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Determination of glycerol in wines by high-performance liquid chromatography: comparison with enzymatic method*

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ABSTRACT

A method for determining glycerol in wines by high-performance liquid chromatography has been developed using three 250 × 8 mm columns based on a sulphonated styrene–divinyl benzene polymer (SHODEX S-801/S,S-802/S,S-802/S) in series This polymer combines cation-exchange mechanisms with molecular exclusion The columns were thermostated at 75°C using water as the mobile phase A refraction index detector was used and the samples were directly injected The method is reproducible (coefficient of variation above 1%) and accurate Linear regression analysis and Student's *t*-test for comparison of the mean show that the results are comparable with those obtained by an enzymatic method (differences less than 4%)

INTRODUCTION

Glycerol is the major component of wine (after water and ethanol) and is a secondary product of alcoholic fermentation It contributes to the sensory properties of wine, such as body, smoothness and sweetness

The official chemical method of analysis [1] requires a long and tedious sample preparation, with non-quantifiable losses of glycerol occurring [2] and anomalous higher values attributable to mannitol and sorbitol However, enzymatic methods [3] are specific, accurate and reproducible [4] and can be applied in segmented continuous-flow analysers [5]

Gas chromatographic determination of glycerol in wines can be carried out by direct injection on packed columns [6,7] However, problems sometimes arise because the desorption is not quantitative when porous polymers are used as stationary phases

These problems are avoided by using capillary columns, but it is necessary to prepare the sample by extraction with organic solvents [8] or the formation of derivatives before analysis [9]

Different procedures have been developed using high-performance liquid chromatography (HPLC) with columns of the amino type [10,11], or based on cation-exchange mechanisms [12], a cation-exchange column (Aminex HPX-87H, Bio Rad, Richmond, CA, USA) has been used by several

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workers [13,14] without sample preparation, although one study [15] suggested passing the wine through ion-exchange resins to avoid interference from the organic acids present, an aspect which has also been reported by Flak [10]

In all these instances a refraction index detector was used Conductivity detectors [16] and post-column reactors with fluorimetric [17] or pulsed amperometric [18] detectors have also described A method using ^{13}C nuclear magnetic resonance spectroscopy has also been reported [19]

An HPLC method of determining glycerol in wines by direct sample injection is presented in this paper Various columns are used, combining the mechanisms of molecular exclusion and cation-exchange chromatography In this way neither compounds with a higher molecular mass nor organic acids (mobile phase, water) are retained Carbohydrates and polyalcohols are also separated

EXPERIMENTAL

Samples and reagents

All the chemicals used were of analytical-reagent grade Glucose, fructose, glycerol and ethanol were supplied by Panreac (Barcelona, Spain), butan-2,3-diol and tartaric, malic, lactic, acetic, citric, succinic and fumaric acids were from E Merck (Darmstadt, Germany), shikimic and citramalic acids were from Fluka (Buchs, Switzerland) and Millipore (Bedford, MA, USA) Milli-Q water was used

The wine samples were injected directly after filtration through 0 2-μm membranes (Dynagard, 0 8 cm^2, Microgon, Laguna Hills, CA, USA)

Chromatographic method

The equipment consisted of a solvent chamber (SEC-4, Perkin-Elmer, Norwalk, CT, USA), a pump (Series-10, Perkin-Elmer), injection valve (7125, Rheodyne, Cotati, CA, USA), a column furnace (220-DW, Croco-Cil, Saint Foy La Grande, France), a refraction index detector (LC-25, Perkin-Elmer) and a data processing system (450-MT2, Kontron Instruments, Milan, Italy)

The samples were injected through a 6-μl loop into a system made up of three columns 250 × 8 mm in series (Shodex S-801/S, S-802/S, S-802/S, Showa Denko, Tokyo, Japan) and a Shodex S-800P pre-column controlled by thermostat at 75°C, using water as the mobile phase, the flow-rate was 1 ml/min and a refractive index detector

Enzymatic method

The enzymatic determinations were carried out with a UV–visible scanning spectrophotometer (GBC 911, GBC Scientific Equipment, Dandenong, Australia)

Boehringer Mannheim enzymatic kits were used The wines were diluted 1 25 to obtain glycerol concentrations between 0 03 and 0 4 g/l [3]

Data treatment

Data were processed using the BMDP statistical package [20], using Student's *t*-test for comparisons of the mean (BMDP3D program) and linear regression (BMDP1R program) These programs were run on a VAX 9200 computer

RESULTS AND DISCUSSION

Chromatographic separation

The three molecular exclusion columns connected in series provided high resolution without an excessively long analysis time

