Journal of Chromatography, 634 (1993) 47-56
Elsevier Science Publishers B.V., Amsterdam

CHROM. 24 789

Ion chromatographic determination of monosaccharides from trace amounts of glycosides isolated from grape musts

P. Pastore*

Department of Inorganic, Metallorganic and Analytical Chemistry, University of Padova, Via Marzolo 1, 35131 Padova (Italy)

I. Lavagnini

Institute of Industrial Chemistry, University of Padova, Via Marzolo 9, 35131 Padova (Italy)

G. Versini

Agricultural Institute of S. Michele all'Adige, Via E. Mach 1, 38010 S. Michele all'Adige (TN) (Italy)

(First received July 22nd, 1992; revised manuscript received November 10th, 1992)

ABSTRACT

An isocratic ion chromatographic procedure for the determination of monosaccharides from the hydrolysis of trace amounts of glycosides present in grape musts is described. A laboratory-made ultramicroband electrode, tested previously with reference compounds, was used in the pulsed amperometric detection of the recovered sugars. The very low detection limits (lower than 1 mg/l) allowed the identification of xylose (not previously detected in grape musts) and apiose. The correctness of using the peak height as the measurement of the sugar concentrations also for partially overlapped peaks was checked by a deconvolution procedure based on the simplex method.

INTRODUCTION

The determination of the constituents of food products present at trace levels is acquiring greater importance in view of the protection of the consumer and the implementation of legislation. A typical example is the characterization of grape musts, which is carried out by utilizing a variety of analytical procedures. Among the most important is the determination of the $^{13}C/^{12}C$ [1] and $^{1}H/^{2}H$ [2] isotope ratios. In this context, attention has recently been devoted to the potential determination of glycosides present in grape musts but their direct determination is cumbersome owing their great variety due to different aglyconic constituents [3], *i.e.*, terpenoids, phenols and other compounds. Therefore, an easier approach is to determine the sugars coming from hydrolysis of the glycosides. This analysis can be completed by the determination of only the principal aglyconic constituents. Owing to the very low concentration of glycosides in musts, trace level analysis is required; consequently, precision, accuracy and reasonable analysis times are difficult to achieve. At present the determination of carbohydrates is achieved by either enzymatic analysis and/or chromatographic (gas and liquid) analysis. The former procedure is fast and sensitive but suffers

* Corresponding author.

0021-9673/93/$06.00

from many interferences and limitations [4,5]. The latter procedures either require previous purification and derivatization steps or are not sufficiently sensitive owing to the lack of suitable detectors [6]. Recently, high-performance anion-exchange chromatography (HPAEC) with pulsed amperometric detection has proved to be a very effective method for carbohydrate determinations at the mg/l concentration level [7]. This approach does not require pretreatment of the sample and, when appropriately operated [8] pulsed amperometric detection gives detection limits below 1 mg/l.

This paper demonstrates (i) the application of anion-exchange chromatography with pulsed amperometric detection to the determination of trace levels of monosaccharides derived from the hydrolysis of glycosides, (ii) the utility of a deconvolution procedure for resolving partially overlapped peaks, to validate the use of the peak heights in quantitative analysis, and (iii) the potential of an ultramicroband gold electrode for the detection of carbohydrates.

EXPERIMENTAL

Chemicals and reagents

Demineralized water, passed through a Millipore (Bedford, MA, USA) cartridge (GS, 0.22 μm) for final purification, was used throughout. Analytical-reagent grade arabinose, rhamnose, galactose, glucose, mannose, xylose, fructose and ribose were used as received. Apiose was obtained by hydrolysis of either 4-*p*-nitrophenylapiose or apiin {7-[(2-O-D-apio-β-D-furanosyl-β-D-glucopyranosyl)oxy]-5-hydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one}. Standard solutions of all sugars, except apiose, were prepared by serial dilutions of 100 mg/l stock solutions made from weighed amounts of the standard reagents. For apiose, approximate concentration values are reported as work is in progress to define the yield of the hydrolysis reaction. The solutions were stored at low temperature to avoid bacterial degradation. The eluent used in the optimized chromatographic tests was a $2 \cdot 10^{-2}$ *M* sodium hydroxide aqueous solution. In the flow-injection (FI) tests, carried out to characterize the ultramicroband electrodes, the eluent was 0.1 *M* H_2SO_4 and the standard samples were either a $1 \cdot 10^{-4}$ *M* dopamine or a $1 \cdot 10^{-4}$ *M* $[Fe(CN)_6]^{3-}$ aqueous solutions.

Apparatus and procedure

In all the experiments a Dionex (Sunnyvale, CA, USA) Model 2010i ion chromatograph equipped with a 20-μl loop was used. In the electrochemical determination of the sugars, an EG & G PAR (Princeton, NJ, USA) Model 400 amperometric detector was used. The working electrodes were either a standard 3.5 mm diameter gold electrode (supplied by EG & G PAR) or a 4 mm $\times$ $5 \cdot 10^{-3}$ mm gold microband electrode made in our laboratories. A stainless-steel counter electrode and an $Ag/AgCl/Cl^-_{sat.}$ reference electrode were employed. In analyses for sugars the detection mode chosen was a triple pulse polarization waveform with a current sampling step at 100 mV of 0.8 s, an anodic polishing step at 650 mV of 0.3 s and a cathodic surface renewing step at -800 mV of 0.160 s.

