 cm long. Other tubing connected to the valve were 30 cm long. All tubing were 0.8 mm id, except the RC that was 0.5 mm id.

The protocol of flow and timing sequence required for the determination of sulfate in wines is given in Table 1.

The analytical cycle can be divided in three parts: sample dilution and acidification, turbidimetric determination of sulfate and MC washing. The first part started with sequential aspiration of acid, sample and acid into the HC. Then, the flow was reversed and the stacked zones were sent into the MC. After a certain period of time,

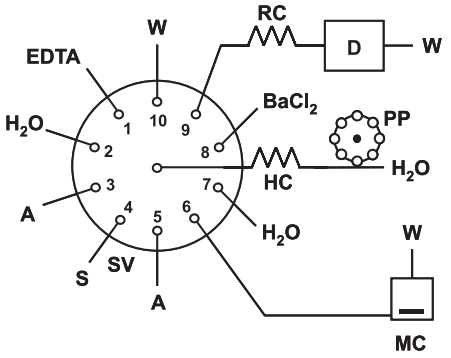

Figure 1. SI manifold for the determination of sulfate in wines. SV: selection valve; PP: peristaltic pump; HC: holding coil; RC: reaction coil; D: detection system; MC: mixing chamber; A: 0.6 mol L^{-1} hydrochloric acid; S: sample or standard; EDTA: EDTA buffer solution; $BaCl_2$: barium chloride reagent; W: waste.

Table 1. Protocol sequence for the determination of sulfate in wines.

Step	Valve position	Operation time (s)	Volume (μL)	Description
a	3	13.4	200	Aspirate acid
b	4	20.1	300	Aspirate sample
c	5	6.7	100	Aspirate acid
d	6	26.8	400	Dispense holding coil content to mixing chamber
e	6	10.1	150	Aspirate mixing chamber content to fill the connection tubing
f	10	25	1480	Flush holding coil
g	1	4.5	267	Aspirate EDTA
h	2	13.5	800	Aspirate water
i	6	6.7	100	Aspirate diluted sample from mixing chamber
j	8 / 7	5	75	Aspirate $BaCl_2$ reagent or water
k	9	50	2959	Propel HC content to detector, signal acquisition
l	6	90	5326	Dispense water to wash mixing chamber

diluted sample was drawn from the MC to fill the connection to the selection valve.

After flushing the HC, the system was ready to perform the turbidimetric determination of sulfate. EDTA solution, water, diluted sample from MC and $BaCl_2$ solution were sequentially aspirated into the HC; after selection of the detector port, the HC content was propelled to the spectrophotometer and absorbance measurements were carried out. These steps could be performed up to six times, considering the volume of diluted sample available in the mixing chamber, allowing several determinations with only one sample aspiration. When blank runs were performed, this procedure was the same, except that $BaCl_2$ solution was replaced by water.

In the last step, MC was washed with water to remove sample remains and to fill it for a new analytical cycle.

Reference procedure

The gravimetric procedure described by OIV[11] was based on precipitation of sulfate as $BaSO_4$. After drying and ashing of the precipitate formed, the mass of $BaSO_4$ was determined by weighing.

Results and Discussion

Development of the SI system

This work aimed the use of a mixing chamber for sample preparation and multiple collection of sample aliquots in a sequential injection system. Hence, after choosing the determination of sulfate in wines and defining the conditions for the physical parameters, the influence of some chemical parameters in the calibration curve was assessed. These studies were performed in a SI system without mixing chamber, using acidified and diluted standards. Finally, sample preparation in the mixing chamber and multiple collection were implemented, placing the chamber in a side port of the selection valve.

Physical parameters. Flow rate was as high as possible (3.55 mL min^{-1}), except in those steps where it could affect repeatability. A lower flow rate (0.90 mL min^{-1}) was applied during the steps performed for sample dilution and acidification (Table 1, steps a to e); along the turbidimetric determination, this low flow rate was only applied to draw diluted sample from the MC (Table 1, step i) and to aspirate $BaCl_2$ reagent (Table 1, step j).

