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A novel method for the determination of total 1,3-octanediols in apple juice via 1,3-dioxanes by solid-phase microextraction and high-speed gas chromatography

Daniel Díaz Llorente[a], Pilar Arias Abrodo[a,*], Enrique Dapena de la Fuente[b], Juan José Mangas Alonso[b], Maria Dolores Gutiérrez Álvarez[a], Domingo Blanco Gomis[a]

[a] Departamento de Química Física y Analítica, Universidad de Oviedo, C/ Julián Clavería 8, E-33006 Oviedo, Spain
[b] Servicio Regional de Investigación y Desarrollo Agroalimentario (SERIDA), Carretera de Oviedo s/n, E-33300 Villaviciosa, Spain

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ABSTRACT

In this work, a novel, simple and fast method based on solid-phase microextraction (SPME) followed by high-speed gas chromatography (HSGC) was developed for the analysis of total 1,3-octanediols in apple juices by means of derivatization reaction to volatile 1,3-dioxanes. The derivatization reaction, SPME conditions, glycosidically bound fraction and 1,3-nonanediol as a surrogate standard were studied. The formation of 1,3-dioxanes from 1,3-diols was confirmed by GC–MS. The method was validated obtaining a regression coefficient (r^2) of 0.9996, precisions between 0.3 and 9.8%, extraction recoveries in the range 94.7–112.2% and LOD of 2.9 $\mu g\,l^{-1}$. Experimental design has been employed in the optimization of extraction factors and robustness assessment. The method was applied to the analysis of 21 Asturian apple varieties finding a double reciprocal relationship between the concentrations of saturated and unsaturated 1,3-octanediol.

1. Introduction

R-octane-1,3-diol and its unsaturated form R-5(Z)-octene-1,3-diol are well-known natural apple constituents present in large amounts in cider apple varieties [1,2]. The β-glycols are present in their free and glycosidically bound form in apples. These antimicrobial β-glycols are effective in controlling microorganisms associated with infections but are harmless to human [3]. During fermentation, the β-glycols react with the considerable amount of acetaldehyde produced by yeast. The cyclic acetals formed are volatile compounds that have been detected in some French ciders [4] and are reported to have unique cidery aroma [5]. Fig. 1 shows the formation of 1,3-dioxanes from 1,3-diols and acetaldehyde.

Solid-phase microextraction (SPME) was introduced in the early 1990s by Arthur and Pawliszyn [6]. This technique is based on the partition of an analyte between the sample matrix and a fused silica fiber coated with a stationary phase. The analyte is then desorbed from the fiber into a suitable separation and detection system, usually a gas chromatograph. The main advantages of this technique are simplicity of operation, speed, its solventless nature, analyte/matrix separation and the preconcentration. Fibers are available in different coating combinations, blends or copolymers, film thickness and fiber assemblies, expanding to certain extent the field of possible applications [7].

High-speed gas chromatography is designed to minimize analysis time without compromising chromatographic resolution; it is usually achieved by reducing the characteristic diameter of the GC column. A major advantage of this technique is that the peak width is small and so the signal-to-noise ratio is larger. In addition to a reduced column diameter, nearly all high-speed GC instruments employ short columns. The concepts of high-speed gas chromatography have been reviewed by Cramers et al. [8]. A recent review by Snow [9] describes the practical implications of the use of short columns on high-speed GC method development, optimization, and resolution. Microbore (0.1 mm i.d.) columns are much more efficient than conventional 0.25 mm i.d. columns; this enables separations to be performed on shorter columns (10–20 m), with less carrier gas consumption and faster analysis times [10].

This paper describes for the first time the development of an analytical method for the determination of total R-octane-1,3-diol and R-5(Z)-octene-1,3-diol in apple juice after derivatization with acetaldehyde based on the natural formation of 1,3-dioxanes

* Corresponding author. Tel.: +34 985103511; fax: +34 985103125.
E-mail address: piarab@uniovi.es (P.A. Abrodo).

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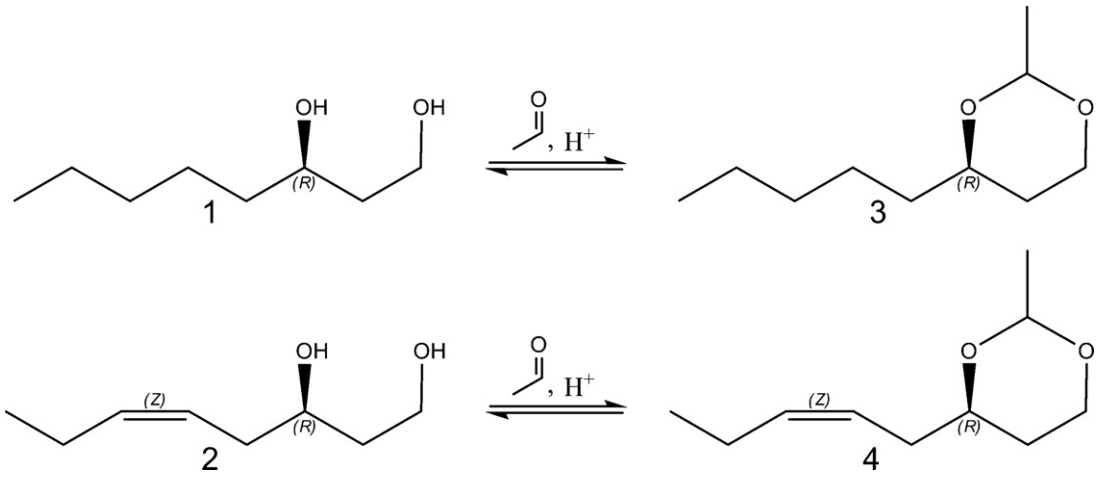