The analytical column was a Dionex Carbopac PA1 anion-exchange column (250 mm x 4 mm I.D.) equipped with a guard precolumn (50 mm x 4 mm I.D.). In the flow-injection tests the analytical column was replaced with a delay tube of 3 m (volume 1.5 ml) in a knitted configuration (Supelco, Bellefonte, PA, USA) to accomplish the minimal dispersion of the injected volume. The signals were recorded with a Hewlett-Packard (Palo Alto, CA, USA) Model 7090A digital plotter connected to an IBM-compatible computer.

Gas chromatographic identification proofs were performed with a Carlo Erba (Milan, Italy) Fractovap 2900 gas chromatograph with flame ionization detection (FID) and a Spectra-Physics (San Jose, CA, USA) Model SP 4270 integrator. The column was a Supelco fused-silica capillary (30 m x 0.32 mm I.D.) with a 0.1-μm silicone OV-1 dimethyl gum stationary phase. In these tests, oxime derivatives were formed to reduce the number of isomers and then derivatized with a mixture of hexamethyldisilazane and trimethylchlorosilane in pyridine [9].

For the deconvolution of the peaks, the sim-

plex algorithm, as a non-linear regression method, was written in TURBO PASCAL 5.5 and run under the DR DOS 5.0 operating system on a personal computer (CPU i80486).

To control accurately the strength of the eluent and to minimize the carbonate content, the following procedure was adopted. An approximately 0.1 *M* NaOH solution was prepared from a 50% (w/w) aqueous solution in which the carbonate content was very low; an aliquot of this solution was then titrated and adjusted to 0.02 *M*. This solution was continuously deaerated with helium to avoid carbon dioxide absorption. The hydrolyses of apiin and *p*-nitrophenylapiose were carried out with 6 *M* hydrochloric acid in closed vials by heating at 80°C for 1 h. Acetonitrile was used to solubilize apigenin produced during the hydrolysis of apiin. The apigenin formed was then removed by precipitating it with water. The apiose solution was injected after filtration using a 0.45-μm Millipore HATF filter and a subsequent cleaning step with a Dionex OnGuard-RP cartridge to remove organic compounds.

Extraction, purification and hydrolysis of glycosides

A l-l volume of must, previously clarified with bentonite, was passed into a 60 cm x 3 cm I.D. glass column partially filled with XAD-2 resin (0.1-0.25 mm) (Serva, Heidelberg, Germany). The resin was washed with methanol, then with diethyl ether and finally with water. After percolation of the sample, the column was washed with 700 ml of deionized water to remove most of the free sugars, major constituents of the matrix. Subsequent elution with a pentane–dichloromethane (2:1, v/v) solution removed from the column the so-called "free compounds", *i.e.*, the non-glycosidic species. Further elution with 700 ml of ethyl acetate allowed the recovery of the required glycosides. This last fraction was made anhydrous with Na_2SO_4, concentrated with a rotating evaporator and filtered on a Millipore GS 0.22-μm filter. Glycosides were separated from traces of polyphenols by eluting the sample with a few millilitres of ethanol in a 5 cm x 1 cm I.D. polyvinylpolypyrrolidone column suitably activated [10]. The resulting solution was dried in a rotary evaporator.

The oily material obtained was again chromatographed on a 25 cm × 2 cm I.D. silica gel column [silica gel 60 (Merck), 0.2-0.5 mm] with 250 ml of ethyl acetate-ethanol (3 : 1, v/v) eluent to remove the last traces of organic compounds. The final solution was dried, filtered and then evaporated to dryness. The resulting yellowish material was washed with chloroform stabilized with ethanol (0.75%, v/v); a white glassy material was obtained.

To validate the recovery procedure adopted and the purity of the compounds obtained, two tests were performed: (i) after elution with ethyl acetate-ethanol the silica gel column was washed with 100 ml of methanol and the resulting solution was analysed for the presence of aglycones coming from the glycosides [11]; (ii) ^1H NMR analysis of the chloroform residuals was used to check for the absence of sugars and the presence of waxy compounds. In the same way, the absence of the waxy compounds in the glassy material was tested. The same hydrolysis method as described above for apiin and *p*-nitrophenylapiose was used for all the recovered glycosides.

Construction of the band microelectrode

The microband electrode assembly is shown in Fig. 1. From a 99.9% gold foil (2.5 cm x 2.5 cm × $5 \cdot 10^{-4}$ cm) (Goodfellow Metals, Cam-

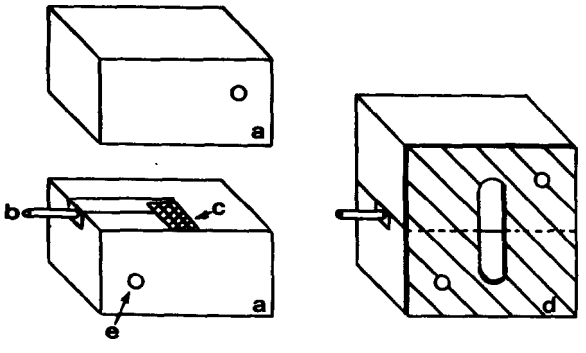

Fig. 1. Ultramicroband electrode assembly. a = Epoxy resin blocks; b = brass external contact; c = gold foil with dimensions of 0.4 cm x $5 \cdot 10^{-4}$ cm (the foil is glued to the electrical contact with silver paint); d = PTFE spacer enclosing the cell volume (50 μm thick); e = hole for the cell fitting.