Previous studies indicated that there was a general tendency for the formed penetrated product zone of barium sulfate to disperse more in the tubing when the tube diameter increases.[13] Considering this, tubing of 0.5 mm of internal diameter was used as reaction coil. The straight configuration was adopted and its length was as short as possible to connect the valve and the flow cell.

Chemical parameters. These studies were performed without the MC; the analytical cycle included steps g to k (Table 1). As both white and red wines absorb radiation in the UV/visible region,[14] sample dilution was regarded as necessary to minimize wine color interference in the detection system. Considering this, the initial studies of chemical variables were performed with standards between 50 and 500 mg L^{-1} of K_2SO_4 and 1% (v/v) ethanol in order to simulate concentration values found in diluted wines. The inclusion of ethanol was regarded necessary as it can decrease the solubility of the reaction product. Standards

also contained 0.1 mol L^{-1} of HCl to ensure a proper acidic medium for reaction occurrence.[15] To avoid the build-up of barium sulfate precipitate in the flow system an alkaline buffer-EDTA solution was used to rinse the tubing between determinations.

The order in which the different sequences of reagents and samples are drawn up and propelled into the detector is very important in SI. Considering this, EDTA and water were first aspirated and the aspiration order of $BaCl_2$ solution and sample was tested, using 200 μL of 150 g L^{-1} $BaCl_2 \cdot 2H_2O$ and 200 μL of K_2SO_4 standards. The sequential aspiration of sample and reagent was chosen; the slope of the calibration curve in this situation was 6 times the value obtained when the aspiration order was reversed.

The concentration of $BaCl_2$ was studied between 25 and 200 g L^{-1} of $BaCl_2 \cdot 2H_2O$, using 75 μL of sample and 200 μL $BaCl_2$. As $BaCl_2$ concentration increased, both slope and intercept of the calibration curve increased. For further studies, the solution containing 200 g L^{-1} was chosen.

Preliminary studies indicated that sensitivity was higher for larger sample volume; the chosen sample volume was 100 μL as a compromise between sensitivity and interference from wine matrix.

The volume of $BaCl_2$ reagent was varied between 50 and 100 μL. Sensitivity increased with volume increasing; the chosen volume was 75 μL as a compromise between sensitivity and reagent consumption.

Mixing chamber introduction. With the previous conditions set, further studies were performed using the MC. For these experiments, concentration of K_2SO_4 varied between 200 and 2000 mg L^{-1}, all standards were 10% (v/v) ethanol and 100 μL of diluted sample were drawn from the MC. The sample volume was varied between 150 and 350 μL and the volume transferred to the MC (step d, Table 1) was equal to the sample volume plus 100 μL. The results are presented in Figure 2. Increasing sample volume, sensitivity increased up to 300 μL. As a larger volume (350 μL) gave the same results, the sample volume chosen was 300 μL.

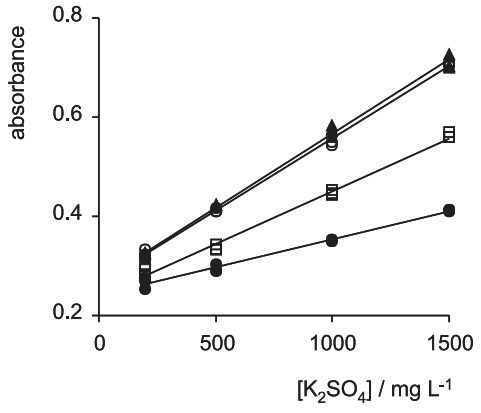

Figure 2. Results obtained from the study of the sample volume propelled to the mixing chamber; the values tested (μL) were: ● 150, □ 250, ▲ 300, ○ 350.