Fig. 1. Formation of 1,3-dioxanes from 1,3-diols in presence of acetaldehyde. (1) R-octane-1,3-diol; (2) R-5(Z)-octene-1,3-diol; (3) 2-methyl-4-(R)-pentyl-1,3-dioxane; (4) 2-methyl-4-(R)-[2′-(Z)-pentenyl]-1,3-dioxane.

during the cider fermentation. These 1,3-dioxanes are volatile compounds that can be isolated in the headspace of an apple juice sample using the SPME procedure and subsequently separated and detected in a high-speed gas chromatograph. The optimization of the main factors affecting the process and the robustness evaluation of the method were carried out by means of experimental design [11].

2. Experimental

2.1. Chemicals and reagents

2-Methyl-1-pentanol (internal standard), sodium chloride (99%), R-nonane-1,3-diol (97%, employed as a surrogate standard), acetobromo-α-D-glucose (>95%) and Dowex-50W (50–100 mesh, H^+ form) were purchased from Sigma–Aldrich (Madrid, Spain). Acetaldehyde (99%), sodium methoxide (0.5 M in methanol) and β-glucosidase from almonds 6 U/mg were purchased from Fluka (Madrid, Spain). Ethanol, hexane, diethyl ether, methanol and ethyl acetate gradient grade were obtained from Merck (Darmstadt, Germany). Silver (I) oxide (>99%) was purchased from Acros Organics (New Jersey, USA). MilliQ water (Millipore, Milford, MA) was used throughout.

2.2. Sample collection

Samples of 21 Asturian apples varieties belonging to the Protected Designation of Origin *Cider from Asturias* were picked by SERIDA at their optimal harvest time as determined by the starch index, a method based on colour development. Fruit to be sampled were visually inspected to ensure no apparent herbivore damage or disease prior to sampling. Apple juices were obtained by mechanic pressure and stored frozen. Samples were defrosted overnight at 4 °C prior to analysis.

2.3. Sample preparation

Apple juices were diluted to different proportions in water and 8 ml was added to a 15 ml SPME vial. Acetaldehyde (10% in water) amounts and pH values between 0–500 μl and 1–8, respectively, were evaluated to achieve the optimal conditions for the derivatization reaction. Reaction times in the range 0–420 min were considered to study the kinetic behaviour of the reaction. Amounts of NaCl between 0 and 4 g were tested to enhance the concentration of analytes in the headspace. Enzymatic hydrolysis was carried out with β-glucosidase from almonds to determinate the glycosidically bound 1,3-diols. Four different fiber coatings were employed to select the more effective one. An extraction time of 5 min (non-equilibrium conditions) was established to avoid long analysis times.

2.4. Solid-phase microextraction

The SPME apparatus was purchased from Supelco (Bellefonte, USA). A 100 μm polydimethylsiloxane (PDMS), 65 μm polydimethylsiloxane/divinylbenzene (PDMS/DVB), 75 μm carboxen/polydimethylsiloxane (CAR/PDMS) and 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber coatings were used. The fiber coatings were activated according to the manufacturer's instructions.

2.5. High-speed gas chromatography

Analyses were performed in a GC-2010 gas chromatograph (Shimadzu, Kyoto, Japan) equipped with a 10 m × 0.10 mm i.d. TRB-FFAP (Teknokroma, Barcelona, Spain) capillary column (film thickness = 0.20 μm) and a flame ionization detector (FID). The injector temperature was set at 250 °C and the detector at 280 °C. Helium was used as carrier gas at a linear velocity of 40 cm/s using constant flow method. The oven temperature was programmed for 80 °C increased at a rate of 70 °C/min to 200 °C and held for 2 min. Total analysis time was 4.14 min. Injections were made in the splitless mode.

2.6. Gas chromatography–mass spectrometry

The volatile 1,3-dioxanes obtained from the derivatization of 1,3-diols presents in apple juice were analyzed using a HP 6890 GC system, coupled with a HP MD5973 quadrupole mass spectrometer. The extracted compounds were separated on an HP-1MS capillary column (60 m × 0.25 mm i.d., 0.25 μm film thickness). Desorption of the SPME fiber was performed in splitless mode for 2 min. The column oven temperature was programmed to rise from an initial temperature of 40 °C for 5 min, followed by a ramp to 300 °C at 10 °C/min. The injection temperature and ion source temperature were 250 and 240 °C, respectively. Helium was used as carrier gas with a flow rate of 1 ml/min. The ionization energy was 70 eV. All data were obtained by collecting the full-scan mass spectra within the scan range of 33–350 amu. The compounds (2-methyl-4-(R)-pentyl-1,3-dioxane and 2-methyl-4-(R)-[2′-(Z)-

pentenyl]-1,3-dioxane) were identified using the previous reported mass spectra [4].