bridge, UK) a rectangle of about 0.4 cm x 0.8 cm was cut, washed with 2 M nitric acid, then with water and finally dried in an oven for 2 h at 120°C. The gold foil was sandwiched between two blocks of epoxy resin (Epofix HQ, Struers, Copenhagen, Denmark) previously polymerized with 12% of triethylenetetramine and suitably ground. External electrical contact was made by inserting a brass pin into the centre of one of the two plastic blocks. The pin was placed in such a way as to make electrical contact with the gold foil (see Fig. 1). The contact, placed exactly in the centre of the block, was made with silver paint, then dried for 30 min in an oven at 50°C. The epoxy resin block containing the brass pin was covered in the upper part by adhesive tape in order to avoid sliding of the silver paint up to the edge of the block. The two blocks were soldered with the same epoxy resin. They were strongly pressed for 3 h to maintain the band in a straight position and then dried for 24 h in the oven at 80°C. The electrode was subsequently smoothed with 220–1000-grit Carborundum sheets supplied by Struers and then with 0.3-μm alumina. Two holes were made in order to attach the electrode to the detector body.

RESULTS AND DISCUSSION

Behaviour of the single microband electrode

Recent papers [12,13] claim the superiority of ultramicroband electrodes as detectors in HPLC systems for several reasons: (i) the peak current should be less dependent on the volume flow-rate than that generated by a conventionally sized electrode, (ii) the response should stabilize more rapidly, thus reducing the drift of the background current, which can affect the effective detection limit [13] and (iii) the signal-to-noise ratio should be larger owing to the lowering of the noise when it is produced predominantly by flow-rate fluctuations [14]. To verify the general behaviour of our laboratory-made gold microband electrodes and the results reported in the literature [12–14], we first tested the electrodes with standard analytes, in the flow-injection mode, and then in the chromatographic analysis of sugars. Figs. 2 and 3 (curves a) show the log-log plots of the current obtained with a continuous flow of the analyte ($1 \cdot 10^{-4}$ M dopamine solution) vs. the volume flow-rate for the disc and the microband electrode, respective-

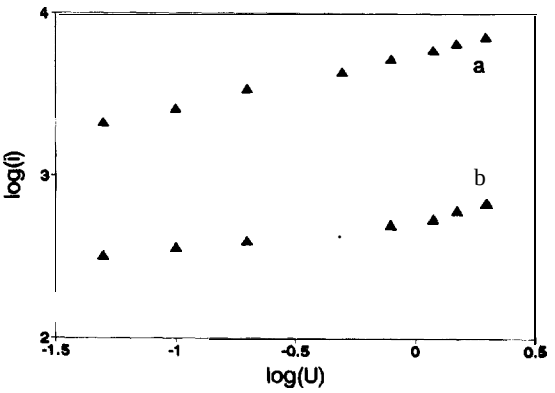

Fig. 2. (a) Log-log plot of the steady-state current (i_{ss}) vs. Row-rate (U) for a conventional-sized electrode under a continuous flow of $1 \cdot 10^{-4}$ M dopamine in 0.1 M sulphuric acid. (b) Log-log plot of the peak current (i_p) vs. U for injections of 20 μl of the same dopamine solution. Experimental conditions: knitted delay tube, 3 m (1.5 ml); polarization potential, 0.600 V vs. $Ag/AgCl/KCl_{sat.}$; eluent for the FI mode, 0.1 M sulphuric acid. Regression parameters: $\log i_{ss} = 3.76\ (\pm 0.01) + 0.330\ (\pm 0.007) \log U$, r = 0.998; $\log i_p = 2.74\ (\pm 0.03) + 0.19\ (\pm 0.01) \log U$, r = 0.95.

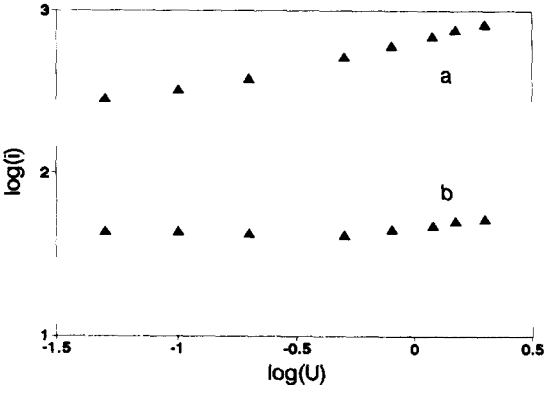

Fig. 3. Same as Fig. 2, but for the microband electrode. (a) Continuous flow of $1 \cdot 10^{-4}$ M dopamine. Regression parameters of the two approximate straight lines fitting the first three and the remaining five points: $\log i_{ss} = 2.773\ (\pm 0.004) + 0.25\ (\pm 0.01) \log V$, $r = 0.998$; $\log i_{ss} = 2.828\ (\pm 0.006) + 0.33\ (\pm 0.01) \log U$, $r = 0.995$. (b) Peak currents relating to 20-μl injections of $1 \cdot 10^{-4}$ M dopamine solution. Regression parameters of the two approximate straight lines fitting the first three and the remaining five points: $\log i_p = 1.625\ (\pm 0.004) - 0.018\ (\pm 0.011) \log U$, $r = 0.75$; $\log i_p = 1.676\ (\pm 0.006) + 0.17\ (\pm 0.01) \log U$, $r = 0.98$.

ly. The responses of the disc agree with the well known equation of Weber and Purdy [15]:

$$i = 1.47nFC^{b}(DA/b)^{2/3}U^{1/3}$$

which shows a dependence of the current on the flow-rate value to the 1/3 power. In this equation n is the number of electrons in the process, F is the Faraday (96487 C), C^{b} (mol/cm^3) is the bulk concentration, D (cm^2/s) is the diffusion coefficient, A (cm^2) is the electrode area, b (cm) is the thickness of the thin-layer channel and U (cm^3/min) is the volume flow-rate.