Interference studies. Some cationic and anionic species normally found in wine samples were studied as potential source of interference. The concentration assayed corresponded to the highest values regularly found.[10] When K^+ (1500 mg L^{-1}), Na^+ (150 mg L^{-1}), Ca^{2+} (80 mg L^{-1}), Mg^{2+} (200 mg L^{-1}), PO_4^{3-} (900 mg L^{-1}), NO_3^- (25 mg L^{-1}), SO_3^{2-} (300 mg L^{-1}) and HCO_3^- (1000 mg L^{-1}) were added separately to 500 mg L^{-1} K_2SO_4 (276 mg L^{-1} SO_4^{2-}) standard, no interference was detected.

Evaluation of the method and its application to wine samples

The procedure described in Table 1 was applied to several wine samples. Considering the absorbance value in the peak maximum, a calibration curve was established. For determination in wines, each sample was subjected to a "blank run", where $BaCl_2$ reagent was replaced by water.

The results obtained were compared to those resulting from the reference procedure.[11] Relative deviations between -21.2 and -8.7 % were obtained for white wines; +6.9 and +28.6 % were the values acquired for red wines. These figures indicated that the proposed methodology was possibly affected by systematic errors, owing to wine color interference.

To investigate this possibility, both standards and samples were subjected to the blank run; peak profiles for 500 and 1000 mg L^{-1} standards and for white and red wine samples are presented in Figure 3. The peak maximum of the determination signal was not obtained at the same time for standards and white wines. Moreover, the time in which maximum absorbance was reached was not the same for blank and determination signal. This situation can explain the negative deviations obtained for white wines as the value considered for interpolation in the calibration curve was underestimated. Hence, absorbance should be read at a fixed time (Figure 3, dotted line) for correct blank signal subtraction.

A new calibration curve was established using standards with concentrations between 300 and 1500 mg L^{-1} of K_2SO_4; the absorbance value considered was the determination signal minus the blank signal; both signals were obtained at a pre-set time. In order to evaluate the accuracy of the proposed methodology, table wine samples were analyzed. The results (C_{SI}), presented in Table 2, were compared with those furnished by the reference procedure (C_r); relative deviations lower than 3.5% were found. A linear