Fig 1 shows the chromatogram of a red wine obtained under the described conditions The first group of compounds (A) observed are those with the highest molecular mass and those with an acid character Glucose, fructose, glycerol, butan-2,3-diol and ethanol (the last peak) are then eluted The next sample can be injected without waiting for the elution of later peaks Peak 4 results from the addition of 0 8 g/l butan-2,3-diol to the initial wine to show its presence more clearly The separation observed between peaks 3 and 4 is not complete, but in practice has no influence on the results for glycerol

Fig 2 is a chromatogram of a standard mixture of acids usually present in wine and shows how they eluted in zone A of Fig 1, and therefore do not interfere in the determination of glycerol This is an advantage compared with other procedures [13–15], in this instance previous preparation of the sample is not necessary

Comparison of the proposed HPLC method and the enzymatic method

Two wines, one red and one white, to which were

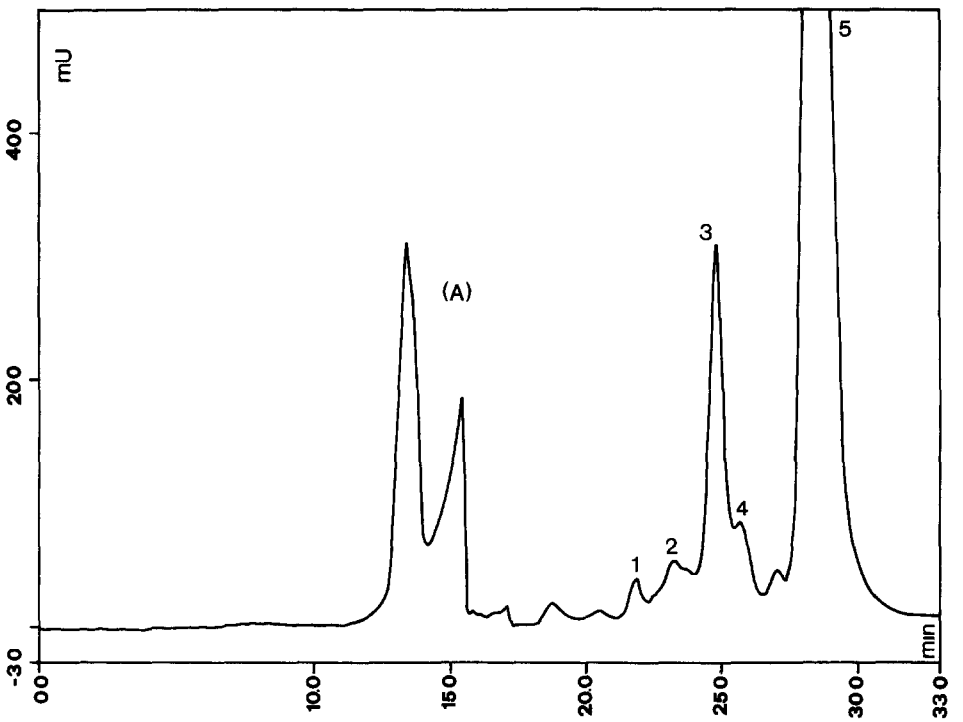

Fig 1 Chromatogram of a red wine using Shodex S-801/S and S-802/S columns at 75°C with a mobile phase of water at a flow-rate of 1 ml/min and using a refraction index detector Peaks A = see text, 1 = glucose, 2 = fructose, 3 = glycerol, 4 = butan-2,3-diol (0 8 g/l added to the initial wine), 5 = ethanol

added increasing amounts of glycerol (0 5, 1 0, 1 5 and 2 0 g/l) were analysed The distribution of the data obtained (mean values, four replicates) is shown in Table I

The regression lines for the results of both methods [y (mean values of glycerol recovered, g/l) *versus* x (g/l of glycerol added)] are $y = 0\,9524x$ ($r^2 = 0\,9962$, $s = 0\,090$) for the HPLC method and $y = 1\,0030x$ ($r^2 = 0\,9998$, $s = 0\,020$) for the enzymatic method

The slopes do not differ significantly from unity in either instance ($p < 0\,05$), a greater dispersion is observed in the results obtained by HPLC (0 09 *versus* 0 02)

Fig 3 shows the regression line (HPLC method *versus* enzymatic method) referred to the whole set of mean values from Table I The data fit fairly well ($r^2 = 0\,9999$), although significant differences ($p < 0\,05$) between the two methods were detected less than 4 2% (mean differences about 2%) according to Student's t-test

When ten different wines (five white and five red) were analysed by the two methods (five replicates), the values shown in Table II were obtained The differences between the values from the two methods by Student's t-test were less than 4%, an acceptable margin in practice

Tables I and II give the standard deviations and the coefficients of variation in each instance The coefficients of variation are not generally greater than 1% in either method