The results obtained with the microband electrode are in agreement with the data obtained in a previous study [16] dealing with the theoretical behaviour of ultramicroband electrodes in flowing solutions. The results of digital simulation procedures indicated in fact an increasing current density effect at the edges of the band, the so-called "edge effect", on reducing the solution flow-rate. This result explains the lowering of the slope of the plot of log i_p *vs.* log U reported in Fig. 3 (curve a), where it changes from 0.33 (high flow-rate) to 0.25 (low flow-rate).

Figs. 2 and 3 (curves b) show the log-log plots of the peak currents, i_p, *vs.* the flow-rate, U, for the same electrodes, disc and band electrodes when samples of 20 μl of dopamine were introduced into the system. The peak currents relative to the ultramicroband electrode exhibit a lower dependence on the flow-rate than that relative to the disc electrode.

It must be noted that our experimental results were obtained under the same dispersion conditions, as literature data [17,18] reported that for conventionally sized electrodes the slope of the log-log plot of peak current vs. flow-rate can vary continuously from positive to negative values depending on the dispersion conditions, i.e., from 1/3 to −1/6 for FI experiments [17] and from 5/6 to −1/6 for chromatographic tests [18]. In any event, the lower slope in Fig. 3b, which relates to results obtained with the band under FI conditions, can be justified by the trend found under continuous flow of the electroactive species.

In general, the microband electrode gave detection limits lower than those given by the conventional electrode either for dopamine or for hexacyanoferrate (II) always operating in the monoamperometric mode. For dopamine the detection limits are cu. 5 and 12 μg/l for the microband and the disc electrodes, respectively. When samples of glucose were analysed in FI experiments, the microband and conventional electrodes produced comparable detection limits (cu. 0.5 mg/l). The particular polarizing sequence adopted to detect carbohydrates and the nature of the electrochemical process (surface reactions) [19] are probably the causes of an increase in the noise with a consequent fall in the performance of the band detector on passing from the amperometric to the pulsed detection mode. However, the gains in the stabilization time, a few minutes instead of at least 30 min (see Fig. 4), and in the signal reproducibility remain unchanged. The reproducibility, measured as the relative standard deviation ($\sigma_C/C \cdot 100$) of ten repeated injections of 20 μl of 2 mg/l glucose solution, was 3%.

Chromatographic analysis of sugar mixtures

The hydrolysis of the recovered glycosides may give mixtures containing rhamnose, arabinose, galactose, glucose, mannose, xylose, ribose, apiose and fructose. It must be pointed out that the presence of fructose in the glycosides has never been reported [20] and therefore it is probably present as a contaminant coming from the must, which is composed mainly of glucose and fructose in the molar ratio 1: 1 [21]. This hypothesis is strongly supported by the fact that more prolonged washing of the resins with water reduced the amount of the fructose found. On the basis that free glucose and fructose follow the same route in the recovery of the glycosides from the must, the amount of glucose found has to be suitably corrected in turn by subtracting from it an amount equivalent to that of the fructose found.

Sugar mixtures similar to that reported above can be analysed either by gas chromatography after derivatization [22] or by gradient HPAEC [23]. In addition to problems of reproducibility and yields, FID is not sufficiently sensitive to detect concentrations below 1.0 mg/l (Fig. 5). With HPAEC the isocratic mode was preferred in order to avoid the postcolumn addition of

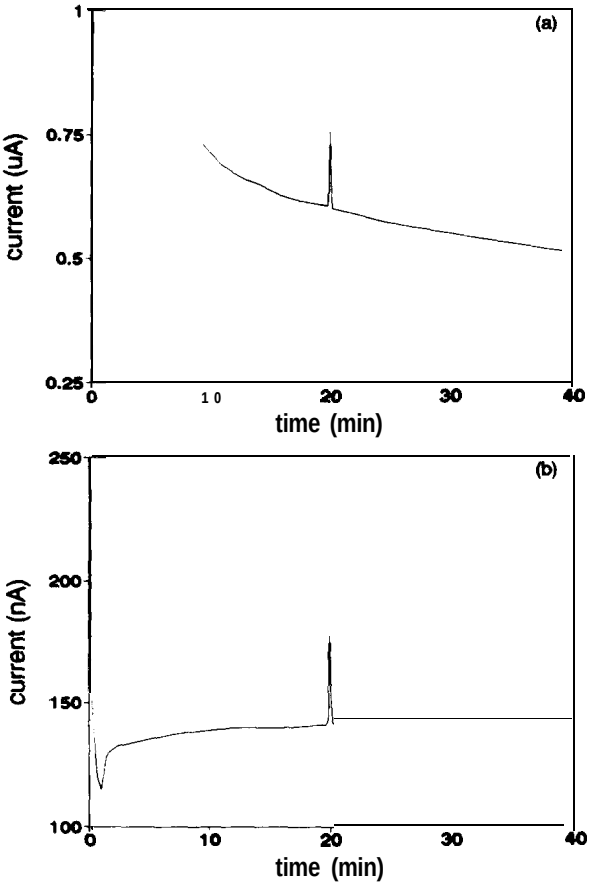

Fig. 4. Baseline drift for (a) disc electrode and (b) band electrode when starting with the polarization. Experimental conditions: eluent, 20 m*M* NaOH; flow-rate, 1 ml/min; sample injected, 20 μl of 1 mg/l glucose solution. Pulsed amperometric detection conditions: E_1 = 100 mV, T_1 = 0.8 s, E_2 = 650 mV, T_2 = 0.3 s, E_3 = -800 mV, T_3 = 0.160 s.

concentrated sodium hydroxide, a potential source of noise. Further, with the isocratic mode the long time required to restore the initial conditions of the analytical column was avoided. It must be noted that sometimes the overall time spent on one analysis, performed with a gradient operating mode, is heavily affected by the recovery time.

