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Determination of calcium, magnesium, sodium and potassium in wines by FIA using an automatic zone sampling system

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In this paper, an automatic flow injection system based on the zone sampling technique for the determination of calcium and magnesium in wines by atomic absorption spectrometry, and sodium and potassium by flame photometry, is described. A reproducible cleavage of a portion of the injected sample plug was achieved by using two computer-controlled rotatory valves. The developed methodology yielded up to a 2000-fold dilution, without a significant decrease in the sampling rate, which was never lower than 90 determinations per h for any of the species analyzed. The quality of the results obtained with the automatic system for several types of wines was evaluated by comparison with those obtained with the reference methods. A relative standard deviation of less than 3% was achieved for all the samples ($n = 15$) and species analyzed. Copyright © 1996 Elsevier Science Ltd.

INTRODUCTION

The concentrations of calcium, magnesium, sodium and potassium are among the most common analyses in wine control laboratories. Their presence does not only affect some production stages (e.g. the fermentation process and storage) but also the stability of the final bottled product (Amerinem & Ough, 1980; Ribéreau-Gayon *et al.*, 1986).

The reference methods for these elements are mainly atomic absorption spectrometry (AAS) for calcium and magnesium and flame photometry (FP) for sodium and potassium. For any of these determinations the end method of determination is performed in a simple and expeditious way. However, the manual pre-treatment of the samples is frequently a tedious time-consuming operation owing mainly to the high level dilutions that are often necessary to adjust the sample's concentration to the linear working range of the detector.

Flow injection analysis (FIA) is a methodology currently used for automating various steps of analytical procedures like sample handling and pre-treatment, including sample dilution. Among the FIA techniques used to obtain high on-line dilutions are the injection of microlitre-volume samples (Fang *et al.*, 1993), stream splitting (Lima & Rangel, 1990), the use of dilution chambers (Ruzicka & Hansen, 1978; Stewart & Rosenfeld, 1982; Garn *et al.*, 1988), dialysis (Lima *et al.*, 1991) and zone sampling (Reis *et al.*, 1981).

An FIA manifold based on the zone sampling technique is very versatile and easily adjusted to the concentration of the sample without a significant decrease in the sampling rate. However, in contrast to what happens with other FIA procedures, its manual operation is extremely uncertain in terms of accuracy since its performance is dependent on the precise timing control of the zone sampling process.

This was the main reason for the development of an automatic FIA system based on zone sampling that could be coupled to an atomic absorption spectrometer and to a flame photometer for the determination of calcium, magnesium, sodium and potassium in wines. Given that the dilution level required for each sample was automatically adjusted by the analytical system, the determinations were carried out independently of the sample's concentration.

MATERIALS AND METHODS

Instrumentation

The determination of calcium and magnesium was carried out using a Pye-Unicam Model SP9 atomic

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absorption spectrometer, and for sodium and potassium a Jencons Model PFP7 flame photometer using a butane flame. A Gilson Model 221 was used as an autosampler.

Reagents and solutions

Chemicals were all of analytical reagent grade and the solutions were prepared using deionized water (conductivity $< 0.1\ \mu S\ cm^{-1}$). The standard solutions used for the preparation of the calibration curves were prepared by dilution of BDH Spectrosol 1000 mg $litre^{-1}$ standard solutions, except for the potassium standard solution which was prepared by the weighing of potassium chloride (Merck, ref. 4936), previously dried at 110°C overnight.

Flow injection manifolds

The propulsion of the solutions was made using Gilson Model Minipuls 3 peristaltic pumps and Tygon pumping tubes of the same brand. Tubing was made of Omnifit PTFE ($\phi = 0.8$ mm) with Gilson end-fittings and connectors, home-made Y-joints (used as confluence points) and pulse dampers constructed of perspex (Alegret *et al.*, 1987). Two Rheodyne Model 5020 injection valves were used for sample injection and for the re-sampling process. These valves were equipped with an electric motor and an interface for microcomputer control.

Hardware and software

A 486DX-based microcomputer was used as the control unit. The communication between the computer and the analytical system was established through a data acquisition and control interface PC-LABCard model PCL-812PG from Advantech.