Influence of butan-2,3-diol

As the chromatographic resolution of the peaks of glycerol and butan-2,3-diol is not complete (Fig 1), a study was carried out to determine the influence of butan-2,3-diol in the quantification of glycerol One white and one red wine, to which were added 0 4 and 0 8 g/l butan-2,3-diol, were analysed in quadruplicate The results suggest that the incomplete separation does not have an appreciable influence, the differences were less than 2 3%

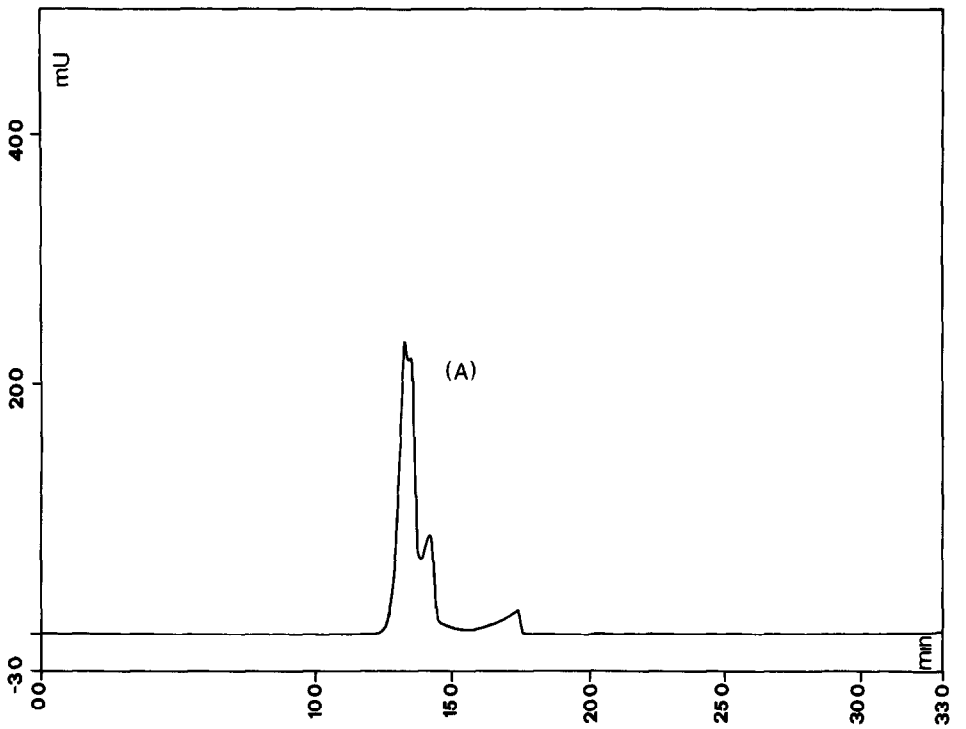

Fig 2 Chromatogram of a standard mixture of tartaric, malic, shikimic, lactic, acetic, citric, succinic, citramalic and fumaric acids in concentrations similar to those of the wines analysed Chromatographic conditions as in Fig 1

TABLE I

COMPARISON OF THE RESULTS FROM THE DETERMINATION OF GLYCEROL BY THE ENZYMATIC METHOD AND THE PROPOSED HPLC METHOD AFTER THE ADDITION OF VARIOUS AMOUNTS OF GLYCEROL

Values in parentheses are coefficients of variation (%)

Glycerol added (g/l)	Glycerol found (mean ± S D , n = 4) (g/l)	
	Enzymatic method	HPLC method
White wine		
0 0	6 08[a] ± 0 01 (1 3)	5 92 ± 0 01 (0 2)
0 5	6 67[a] ± 0 08 (1 3)	6 39 ± 0 04 (0 6)
1 0	7 15[a] ± 0 06 (0 8)	7 05 ± 0 02 (0 3)
1 5	7 64[a] ± 0 04 (0 5)	7 42 ± 0 06 (0 8)
2 0	8 21[a] ± 0 06 (0 7)	8 01 ± 0 10 (1 2)
Red white		
0 0	7 64 ± 0 05 (0 7)	7 60 ± 0 01 (0 1)
0 5	8 18 ± 0 03 (0 4)	8 23 ± 0 05 (0 6)
1 0	8 70 ± 0 04 (0 4)	8 55 ± 0 17 (2 0)
1 5	9 16[a] ± 0 00 (0 0)	8 96 ± 0 05 (0 6)
2 0	9 71[a] ± 0 04 (0 4)	9 48 ± 0 05 (0 5)

[a] Significant differences ($p < 0\ 05$) between the mean values obtained by the two methods