2.7. Optimization procedure and robustness evaluation by experimental design

A 2^4 two-levels full factorial design (FFD) was performed to investigate the effects of temperature of extraction (T), time of extraction (t), NaCl amount and pH in order to obtain the maximum sensitivity. Low and high levels were selected based on preliminary tests: T = 25–35 °C, t = 1–5 min, NaCl = 0.5–3 g and pH 1–5. Four centre-points were added to ensure enough degrees of freedom for error evaluation. The order of the experiments was fully randomised in order to avoid possible memory effects of the analytical apparatus. This experimental plan allows the evaluation of the effects of the main factors and of their interactions.

In order to test the method robustness some factors which represent potential sources of variability were selected and examined in an interval around the nominal level using experimental design. In this case the selected factors related to the operating procedure employed were quantitative and were defined as temperature of extraction (T = 23–27 °C), time of extraction (t = 4.9–5.1 min), NaCl amount (NaCl = 2.9–3.1 g) and pH (0.9–1.1). The experimental ranges selected were representative of the variations which can be expected when the method is transferred. In all cases, data analysis was performed by means of the statistical package Statgraphics Centurion XV for Windows Version 15.2.06.

3. Results and discussion

The analysis of 1,3-octanodiols has a remarkable importance in the cider production field due to its contribution in cider aroma. These β-glycols have been traditionally analyzed by GC-FID and GC–MS with high analysis times (1–2 h) [12]. Moreover, these methods involved high time-consuming liquid–liquid extractions with large amounts of organic solvents such as dichloromethane. High-speed gas chromatography has allowed us to reduce the analysis time to 4.14 min. In addition, SPME avoided the use of organic solvents reducing considerably the sample treatment time. Because the standard compounds of 1,3-octanediols are not commercially available, 1,3-nonanediol has been tested as a surrogate standard in the determination of 1,3-octanediols by means of SPME analysis validation with a liquid–liquid extraction method. In this study, no differences between the extraction methods have been found. Thanks to the use of GC-FID the 1,3-diols effective carbon numbers (ECNs) and Relative Response Factors (RRFs) have been estimated with the aim to quantify this compounds constructing a calibration curve for 1,3-nonanediol.

3.1. Optimization of derivatization reaction

In this section the different parameters that can affect the yield of derivatization reaction of 1,3-diols to form volatile 1,3-dioxanes have been investigated. These parameters were apple juice and acetaldehyde amounts, pH value and reaction time.

In a first approach, several reactions were carried out using different sample amounts of a reference apple juice, consisting of a mixture of Asturian cider apple juices. The amounts employed were 100, 200, 300, 400 and 500 μl in 8 ml of MilliQ water. Moreover, 3 g NaCl and 100 μl of a solution containing 10% acetaldehyde in water were added. Samples were acidified to pH 3.5 and the mixture was placed at 25 °C overnight (14 h) under magnetic stirring to complete the reaction. SPME extractions employing a PDMS/DVB fiber coating were performed at 25 °C for 5 min in a replicate analysis (n = 3) with desorption time of 2 min. A linear relationship between the amount of apple juice and the chromatographic response was observed. The regression coefficient (r^2) was 0.998. Using 100 μl of apple juice high peak areas were obtained in the chromatographic system. Analysis of samples containing 200 μl or bigger quantities resulted in excessively high and not well defined peaks, and, hence, 100 μl was regarded as the optimal apple juice amount in subsequent experiments.

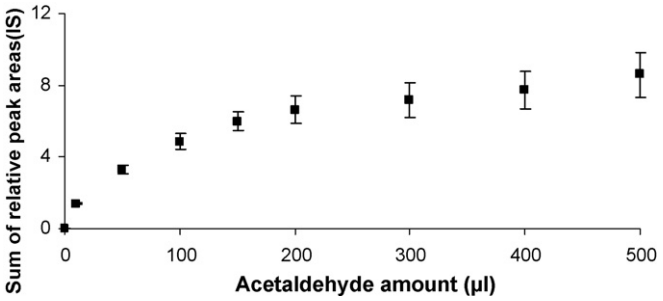

Fig. 2. Influence of acetaldehyde amount on 1,3-dioxanes relative peak areas.

Next, the amount of acetaldehyde added was studied. 2-Methyl-1-pentanol was employed as internal standard because it improves the method by means of a decrease in CVs. For this purpose, amounts between 0 and 500 μl of a solution containing 10% acetaldehyde in water were tested. We employed water solution because of the use of pure acetaldehyde involved losses of this reagent. The sum of peak areas (n = 3) relative to internal standard (IS) as a function of acetaldehyde amount is shown in Fig. 2. In this figure we can distinguish two regions, being the first between 0 and 150 μl a region with a sharp increase in relative peak areas as a function of acetaldehyde amount. In the second region, between 200 and 500 μl, the increase is less than previous one. Therefore, 200 μl was chosen as the acetaldehyde solution amount.