The control software was developed using Microsoft QuickBASIC version 4.5. In data acquisition from the PCL-812PG, card routines furnished by Advantech were also used and integrated in the software. The software developed for the analytical system (Fig. 1) controlled the peristaltic pumps, injection valves, sampler and data acquisition during processing. The software also managed data storage and concentration calculations. The data collected was processed using a least-squares regression.

Reference procedures

The results obtained with the developed methodology were evaluated by comparison with those obtained with

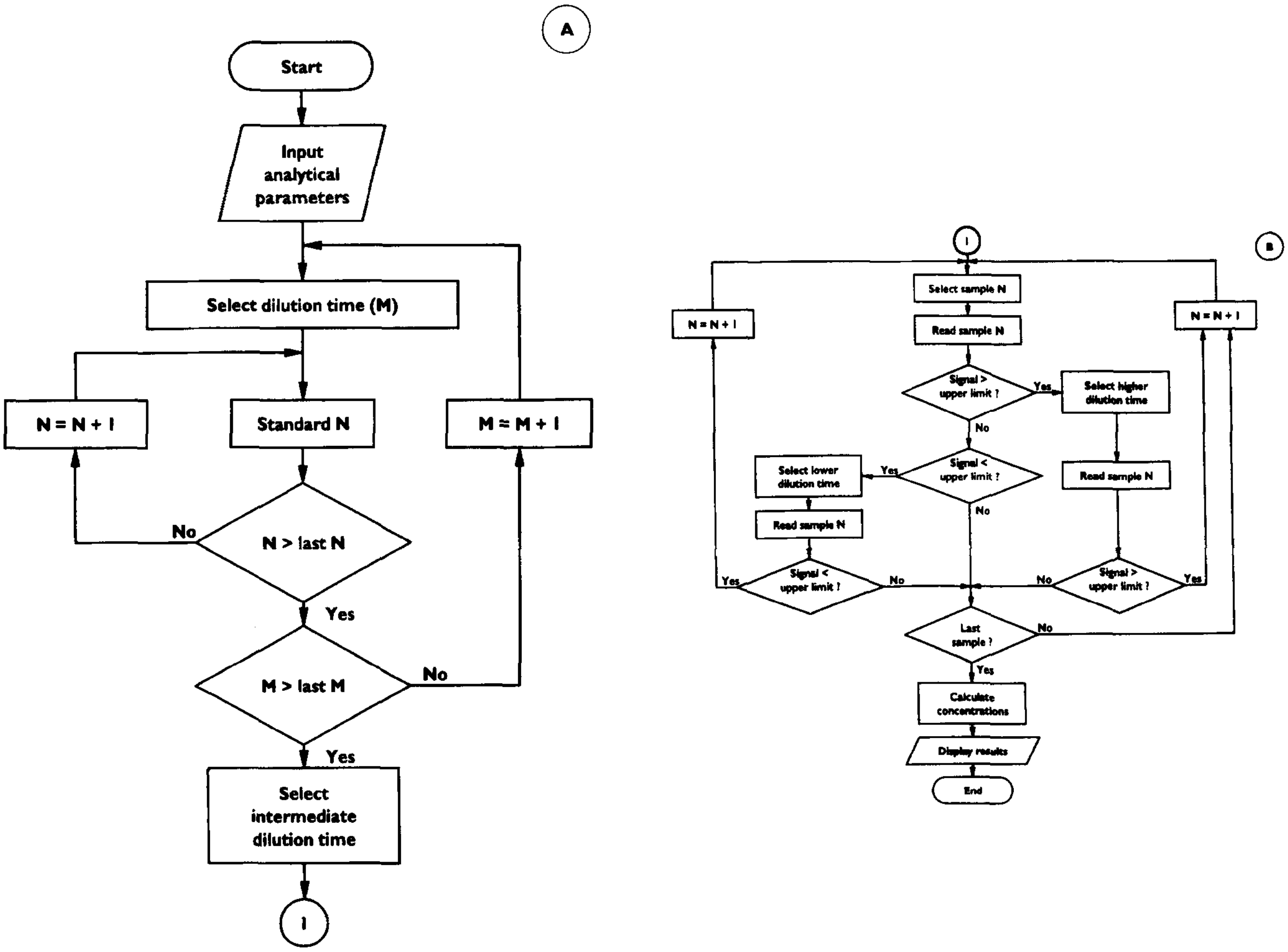

Fig. 1. Flowchart of the analytical system software: (A) calibration routine; (B) sample measurement routine.

the reference methods. Calcium and magnesium were determined by AAS as recommended by the Office International de la Vigne et du Vin (OIV, 1986). Sodium and potassium were determined by FP according to the Association of Official Analytical Chemists (AOAC, 1984).