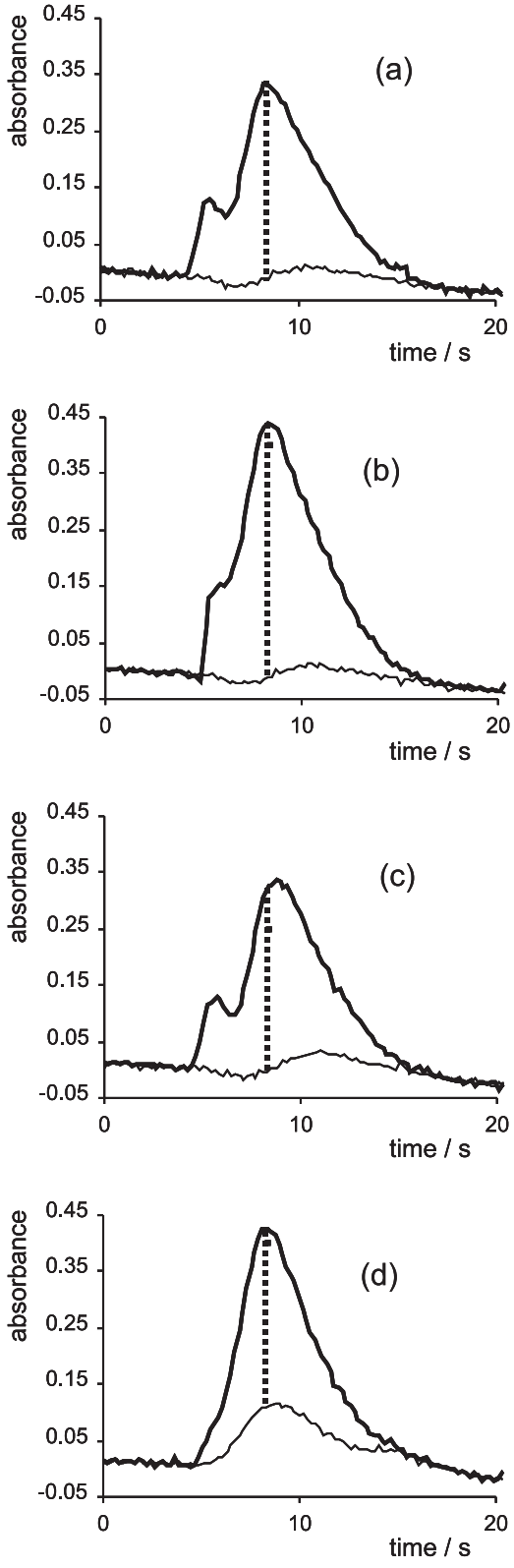

Figure 3. Peak profile (absorbance versus time/s) obtained from turbidimetric determination (bold line) and blank run (normal line) for standards and wine samples. The dotted line corresponds to the difference between determination signal and blank signal at 8.3 s. (a) 500 mg L^{-1} standard; (b) 1000 mg L^{-1} standard; (c) white wine; (d) red wine.

relationship between C_{SI} and C_r was established; the equation found was

$$C_{SI} = 6(\pm 47) + 0.99(\pm 0.09) \times C_r$$

where the values in parenthesis are 95% confidence limits. From these figures it is clear that the estimated slope and intercept do not differ from the values 1 and 0, respectively. Thus, there is no evidence for systematic differences between the two sets of results[16] obtained by the proposed methodology and by the reference procedure.

Table 2. Results (mg L^{-1}) obtained by the SI methodology (C_{SI}) and by the reference procedure (C_r) for the determination of sulfate. RD: relative deviation. 1-6: white wines; 7-10: red wines.

Sample	C_r	C_{SI}	% RD
1	443 ± 3	433 ± 86	-2.3
2	421 ± 6	416 ± 41	-1.2
3	497 ± 16	497 ± 35	0.0
4	411 ± 6	421 ± 42	2.4
5	418 ± 10	419 ± 31	0.2
6	367 ± 10	380 ± 31	3.5
7	722 ± 10	712 ± 13	-1.4
8	648 ± 7	671 ± 37	3.5
9	596 ± 4	608 ± 38	2.0
10	579 ± 10	559 ± 43	-3.5

Repeatability. It was estimated by calculating the relative standard deviation; in the present work, as the absorbance value results from an arithmetic operation, standard deviation (s) was calculated as

$$s = \sqrt{s_D^2 + s_B^2}$$

where s_D is the standard deviation of determination signal and s_B is the standard deviation of blank signal.[16] For the analyzed samples, relative standard deviations of absorbance values were lower than 3.5%, except for sample 1; the values obtained for calculated concentrations were lower than 10%.

Detection limit. The detection limit calculation was based on three consecutive performances of the analytical cycle using a 10% (v/v) ethanol solution. The calculated value was the concentration corresponding to the absorbance value (determination signal minus blank signal) plus three times its standard deviation. The standard deviation was calculated as indicated above. The value obtained was 154 mg L^{-1} of K_2SO_4.

Sample throughput. The analytical cycle of the present methodology can be divided in three parts: sample preparation (Table 1, steps a to f), turbidimetric determination of sulfate (Table 1, steps g to k) and MC

washing (Table 1, step l). Considering that the time required for the proper port selection must also be accounted, it took 112 s for sample preparation, 89 s for each determination and 97 s for MC washing. For sample analysis, the whole procedure took 743 s if the turbidimetric determination of sulfate was repeated six times. In this case, the sample frequency was about 5 per hour.

Conclusions

The mixing chamber, placed in one of the side ports of the selection valve, was used to dilute and acidify the sample. Moreover, it was possible to draw up to six aliquots from the diluted sample, enabling replicate and blank measurements from the same sample portion. This feature also allowed time saving since washing of the mixing chamber was not required between several determination cycles.