Then, the optimal pH value was evaluated to enhance the derivatization reaction rate by means of the catalytic effect produced by acid media in the acetylation reaction. For this purpose, pH values between 1 and 8 were tested. The relative peak areas for both compounds increase noticeably at low pH values except for unsaturated 1,3-octanediol at pH 1 where a slight reduction in peak area occurs. Nevertheless at pH 1 the reaction rate is greatly amplified and, consequently, this pH value was selected as the optimum.

Finally, with the aim of study the kinetic behaviour of the reaction, extractions (n = 3) were carried out within an interval between the reaction start and 420 min (Fig. 3). As can be observed an increase in relative peak areas takes place from the starting point to 60 min. Thus, 60 min was selected as most favourable reaction time.

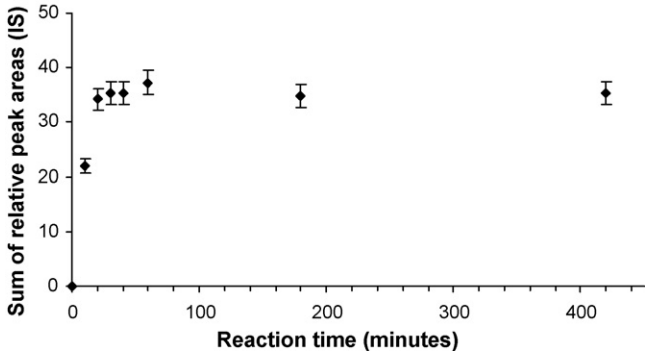

Fig. 3. Evolution of the relative peak areas with the reaction time.

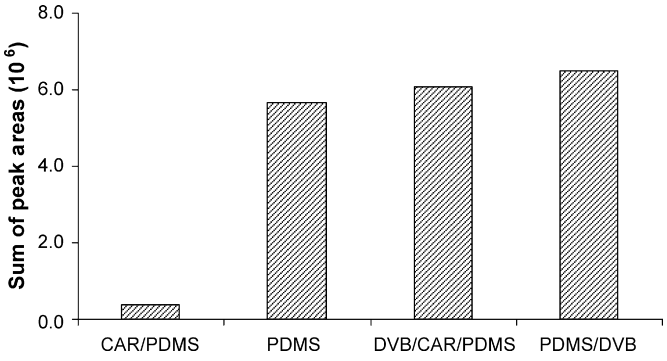

Fig. 4. Influence of the investigated fiber coatings on the SPME efficiency.

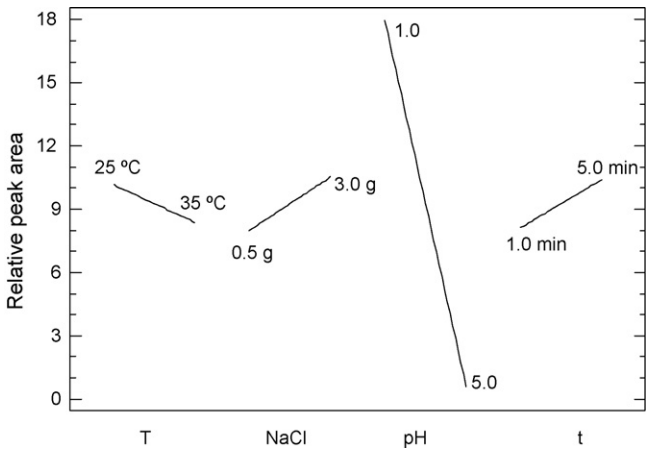

Fig. 5. Main effects influence on the solid-phase microextraction of 1,3-dioxanes.

3.2. Optimization of SPME conditions

The choice of an appropriate coating is necessary for a SPME method. The extraction efficiency depends on the molecular mass and polarity of the analytes. Four commercially available SPME fiber coatings, with different polarity and inner structure, were tested for the efficiency of SPME extraction of 1,3-dioxanes in apple juice as mentioned above. Reference apple juice was used to optimize the SPME conditions. In Fig. 4 the effect of fiber coating on the sum of peak areas of the 1,3-dioxanes can be observed. As seen from this figure, the best extraction efficiency was obtained by using PDMS/DVB fiber. Therefore, the PDMS/DVB was regarded as the optimal fiber coating and used in the work.

Then, NaCl amounts between 0 and 4 g in 8 ml sample were evaluated. In this study, an increase in relative peak areas was observed between 0 and 3 g. This was due to the salting out effect produced by an increase in the ionic strength of the sample. After that, the relative peak areas remained without important differences. The formation of a NaCl precipitate was detected between 3 and 4 g caused by the saturation of NaCl in water (solubility 3.6 g/10 ml).

Extraction time of 5 min was established to avoid high analysis times, because our previous works indicated that equilibrium between fiber coating and headspace was not reached in a reasonable extraction time regarding the chromatographic analysis time (4.14 min). Desorption time of 2 min at 250 °C was fixed since we confirmed that the total analytes amount was desorbed under this conditions.

So as to assess the interactions among the studied effects, i.e., temperature of extraction, time of extraction, NaCl amount and pH, the optimization of these factors was carried out by means of experimental design. Response was evaluated in terms of 1,3-dioxanes relative peak areas. The analysis of these results showed that not all the initially selected variables produced significant effects and that no significant interactions between factors were apparent. Fig. 5 shows the main effects plot for SPME of 1,3-dioxanes in apple juice samples. The main factor plot shows the estimated variable as a function of each experimental factor. In each plot the factor of interest varies from its lowest level to its highest level, while all the factors remain constant at their central values.