RESULTS AND DISCUSSION

In the developed zone sampling system (Fig. 2) the concentrated sample is injected by an injection valve (L_1) into a first carrier stream (Q_2). Following a dispersion on a dilution coil (R_1), the sample reaches and fills the loop of a second valve (L_2) before going to waste (W). By means of rotation of L_2 after a time interval (t_d), an aliquot of the dispersed sample plug is introduced into a second carrier stream (Q_3) and directed toward the detector (D). The rotation of both valves L_1 and L_2 and the selection of the dilution time (t_d) for the analysis are controlled by the microcomputer under a timer control.

It is the size and concentration of the aliquot taken from the original sample that dictates the extension of the dilution inside the manifold.

The dilution time (t_d) of the analysis is established in order to ensure that an aliquot of the trailing edge of the sample plug instead of its front part is selected, because the higher dispersion of the former, with a lower concentration profile, gives less variations in the volume of the sample portion sent to the detector during the re-sampling process (Reis *et al.*, 1981)

The extent of dilution required for the determination of Ca, Mg, Na and K in wine samples is conditioned by both the concentration of the sample and the short linear working range of the AAS and the FP used as detectors. Moreover, since these cations could be present within a wide range of concentrations, the range of dilutions provided by the developed methodology should enable the determination of these species without any modification in the manifold configuration.

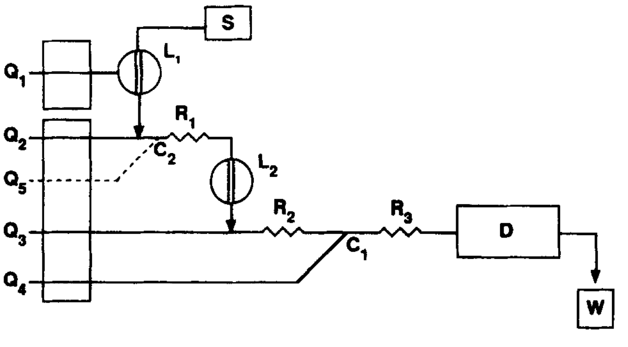

Fig. 2. Zone sampling manifold used for the determination of calcium. magnesium, sodium and potassium in wines. Dashed lines represent alternative channels (see text). S, sample; D, detector; W, waste; flow-rate: $Q_1 = 7.0$ ml min^{-1}; Q_3 and $Q_4 = 4.8$ ml min^{-1}; $Q_2 = 4.8$ ml min^{-1} and $Q_5 = 0.0$ ml min^{-1} or $Q_2 = 2.4$ ml min^{-1} and $Q_5 = 2.4$ ml min^{-1}. Coil length: $R_1 = 180$ cm, $R_2 = 33$ cm; $R_3 = 64$ cm. L_1 and L_2: injection valves (loop length, $L_1 = 16$ or 8 cm and $L_2 = 70$ cm). C_1 and C_2: confluence points.

Considering the highest concentration that are usually found in wines (200 mg litre^{-1} for calcium, 150 mg litre^{-1} for magnesium, 100 mg litre^{-1} for sodium and 1500 mg litre^{-1} for potassium) (Amerinem & Ough, 1980; Ribéreau-Gayon *et al.*, 1986) and also the linear working range of the AAS and the FP that were used, the developed system had to be able to provide up to 2000-fold dilutions without a significant decrease in the sampling throughput and in the reproducibility of the results.

During the optimization assays of the various factors that could influence the performance of the system special attention was given to the flow rates, coil length (R_1) and initial sample volume.