The best operating conditions were obtained with appropriate values of the eluent concentration, 20 m*M* NaOH, and the column temperature, 15°C. These values were chosen on the basis of a three level-two factor (10, 20 and 30°C and 15, 20 and 30 m*M* NaOH) factorial design. The influence of these factors comes from the

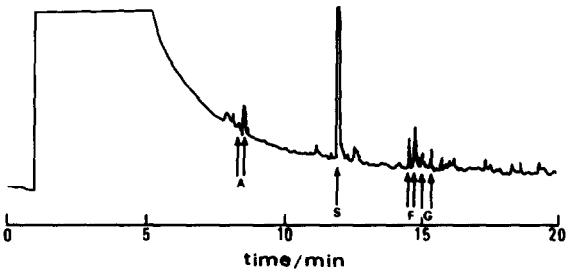

Fig. 5. Gas chromatographic response of a mixture of monosaccharides. Chromatographic conditions: carrier gas, hydrogen; flow-rate, 2 ml/mm; injection volume, 3 μl; splitting ratio, 1: 5; temperature programme, 130°C for 8 min then increased to 200°C at 6°C/min; injector and detector temperatures, 300°C. Analyte concentrations: (A) arabinose isomers 2.0 mg/l, (G) glucose isomers 2.1 mg/l, (F) fructose isomers 2.2 mg/l, (S) methyl-u-D-mannopyranose 40 mg/l as internal standard. All the sugars are separated as *cis–trans* isomers of the corresponding silanized oximes.

anionic nature of the eluted sugars and from the different dependences of their capacity factors on temperature. Fig. 6 shows in particular that lowering of the temperature affects the capacity factors of glucose, mannose and xylose in different ways, giving the greatest relative difference at 10°C.

Table I reports the resolution values obtained at 10, 15 and 20°C for the three poorly resolved sugars. It is evident that at 20°C xylose and mannose are not resolved whereas at 10°C the

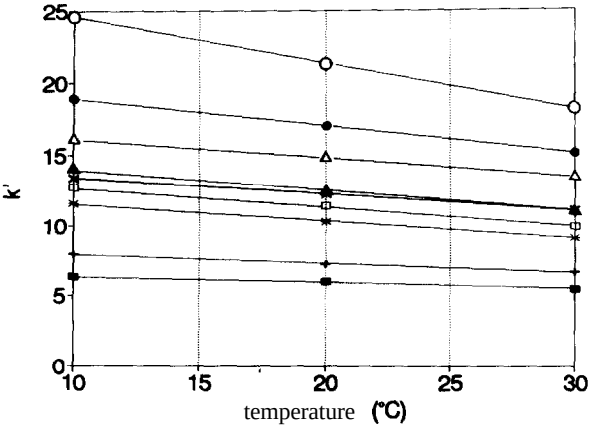

Fig. 6. Effect of column temperature on the capacity factors (k') of sugars. Chromatographic conditions: flow-rate, 1 ml min^{-1}; sample loop, 20 μl; column, Dionex CarboPac PA1 plus a CarboPac guard column; mobile phase, 20 m*M* NaOH in water; working electrode, gold band. ■ = Rhamnose; + = arabinose; * = galactose; □ = glucose; x = mannose; A = xylose; A = fructose; ● = ribose; 0 = apiose.

TABLE I

RESOLUTION VALUES" (R) AT DIFFERENT TEMPERATURES FOR THE THREE PEAKS UNDER DECONVOLUTION PROCEDURE

Resolution	Temperature (“C)		
	10	15	20
$R_{Glu/Man}$	1.15	1.08	1.48
$R_{Man/Xyl}$	0.82	0.82	0.31

[a] **Resolution calculated with: $R_{i,i-1} = (t_i - t_{i-1})/[0.5(w_i + w_{i-1})]$, where t_i and t_{i-1} are the retention times of the ith and $(i-1)$th peaks and w_i and w_{i-1} the corresponding baseline widths (w = 4σ for a Gaussian peak).**

best resolution is achieved for the three peaks. At 15°C the procedure gave resolution values similar to those obtained at 10°C but led to lower retention times, in particular for apiose, which is the most retained and broadened peak.

For the choice of the NaOH concentration not only the resolution among glucose, mannose and xylose was taken into account, but also the retention times of the other sugars, in particular that of apiose, which is the most retained and broadened sugar. Concentrations of NaOH lower than 20 m*M* produced impracticably long retention times.