In the present work, determination of sulfate was carried out directly in both white and red wines. This feature was an improvement compared to previously reported segmented flow analysis systems,[17] which could only be applied to white wines or required pre-treatment with charcoal for red wines. In the proposed system, the sample color interference was eliminated by conjugating absorbance measurement at a fixed time and subtraction of the absorbance value from a blank run.

The sampling frequency was about 5 samples per hour, which can also be considered an improvement when compared to the time-consuming gravimetric procedure.[11] Hence, the application of the proposed methodology as a screening technique is recommended, despite its lower repeatability when compared to that obtained in the reference procedure.

Acknowledgements

The authors acknowledge the financial support from PRAXIS XXI/Agência de Inovação through Project P076-P31B-09/97-INSIA. M. A. Segundo thanks FCT for the grant PRAXIS XXI BD/13648/97.

References

1. Ruzicka, J.; Marshall, G. D.; *Anal. Chim. Acta* **1990**, *237*, 329.
2. Cerdà, V.; Estela, J. M.; Forteza, R.; Cladera, A.; Becerra, E.; Altimira, P.; Sitjar, P.; *Talanta* **1999**, *50*, 695.
3. Rocha, F. R. P.; Nóbrega, J. A.; Fatibello Fº, O.; *Green Chem.* **2001**, *3*, 216.
4. Taljaard, R. E.; van Staden, J. F.; *Lab. Robot. Autom.* **1998**, *10*, 325.
5. Masini, J. C.; Baxter, P. J.; Detwiler, K. R.; Christian, G. D.; *Analyst* **1995**, *120*, 1583.
6. Crespí, A. R.; Cerdà, V.; Forteza, R.; *Lab. Robot. Autom.* **1995**, *7*, 245; Araújo, A. N.; Costa Lima, J. L. F.; Saraiva, M. L. M. F. S.; Sartini, R. P.; Zagatto, E. A. G.; *J. Flow Injection Anal.* **1997**, *14*, 151; Taljaard; R. E.; van Staden, J. F.; *Anal. Chim. Acta* **1998**, *366*, 177.
7. Hansen, E. H.; Willumsen, B.; Winther, S. K.; Drabøl, H.; *Talanta* **1994**, *41*, 1881; Schindler, R.; Lendl, B.; *Anal. Chim. Acta* **1999**, *391*, 19.
8. Guzman, M.; Pollema, C.; Ruzicka, J.; Christian, G. D.; *Talanta* **1993**, *40*, 81; Sultan, S. M.; Suliman, F. E. O.; *Analyst* **1996**, *121*, 617.
9. Baxter, P. J.; Christian, G. D.; Ruzicka, J.; *Analyst* **1994**, *119*, 1807.
10. Amerine, M. A.; Ough, C. S.; *Methods for Analysis of Musts and Wines*, Wiley: New York, 1980.
11. Office International de la Vigne et du Vin (OIV); *Recueil des Méthodes Internationales d'Analyse des Vins et des Moûts*, OIV: Paris, 1990.
12. Tóth, I. V.; *PhD Thesis*, ESB-Universidade Católica Portuguesa, Portugal, 2000.
13. van Staden, J. F.; Taljaard, R. E.; *Anal. Chim. Acta* **1996**, *331*, 271.
14. Usseglio-Tomasset, L.; *Chimie Œnologique*, Technique & Documentation: Paris, 1995.
15. Baban, S.; Beetlestone, D.; Betteridge, D.; Sweet, P.; *Anal. Chim. Acta* **1980**, *114*, 319.
16. Miller, J. C.; Miller, J. N.; *Statistics for Analytical Chemistry*, 3rd ed., Ellis Horwood: Chichester, 1993.
17. Houtman; A. C.; Du Plessis, C. S.; *Agrochemophysica* **1975**, *7*, 21; Du Plessis, C. S.; Houtman, A. C.; *Agrochemophysica* **1975**, *7*, 47.

Received: February 2, 2002
Published on the web: November 29, 2002