The flow rate (Q_1) of the channel responsible for filling the loop of the first valve (L_1), that introduces the samples in the system, was set to 7.0 ml min^{-1}, because the sampling process had to be performed immediately after the system had decided whether the analysis would be repeated at a different t_d or a new sample would be analysed. This had no effect on the sampling rate. Moreover, since the pump rotated only during a brief time, the flow rate had to be sufficiently high to remove any residue of the preceding sample. During the remainder of the analysis the pump was stopped allowing sample saving.

The flow rate of the carrier solution (Q_2) dictates the residence time and thus the dispersion of the sample plug inside the dilution coil (R_1). An excessive flow rate would decrease the dispersion, creating a sampling zone with a higher concentration profile that could give rise to fluctuations in the cleavage of the sample aliquot, and thus decrease the reproducibility of the analysis. However, an insufficient flow rate would affect the sampling throughput. The length of the coil (R_1) between the two valves is also a crucial parameter since it has a similar influence on the dispersion and therefore on the concentration profile of the sampling zone. Accordingly, it was verified that setting Q_2 and Q_3 to 4.8 ml min^{-1} and the length of R_1 (Fig. 2) to 180 cm it was possible to obtain a sufficiently wide range of dilutions by using t_d values from 17 to 25 s.

The utilization of small sample volumes (80 μl for calcium, magnesium and sodium; 40 μl for potassium owing to a higher sample concentration), gave an adequate dispersion of the sample plug along the coil R_1, which arrived at the second valve (L_2) with a dilution level and a concentration profile more convenient for the cleavage. The loop's length of L_2 was set to 70 cm in order to guarantee that, if necessary, it could hold the entire dispersed sample injected in L_1. This way it would be possible to re-sample the whole sample (and not only an aliquot of it) which would be required when the sample had a very low concentration.

A fourth channel (Q_4) was incorporated in the manifold to promote an additional dispersion of the sample portion during its transport toward the detector and this was also used to add a lanthanum (III) solution that acted as an interference suppresser in the determination of calcium and magnesium. This fourth channel was also used to impose a flow rate at the entry of the nebulizer slightly higher than recommended by the manufacturer, which creates a detector (AAS and FP) inlet overpressure. This overpressure minimizes the physical interferences of the matrix, reduces the efficiency of the nebulization acting as an additional on-line dilution by diminishing the analytical signal and improves the reproducibility of the results (Lima & Rangel, 1991).

When using the zone sampling technique a calibration curve must be constructed prior to the analysis, which involves the preparation of appropriate standard solutions and the recording of their signals at a pre-set dilution time (t_d). Performing the analysis at this dilution time means that all the samples independent of their concentration, will endure the same dilution. However, if the dilution time is not adequate for the analysis, owing to a higher than expected sample concentration, an increased t_d must be used and a new calibration curve must be constructed. However, if the sample is poorly concentrated the same t_d could be too high, yielding an overdilution that will result in a loss of accuracy of the analysis or even make the detection unavailable.

In the system developed this problem was overcome by a calibration step (Fig. 1A) which involves the execution of two or three calibration curves with a similar number of dilution times and of sets of standards with different concentration ranges. At the lower t_d, which produces inferior dilutions, less concentrated standards are injected, establishing the lower concentration limit for sample analysis. For the higher concentrated standards, which undergo a higher dilution similar to the more concentrated samples, the higher t_d is used. A third calibration curve could be made, using an intermediary t_d that will cover the medium concentration range. The calibration curves and the corresponding dilution times are stored in the computer and their selection for the analysis of each sample is defined automatically by the system. This way, during the analysis (Fig. 1B) the system uses initially the intermediary t_d: if the analytical signal is outside the limits of the calibration curve for this dilution time, the system repeats the analysis at the lower t_d if the signal is too low or at the higher t_d if the signal is too high.