As it can be observed, pH was the factor with greatest effect (in fact, the only statistically significant factor). As can be expected, the relative peak area for 1,3-dioxanes increases when the pH decreases due to the catalytic effect of H^+ in the acetylation reaction (Fig. 1). With these results temperature of extractions of 25 °C, NaCl amount of 3 g, pH of 1 and extraction time of 5 min were fixed to obtain the higher results.

3.3. Study of glycosidically bound 1,3-diols

Enzymatic hydrolysis was carried out with β-glucosidase from almonds to determinate the glycosidically bound 1,3-diols. In this comparative study, reference apple juice was analyzed without hydrolysis and after enzymatic hydrolysis using β-glucosidase. 20 mg of β-glucosidase was added to 5 ml apple juice at pH 5, adjusted with NaOH 1 M, and incubated at 35 °C for 24 h. Finally, both apple juices were analyzed with the method exposed below. The analysis showed that there is no difference between 1,3-dioxanes peak areas using β-glucosidase as hydrolyzing agent. This indicate that both free and glycosidically bound 1,3-octanediols could react to form 1,3-dioxanes under the conditions exposed below, due to the fact that glycosidically bound forms are present in all apple varieties [13]. With the aim to confirm the derivatization of glycosidically bound forms in cider apples, a synthesis of 3-hidroxy-nonyl-β-D-glucoside was carried out according to Ref. [13] conditions and characterized by ^{1}H NMR. Next, this glucoside was derivatized and extracted under the conditions exposed below. A chromatographic peak corresponding to 2-methyl-4-(R)-hexyl-1,3-dioxane was found confirming that the glycosidically bound forms of 1,3-diols react under these conditions to form 1,3-dioxanes. For this reason, the proposed method is a total 1,3-octanediols analysis method that determine free and glycosidically bound forms as the same compound.

3.4. Identification of 1,3-dioxanes by GC–MS analysis

To verify the formation of 1,3-dioxanes under the conditions exposed below, a reference apple juice sample was derivatized, extracted by SPME and introduced in a GC–MS instrument. The mass spectral data obtained (Fig. 6) were in concordance with the previous reported for 2-methyl-4-(R)-pentyl-1,3-dioxane (**3**) and 2-methyl-4-(R)-[2′-(Z)-pentenyl]-1,3-dioxane (**4**) in Ref. [4]. With these evidences, we can confirm the correct development of a method for the formation of 1,3-dioxanes from 1,3-diols present in the apple juices.

3.5. Method validation

Because the standard compounds of 1,3-octanediols are not commercially available, 1,3-nonanediol has been tested as a surrogate standard in the determination of 1,3-octanediols by means of a comparative study between SPME and liquid–liquid extraction. In this study we could evaluate the selectivity of fiber coating between 1,3-octanediols and 1,3-nonanediol. For this purpose, we analyzed the same apple juice sample by two different methods.