Fig. 7a shows a typical chromatogram obtained under the described experimental conditions. It can be observed that in spite of the conditions adopted, partial overlap among glucose, mannose and xylose still remains. To verify whether the incomplete resolution could affect the accuracy of the quantitative analysis based on the peak heights in the concentration range of interest (a few mg/l), a deconvolution procedure involving a suitable mathematical model and the simplex method, as a regression procedure, was performed to measure the level of interference at the peak retention times. The model used is expressed by

$$Y_{mod.} = G_1(\sigma_1, h_1) + G_2(\sigma_2, h_2) + G_3(\sigma_3, h_3) + aX + b$$

which is constituted by three Gaussian curves (G) describing the peaks and a straight line describing the baseline drift. The number of

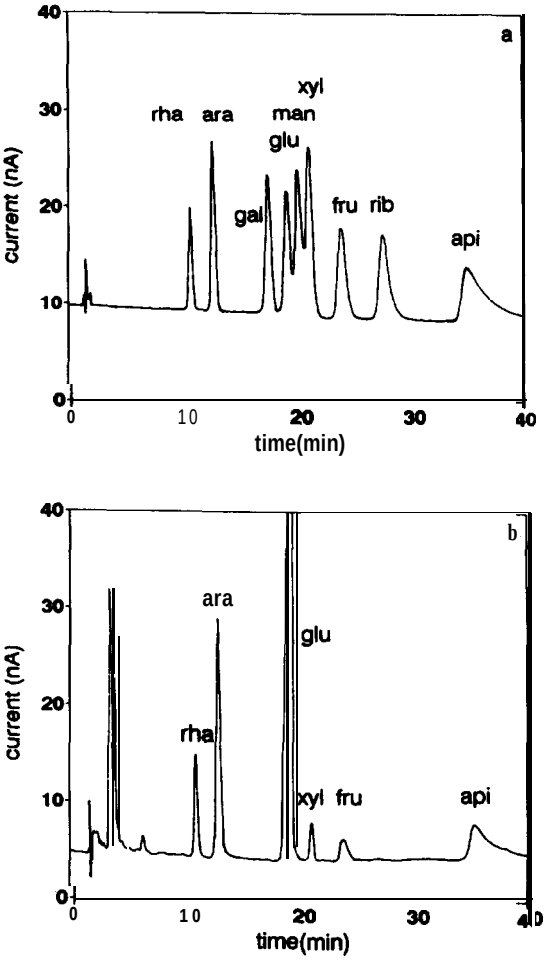

Fig. 7. (a) Anion-exchange elution profile of monosaccharide standards. Chromatographic conditions as in Fig. 6. Column temperature, 15°C. Approximate analyte concentrations: rhamnose 0.5, arabinose 0.5, galactose 0.5, glucose 0.5, mannose 1.0, xylose 0.8, fructose 0.8, ribose 0.8, apiose 1.5 mg/l. (b) Anion-exchange ehttion profile of monosaccharides obtained from hydrolysis of glycosides extracted from a real grape must. Chromatographic conditions as in (a).

parameters to be found is eight: one height (h) and one standard deviation (a) for each Gaussian curve and the slope (a) and the intercept (b) of the straight line. Fig. 8a indicates that the model adopted is correct and Fig. 8b demonstrates that the assumption of using the peak heights also for these partially overlapped peaks is correct, the interferences being negligible.

To verify whether different concentrations of the three sugars, always in the concentration range of interest (C < 10 mg/l), could affect the

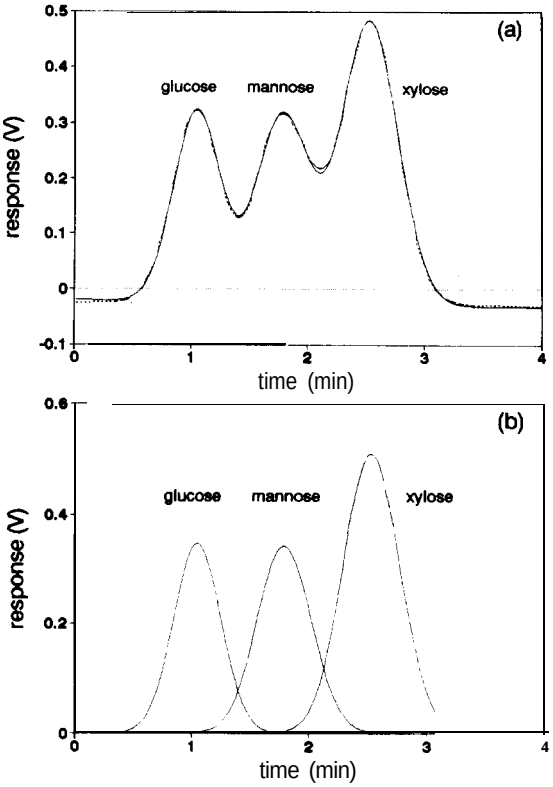

Fig. 8. (a) (Dashed line) experimental and (solid line) fitted chromatograms of glucose, mannose and xylose. (b) Deconvoluted peaks relative to these three sugars. Chromatographic conditions as in Fig. 7. Analyte concentrations: glucose 6, mannose 7, xylose 10 mg/l.

correctness of the hypothesis underlying our quantitative procedure, different analyses were simulated. The standard deviations (a) and the retention times (t) introduced in the model were those found for the three peaks previously deconvoluted ($\sigma_{glucose}$ = 12.5 s, $\sigma_{mannose}$ = 14.13 s, σ_{xylose} = 14.13 s, $t_{glucose}$ = 19.1 min, $t_{mannose}$ = 20.1 min, t_{xylose} = 20.9 min). This represents the most difficult choice as in real mixtures the concentration values were usually lower than those relating to Fig. 8a. The sensitivities were taken as equal on the basis of the experimental calibration lines.