Determination of calcium and magnesium

In the calcium determination, dilution times from 20 to 22 s and from 10.0 to 140.0 mg litre^{-1} calcium standard solutions were used. In the magnesium determination owing to the higher dilutions required, dilution times from 21 to 23 s and from 50 to 200 mg litre^{-1} magnesium standard solutions were used. Despite the high dispersion achieved a good reproducibility was obtained with a relative standard deviation (RSD) of $<1.6\%$ for calcium and $<2.7\%$ for magnesium. The results obtained by the FIA methodology (C_f) (Table 1), when compared with those obtained by the reference methods (C_r), showed for calcium a relative deviation (in percentage) from 0.1 to 1.8% for samples with calcium concentrations between 83.2 and 115.7 mg litre^{-1}. For magnesium, a relative deviation from 0.3 to 4.4% for samples with magnesium concentrations between 38.8 and 100.0 mg litre^{-1} was obtained. A relation of the type $C_f = C_0 + SC_r$ that was established for the two species showed a good agreement between the results obtained by both methodologies (Table 2). The sampling rates were about 120 determinations per h for both species.

Determination of sodium and potassium

In the determination of sodium and potassium the high concentration of the samples, especially for potassium,

Table 1. Results obtained in the determination of calcium, magnesium, sodium and potassium in wine samples, by the developed system (C_f) and by the reference methods (C_r)

	Ca			Mg			Na			K		
Sample	C_f	C_r	RD (%)*	C_f	C_r	RD (%)*	C_f	C_r	RD (%)*	C_f	C_r	RD (%)*
1	85.6	86.2	−0.7	45.0	44.4	1.4	246	234	5.1	964	952	1.3
2	114.3	115.7	−1.2	39.5	38.8	1.8	174	170	2.4	1160	1105	5.0
3	83.3	84.1	−1.0	60.9	61.1	−0.3	184	186	−1.1	1325	1258	5.3
4	84.7	85.8	−1.3	64.9	66.7	−2.7	210	207	1.4	756	742	1.9
5	84.7	84.3	0.5	42.7	44.4	−3.8	178	171	4.1	673	682	−1.3
6	87.8	88.2	−0.5	57.7	55.6	3.8	286	290	−1.4	640	649	−1.4
7	100.1	99.1	1.0	44.3	44.4	−0.2	229	227	0.9	1018	972	4.7
8	88.7	89.1	−0.4	37.1	38.8	−4.4	205	207	−1.0	1229	1195	2.8
9	84.7	83.2	1.8	61.3	61.1	0.3	192	184	4.3	762	756	0.7
10	87.8	87.9	−0.1	61.3	61.1	0.3	170	164	3.7	562	574	−2.1
11	83.3	84.2	−1.1	92.5	94.4	−2.0	109	111	−1.8	916	914	0.3
12	103.7	102.8	0.9	98.1	100.0	−1.9	109	107	1.9	606	591	2.5
13	88.7	89.1	−0.4	60.9	61.9	−1.6	113	116	−2.6	452	459	−1.4
14	97.8	99.5	−1.7	55.4	55.6	−0.4	122	120	1.7	496	508	−2.5
15	96.6	97.3	−0.7	56.1	55.6	0.9	108	107	0.9	686	707	−3.0

*Relative deviation expressed in percentage of the FIA methodology to the reference methods.

Table 2. Results obtained in the determination of calcium, magnesium, sodium and potassium in wine samples ($n = 15$)

Element	Equation parameters C_o	S	R^*	$t_{0.025}$†	$t_{0.025}$§
Calcium	1.403	0.9813	0.9952	1.333	2.145
Magnesium	1.318	0.9706	0.9981	1.328	2.145
Sodium	0.7448	1.008	0.9966	−1.926	2.145
Potassium	−19.34	1.031	0.9963	−0.8490	2.145

*Correlation coefficient.
†Calculated values for a two-tail *t*-test.
§Tabulated values (95% confidence level).

required higher dilutions than those used for calcium and magnesium. Moreover, owing to the higher concentration profile, the cleaving had to be executed in a more concentrated sample zone and any variation in the aliquot re-sampled caused a decrease in the reproducibility. This problem was exacerbated by the extremely high dilution factors involved.

In order to overcome this problem a second confluence (C_2) (Fig. 2) was placed in the manifold to introduce a new flow of deionized water (Q_5) that increases the initial dispersion of the sample and therefore reduces the gradient of concentration of the sampling zone.