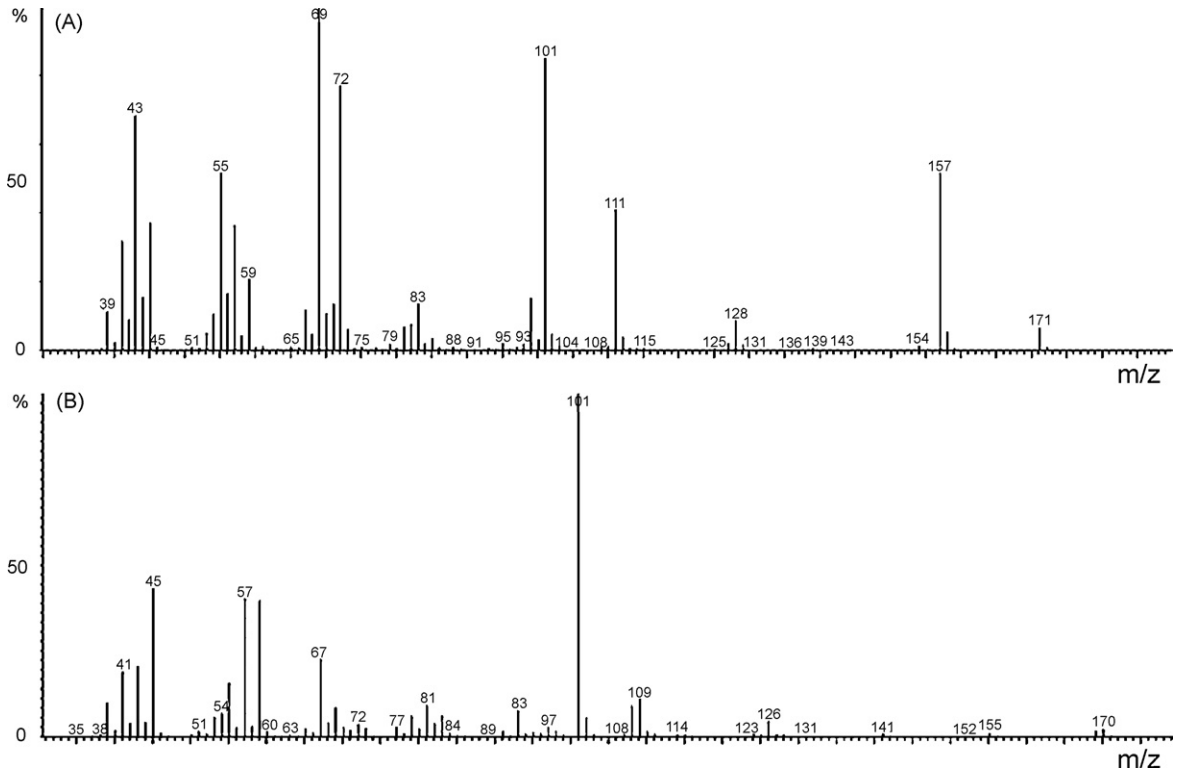

Fig. 6. Mass spectral data of **3** (A) and **4** (B).

In both cases we employed 1,3-nonanediol to construct the calibration curve. In the first analysis, the method developed in this work was used and an external calibration curve at five different concentrations between 5 and 1500 $\mu g\,l^{-1}$ was constructed. In the second analysis, a liquid–liquid extraction method was developed using 10 ml apple juice at pH 1, 10 ml MilliQ water, 10 ml solution containing 10% acetaldehyde in water and 2-metyl-1-pentanol as internal standard. This mixture was placed at 25 °C overnight under magnetic stirring to complete the reaction. Then, a liquid–liquid extraction with 10 ml ethyl acetate in three steps was carried out. Finally the organic layer was injected into the GC-FID system with a split ratio of 1:100. An external calibration curve at five different concentrations between 1 and 80 $mg\,l^{-1}$ was constructed. Three replicates for each concentration were performed in the two methods employed. The calibration curves showed regression coefficients of 0.997 and 0.998, respectively. Table 1 shows the 1,3-diol concentrations expressed in $mg\,l^{-1}$ and determined by the two proposed methods. As we can see, the agreement between methods indicates that the SPME procedure with PDMS/DVB coating is an adequate method since the fiber does not discriminate 1,3-octanediol dioxanes from 1,3-nonanediol dioxane.

The response of the flame ionization detector (FID) is proportional to the carbon number of hydrocarbon molecules; however, heteroatoms attached to the carbon chain can decrease the detector's response. The concept of an effective carbon number (ECN) was introduced to estimate the relative response for any compound. The set of parameters used to calculate effective carbon numbers have been tabulated [14]. Thus, the ECNs for 1,3-octanediol dioxanes and 1,3-nonanediol dioxane have been estimated according to the tabulated values. Next, the 1,3-nonanediol Relative Response Factors (RRFs) for 1,3-octanediols have been computed according to the following expression RRF = (MW of compound)(ECN of standard)/(MW of standard)(ECN of compound) [15]. In Table 2 the ECNs and RRFs for each analyzed compound can be seen. From this time we employed the RRFs as correction factors for the interpolation in the calibration curve, constructed with 1,3-nonanediol dioxane, of the area values obtained for 1,3-octanediol dioxanes.

Calibration curve of 2-methyl-4-(R)-hexyl-1,3-dioxane was constructed with the SPME proposed method at five different concentrations between 5 and 1500 $\mu g\,l^{-1}$. Three replicates for each concentration were performed, obtaining relative standard deviations (RSDs) ranging from 2.8 to 11.7% for run-to-run precision. The regression equation was (area/area IS) = 4.8823[standard] − 0.0092 and it showed a regression coefficient of 0.9996. This equation was used to determinate the concentration of 1,3-diols in apple juices employing the RRFs to correct the peak areas of analytes.

Precision of the overall analytical procedure has been evaluated as repeatability, by means of replicate real sample analysis ($n = 3$), and it was expressed as RSD (%) of the calculated concentrations. The method has been found to have satisfactory precision, with coefficients of variation between 0.3 and 9.8% for the studied compounds (Table 3).

Table 1
Concentrations of 1,3-diols calculated by the proposed methods (CV = coefficient of variation).

Compound	Concentration ($mg\,l^{-1}$)		
	Method A (SPME)	Method B (L–L extraction)	% CV
R-Octane-1,3-diol	25.26	26.18	2.55
R-5(Z)-Octene-1,3-diol	4.28	4.16	2.01

Table 2
Relative Response Factors of 1,3-dioxanes calculated by means of effective carbon numbers.

Compound	ECN	RRF
2-Methyl-4-(R)-hexyl-1,3-dioxane	9	1
2-Methyl-4-(R)-pentyl-1,3-dioxane	8	1.0403
2-Methyl-4-(R)-[2′-(Z)-pentenyl]-1,3-dioxane	7.9	1.0412

Table 3
Determination of 1,3-octanediols in 21 Asturian cider apple varieties (mg l^{-1}).