Fig. 9 validates the procedure suggested, i.e., the use of the peak heights, indicating that in the worst situation, i.e., with ratios 10 : 1: 10 of the three sugars (Fig. 9b), the interference between glucose and mannose is virtually zero whereas that between mannose and xylose is about 4%. Consequently, only the errors coming from the confidence intervals of the calibration lines would be taken into account.

Table II reports the detection limits, computed as three times the standard deviation of the baseline noise, for the searched compounds at the two types of electrodes used. It is evident that both disc and band electrodes satisfy our requirements, i.e., detection limits below 1 mg/l. Even if the sugars give linear i_p vs. C plots over a wide concentration range [8], a drawback was found in the use of an external calibration plot. With the chosen NaOH concentration an appreciable, common, decrease in the retention times of the different analytes was found during the day with a concomitant narrowing of the peaks and an increase in the peak heights. This is due to the progressive, irreversible replacement of the hydroxide of the column by the strongly retained carbonate ions, always present in the eluent. For this reason, the correct performance of the column has to be restored by washing it every morning with 0.4 *M* acetic acid solution for 10 min and then with deaerated water. The change in the retention times during the day with the concomitant change in the peaks suggested that an external calibration procedure should not be adopted, but rather the response of the

TABLE II

DETECTION LIMITS (DL) CALCULATED AS PEAK HEIGHTS EQUAL TO THREE TIMES THE STANDARD DEVIATION OF THE BASELINE NOISE FOR THE DIFFERENT SUGARS

Sugar	DL (mg/l)	
	Disc	Band
Rhamnose	0.087	0.242
Arabinose	0.044	0.106
Galactose	0.072	0.126
Glucose	0.072	0.121
Mannose	0.195	0.256
Xylose	0.069	0.117
Fructose	0.175	0.261
Ribose	0.408	0.500
Apiose	–[a]	

[a] Standard not available.

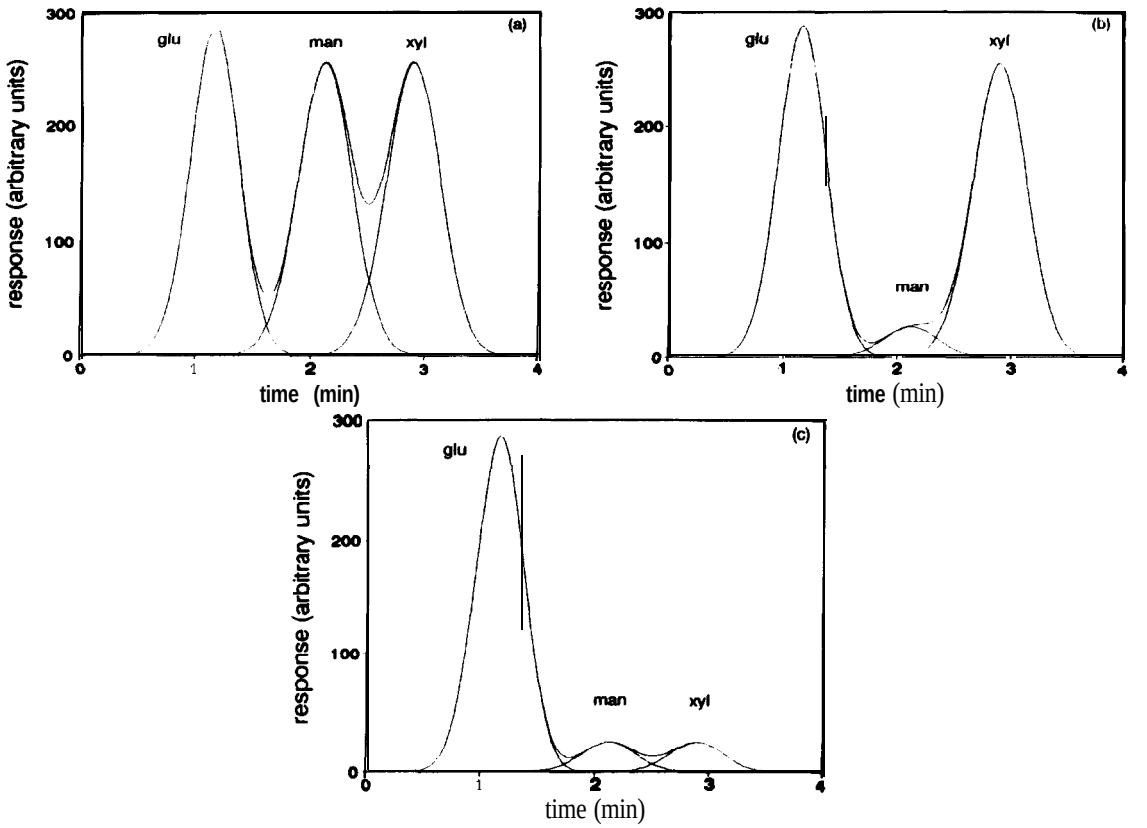

Fig. 9. Simulated peaks of glucose, mannose and xylose in the ratios (a) 10: 10: 10, (b) 10: 1: 10 and (c) 10: 1: 1. Standard deviations and retention times employed in the model: $\sigma_{glucose}$ = 12.5 s, $\sigma_{mannose}$ = 14.1 s, σ_{xylose} = 14.1 s, $t_{glucose}$ = 19.1 min, $t_{mannose}$ = 20.1 min, t_{xylose} = 20.9 min.

analytical system should be checked frequently with standard mixtures of the sugars with concentrations near those found for the analysed samples. The relative standard deviation was found to be about 5% by repeating six injections for every sugar.