The determination of sodium was made using dilution times from 20 to 22 s and sodium standard solutions from 50 to 250 mg litre^{-1} (see Fig. 3). In the determination of potassium dilution times from and 25 to 28 s and from 500 to 1500 mg litre^{-1} potassium standard solutions were used. The RSD obtained was $< 1.4\%$ for sodium and $< 3.2\%$ for potassium.

The results obtained with the developed system (C_f) (Table 1) compared with those furnished by the reference methods (C_r) presented, for sodium, a relative deviation (in percentage) from 0.9 to 5.1% for samples with sodium concentrations between 107 and 290 mg litre^{-1} and, for potassium, a relative deviation from 0.3 to 5.3% for samples with potassium concentrations from 459 to 1258 mg litre^{-1}. The relation $C_f = C_0 + SC_r$ showed a good agreement between the results obtained by both methodologies with slopes and correlation coefficients close to 1 (Table 2). The apparently significant value of C_0 obtained for the potassium correlation is irrelevant because, for the range of concentrations measured, C_0 is lower than the highest deviation obtained, which was itself very small. The sampling rates were about 120 determinations per h for sodium and about 90 determinations per h for potassium.

CONCLUSIONS

The results obtained with the developed methodology applied to the determination of Ca, Mg, Na and K in several types of wine show a good agreement with those obtained by the reference methods. These results show that the methodology could be used as an advantageous alternative to the conventional manual dilution, and even to other FIA dilution techniques.

The analytical system exhibited a high versatility and enabled the attainment, in a reproducible way, of a wide range of dilutions up to 2000-fold. Moreover, it permitted for each sample, and during the same analytical procedure, the most appropriate dilution level according to the sample concentration, avoiding the execution

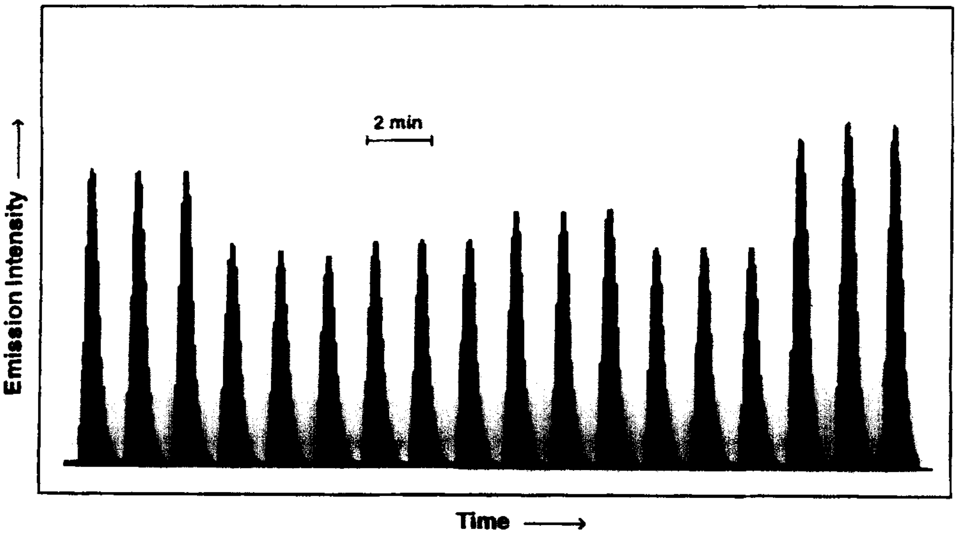

Fig. 3. Routine determination of sodium ion in wines. The record peaks correspond to a triplicate measurement of six wine samples at a dilution time (t_d) of 20 s.

of an identical dilution for samples with different concentrations. This is performed by the control unit (microcomputer) without interference from the system's operator.

Considering the results obtained with the detectors used in this work (atomic absorption spectrometry and flame photometry) it is possible to foresee that the developed system could be associated to an extensive range of detectors commonly used in flow analysis if an acquisition card and a microcomputer are available, and also that its application could be extended to the analysis of other matrices.

The implementation of these FIA system in laboratories where these determinations are currently carried out is accessible, since the materials are simple, easily maintained and is easily operated as it is only necessary for an operator to enter the parameters required to control the methodology and the system will run automatically

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