Apple variety	R-Octane-1,3-diol	% CV	R-5(Z)-Octene-1,3-diol	% CV
Solarina	13.84	3.2	1.89	3.7
Clara	94.45	6.0	11.48	3.4
Verdialona	8.22	3.3	1.37	2.2
Meana	29.48	3.7	9.84	3.6
San Roqueña	2.34	9.8	0.63	2.4
Limón Montes	55.00	2.0	8.33	1.0
Xuanina	11.60	4.7	2.66	5.4
Durona de Tresali	20.31	2.4	3.16	1.2
Ernestina	7.37	7.1	1.15	3.4
Carrió	16.05	3.6	2.25	3.8
Collaos	31.24	2.7	11.22	3.2
Panquerina	2.46	0.4	0.87	5.6
Prieta	35.03	3.6	2.67	1.8
Blanquina	4.81	4.1	1.18	5.3
Regona	58.00	1.9	8.48	2.7
Perezosa	5.30	2.1	1.23	1.4
Teórica	46.43	3.1	7.05	4.8
Coloradona	41.83	2.9	5.45	4.7
De la Riega	9.91	4.4	1.65	5.8
Raxao	4.93	0.3	1.47	0.3
Perico	17.16	2.0	3.41	2.9

The recovery of the method has been studied by analyzing spiked apple juice samples at three concentrations of 1, 50 and 100 mg l^{-1} for 1,3-nonanediol. Extraction recoveries ranging from 94.7 to 112.2% ($n = 3$) were obtained, thus showing the good efficiency of the developed method in terms of accuracy.

Limit of detection (LOD), defined as the lowest concentration that the analytical process can differentiate from background levels, was estimated for a signal-to-noise ratio (S/N) of 3 from the chromatograms corresponding to the extraction of real samples. The method allowed reaching limit of detection of 2.8 μg l^{-1} for 1,3-nonanediol, that involve detection limits of 2.9 μg l^{-1} for 1,3-octanediols.

Finally, the robustness of the method was evaluated by means of experimental design. A full factorial design with the factors and ranges explained below was selected for this research. The chosen design included 20 experiments, four of which were performed at the centre point to estimate the experimental repeatability. The experiments were performed, in a random sequence, using different aliquots of the same apple juice. The responses measured were expressed as 1,3-dioxanes peak areas. An analysis of variance (ANOVA) was performed on the design to assess the significance of the model. The analysis of the results from ANOVA showed that the studied variables were not significant with regard to the response. The Pareto chart of effects is shown in Fig. 7. In this chart, the bar lengths are proportional to the absolute value of the estimated main effects. This figure also includes a vertical line corresponding to the

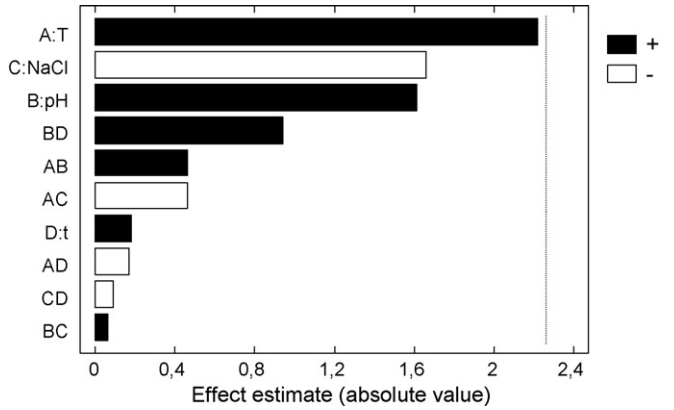

Fig. 7. Pareto chart of the main effects obtained from 2^4 full factorial design.

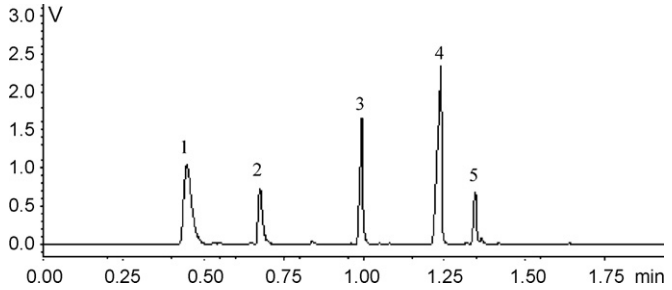

Fig. 8. Chromatogram of 1,3-octanediols in apple juice by SPME-GC-FID. Peak identification: (1) acetaldehyde; (2) ethanol; (3) 2-methyl-1-pentanol (IS); (4) 2-methyl-4-(R)-pentyl-1,3-dioxane; (5) 2-methyl-4-(R)-[2′-(Z)-pentenyl]-1,3-dioxane.

95% confidence interval. An effect, which exceeds this reference line, may be considered statistically significant for the response. The sign of the main effects showed that the response would be improved or not on passing a given factor from the lower to the high level. As can be seen from this figure, the studied factors and their interactions have no significant influence in the analytical response of the method for the selected intervals and, consequently, the robustness of the method has been established.