The procedure has been applied to a variety of grape musts and a typical and interesting chromatogram is shown in Fig. 7b. Among the different sugars detected, the presence of apiose and xylose must be stressed. Apiose has recently been identified as a component of the glycosides [24], whereas this is the first time that xylose has been detected as a component of grape glycosides.

In conclusion, the procedure described here for the determination of the sugars is fairly rapid, sensitive and accurate even at trace levels,

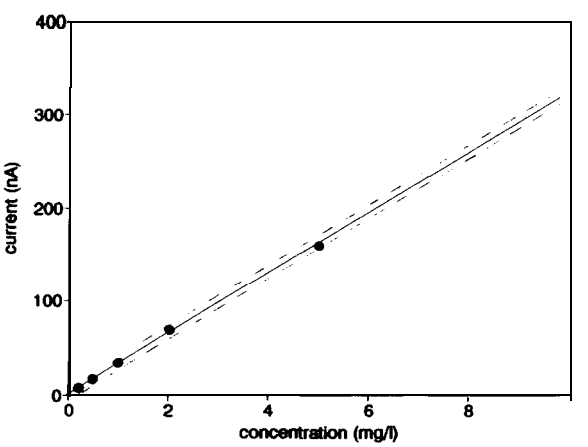

Fig. 10. (Solid line) calibration plot with (dashed lines) 95% confidence interval relative to arabinose. Chromatographic conditions as in Fig. 7. Regression parameters: $i = 1 (\pm 3) + 32.7 (\pm 0.4) C$, r = 0.9995.

as demonstrated by the calibration line for arabinose reported in Fig. 10 as a typical example. It does not require the use of toxic reagents and, when coupled to a suitable recovery procedure for the sugars, is an effective method to furnish data suitable for chemometric analysis. Work is in progress to obtaining a complete data set.

ACKNOWLEDGEMENTS

The financial support of the National Council of Research (CNR) and the Ministry of University and Scientific Research (MURST) is acknowledged with gratitude. The authors thank Professor Franco Magno for valuable comments.

REFERENCES

1 H. Craig, *Geochim. Cosmochim. Acta*, 12 (1957) 133.
2 G. Martin, B. Li Zhang, N. Naulet and M. Martin, *J. Am. Chem. Soc.*, 108 (1986) 5116.
3 S. Bitteur, Z. Gunata, J.M. Brillouet, C. Bayonove and R. Cordonnier, *J. Sci.* Food Agric., 47 (1989) 341.
4 B.L. Slomiany, A. Slomiany and A. Zdebska, *J. Biol. Chem., 257 (1984) 2863.*
5 Y.T. Li, S.C. Li, M.I. Horowitz and W. Pigman, The *Glycoconjugates*, Vol. 1, Academic Press, New York, 1977, pp. 52-67.
6 J.D. Olechno, S.R. Carter, W.T. Edwards and D.G. Gillen, *Am. Biotechnol. Lab.,* 5 (1987) 38.
7 D.C. Johnson and W.R. La Course, Anal. *Chem.*, 62 (1990) 589A.
8 W.R. LaCourse and D.C. Johnson, Carbohydr. *Res.*, 215 (1991) 159.
9 *G.* Versini, A. Dalla Serra and G. Margheri, *Vigne Vini*, 11 (1984) 41.
10 Y. Glories, *Connaiss. Vigne Vin,* 1 (1976) 51.
11 G. Versini, A. Dalla Serra, M. Dell'Eva, A. Scienza and A. Rapp, in P. Schreier (Editor), *Bioflavour '87, Proceedings of the International Conference, Wurzburg–RFG–Sept. 26–30, 1987,* Walter de Gruyter, Berlin, 1987, p. 161.
12 J.M. Zadeii, R. Mitchell and T. Kuwana, *Electroanalysis, 2* (1990) *209.*
13 J.W. Bixler, M. Fifield, J.C. Poler, A. M. Bond and W. Thormann, *Electroanalysis,* 1 (1989) 23.
14 W.L. Caudill, J.U. Howell and R.M. Wightman, *Anal. Chem., 54 (1982) 2532.*
15 *S.G.* Weber and W.C. Purdy, *Anal. Chim. Acta*, 100 (1978) 531.
16 P. Pastore, F. Magno, I. Lavagnini and CA. Amatore, *J. Electroanal. Chem., 301* (1991) 1.
17 P.L. Meschi and D.C. Johnson, *Anal. Chim. Acta, 124 (1981) 303.*
18 *S.* Prabhu and J.L. Anderson, *Anal. Chem., 59 (1987) 157.*
19 L.A. Larew and D.C. Johnson, *J. Electroanal. Chem.*, 262 (1989) 167.
20 S.G. Viorin, R.L. Baumes, SM. Bitteur, Z.Y. Gunata and C.L. Bayonove, *J. Agric. Food Chem., 38* (1990) 1373, and references cited therein.
21 S. Cancellier, A. Calo and A. Costacurta, *Proceedings of the Vth International Symposium on Grape Breeding, St. Martin, September 12-16, 1989, Vitis-Special Issue, 1990,* p. 81.
22 G. Eklund, B. Josefsson and C. Roos, *J. Chromatogr., 142 (1977) 575.*
23 *Carbohydrate Determinations by Anion Exchange with Pulsed Amperometric Detection; Technical Note No. 20,* Dionex, Sunnyvale, CA, 1987.
24 J.M. Brillouet, Z. Gunata, S. Bitteur, R. Cordonnier and C. Bosso, *J. Agric. Food Chem.*, 37 (1989) 910.