3.6. Analysis of 1,3-octanediols in different Asturian apple juices

The proposed method was applied to the analysis of 1,3-octanediols in 21 juices corresponding to apple varieties belonging to the Protected Designation of Origin *Cider from Asturias*. The high-speed gas chromatogram of 1,3-octanediols in an apple juice is shown in Fig. 8. As seen from this figure, two 1,3-octanediol dioxanes, 2-methyl-1-pentanol (IS), ethanol from IS solution and exceeded acetaldehyde have been resolved in less than 1.5 min. The analytical data obtained in GC-FID from apple juices are reported in Table 3, where apple variety and concentrations of 1,3-octanediols, expressed in mg l^{-1}, are shown. As can be seen, Clara variety is shown as having the greatest concentration in the saturated and unsaturated 1,3-octanediols with amounts of 96.45 and 11.48 mg l^{-1}, respectively. By contrast, San Roqueña variety with 2.34 and 0.63 mg l^{-1} shows the smallest amount of 1,3-diols. With these results it is important to highlight the great differences observed in the 1,3-diol concentrations of different Asturian cider apple varieties. These differences could affect to the organoleptic properties of cider, mainly its aroma and will allow us to typify the different apple varieties. Concentration of saturated β-glycol was higher than unsaturated one in all apple cultivars studied. As it is well known, linoleic and linolenic acids are the precursors of saturated and unsaturated β-glycols, respectively, from a mechanism of β-oxidation of fatty acids [16], and apple contains higher levels of linoleic acid than linolenic acid [17,18].

Additionally, the relationship between the saturated and unsaturated 1,3-octanediol has been evaluated using Statgraphics Centurion XV software. In this study we found that there was a double reciprocal relationship between saturated and unsaturated. The equation of the fitted model was: unsaturated = 1/(0.00982835 + 3.59701/saturated). Since the *P*-value in the ANOVA test was less than 0.01, there was a statistically significant relationship between unsaturated and saturated at the 99% confidence level. The *r*-squared statistic indicated that the fitted model explains 90.2% of the variability in unsaturated after transforming to a reciprocal scale to linearize the model. The correlation coefficient was 0.95, indicating a relatively strong relationship between the variables, which can be explained by both molecules are probably biosynthetised from the same mechanism of C18 unsaturated fatty acids degradation.

4. Conclusions

A method based on solid-phase microextraction (SPME) followed by high-speed gas chromatography has been developed for the determination of total 1,3-octanediols responsible of cider aroma in apple juices by means of 1,3-dioxanes formation. The method has been validated showing satisfactory values and 1,3-nonanediol has been demonstrated to be a good surrogate standard for the 1,3-octanediols determination. In addition, free and glycosidically bound 1,3-octanediols react under these conditions to form 1,3-dioxanes.

The developed method was applied to the analysis of 21 apple juices belonging to different Asturian apple varieties. Clara variety is shown as having the greatest concentration in the 1,3-octanediols and San Roqueña variety shown the smallest amounts. Moreover, great differences between varieties that could affect to the organoleptic properties of cider have been found, and a higher concentration of saturated β-glycol with respect to unsaturated one was detected in all samples, which is related to the higher concentration of linoleic acid in apple. Additionally, we found a double reciprocal relationship between saturated and unsaturated 1,3-diols.

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References

[1] G. Brulé, Ann. Technol. Agric. 22 (1973) 45.
[2] T. Beuerle, W. Schwab, Lipids 34 (1999) 617.
[3] D.L. Wright, J.W. Frankenfeld, United States Patent, 3,836,672, September 17 (1974).
[4] C. Dietrich, T. Beuerle, B. Withopf, P. Schreier, P. Brunerie, C. Bicchi, W. Schwab, J. Agric. Food Chem. 45 (1997) 3178.
[5] A.G.H. Lea, J.R. Piggot (Eds.), Fermented Beverage Production, Blackie Academic & Proffesional, London, 1995, p. 66.
[6] C.L. Arthur, J. Pawliszyn, Anal. Chem. 62 (1990) 2145.
[7] C. Dietz, J. Sanz, C. Cámara, J. Chromatogr. A 1103 (2006) 183.
[8] C.A. Cramers, H. Janssen, M.M. Van Deursen, P.A. Leclercq, J. Chromatogr. A 856 (1999) 315.
[9] N.H. Snow, J. Liquid Chromatogr. R.T. 27 (2004) 1317.
[10] A.R. Boden, G.E. Ladwig, E.J. Reiner, Polycycl. Aromat. Comp. 22 (2002) 301.
[11] S.N. Deming, S.L. Morgan, Experimental Design: A Chemometric Approach, Elsevier, Amsterdam, 1993.
[12] P.M. Brunerie, United States Patent, 6,384,243 B1, May 7 (2002).
[13] W. Schwab, G. Scheller, D. Gerlach, P. Schreier, Phytochemistry 28 (1989) 157.
[14] R.L. Grob, Modern Practice of Gas Chromatography, John Wiley and Sons, New York, 1995.
[15] A.R. Katriztzky, E.S. Ignatchenko, R.A. Barcock, V.S. Lobanov, M. Karelson, Anal. Chem. 66 (1994) 1799.
[16] G.R. Dettweiler, R.G. Berger, F. Drawert, Deutsche Lebensmittel-Rundschau 86 (1990) 174.
[17] T.N. Prabha, P.L. Raina, M.V. Patwardhan, J. Biosci. 13 (1988) 33.
[18] J. Song, F. Bangerth, Postharvest Biol. Technol. 30 (2003) 113.