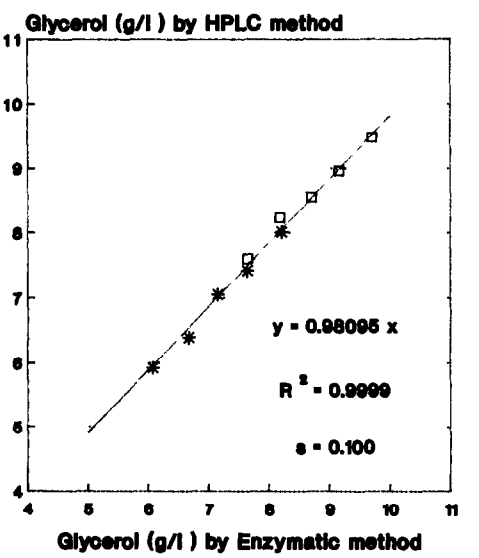

Fig 3 Regression line for the comparison of the results from the determination of glycerol by the HPLC method and by the enzymatic method in the wines listed in Table I (∗) White wine, (□) red wine

TABLE II

COMPARISON OF THE RESULTS OF THE DETERMINATION OF GLYCEROL BY THE ENZYMATIC METHOD AND THE PROPOSED HPLC METHOD IN FIVE WHITE WINES AND FIVE RED WINES

Values in parentheses are coefficients of variation (%)

Wine type	Glycerol found (mean ± S D , n = 5) (g/l)	
	Enzymatic method	HPLC method
White wine		
1	6 08[a] ± 0 01 (1 3)	5 92 ± 0 01 (0 7)
2	8 24[a] ± 0 06 (1 3)	8 48 ± 0 03 (1 0)
3	5 69[a] ± 0 05 (0 8)	5 92 ± 0 03 (0 9)
4	5 44[a] ± 0 05 (0 5)	5 52 ± 0 04 (0 9)
5	5 96[a] ± 0 03 (0 7)	6 17 ± 0 02 (0 5)
Red white		
1	7 64 ± 0 05 (0 7)	7 60 ± 0 01 (0 1)
2	7 11[a] ± 0 07 (1 0)	7 31 ± 0 05 (0 7)
3	6 39[a] ± 0 04 (0 7)	6 49 ± 0 05 (0 8)
4	7 02 ± 0 10 (1 4)	7 07 ± 0 03 (0 4)
5	7 10[a] ± 0 04 (0 5)	7 19 ± 0 04 (0 6)

[a] Significant differences ($p < 0\ 05$) between the mean values obtained by the two methods

Other applications

This HPLC method can also be applied to the simultaneous determination of ethanol in wine, the coefficients of variation found were 0 4% for ten determinations of the same wine and the recovery values ranged from 98 to 102%, giving a good correlation with the official method of alcoholic degree analysis by distillation The method is also applicable to the determination of sucrose at concentrations greater than 2 g/l In the same way it can be used to determine the glucose/fructose ratio in musts and has been used to follow the kinetics of alcoholic fermentation in cellars [21]

CONCLUSIONS

The proposed method of determining glycerol in wines is reproducible and gives results comparable with those obtained by the enzymatic method, the differences are about 2%

As a result of the two separation mechanisms used in this study, interference from the organic acids present in wine is avoided and highly retained compounds are not eluted after the chromatogram has been completed This allows the sample to be injected without previous preparation This method has the advantage, characteristic of chromatographic methods, of allowing various compounds to be analysed simultaneously

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REFERENCES

1 *Recueil des Methodes Internationales d'Analyse des Vins*, Office International de la Vigne et du Vin, Paris, Annexe A21, 1969
2 A Joyeux, M Lafitte and S Lafon-Lafourcade, *Office International de la Vigne et du Vin*, F V 643 (1977) 1–5
3 *Methods of Enzymatic Food Analysis 82/83*, Boehringer Mannheim, Munich, 1982
4 E Walter and P Kohler, *Z Lebensm -Unters -Forsch* , 180 (1985) 121
5 A S Curvelo-Garcia and M C Godinho, *Rev Fr Oenol* , 123 (1990) 47
6 W Postel, L Adam and M Rustler, *Dtsch Lebensm -Rundsch* , 78 (1982) 170
7 M A Lage Yusty, C M Garcia Jares, J Simal Lozano and M E Alvarez Piñeiro, *An Bromatol* , 41 (1989) 375
8 N Gelsomini, F Capozzi and C Faggi , *J High Resolut Chromatogr* , 13 (1990) 352
9 E G Moruno and R Di Stefano, *Vini Ital* , 31 (1989) 41
10 W Flak, *Mitt Klosterneuburg*, 31 (1981) 204
11 R Piccaglia and G C Galletti, *J Sci Food Agric* , 45 (1988) 203
12 A Rapp and A Ziegler, *Dtsch Lebensm -Rundsch* , 75 (1979) 396
13 P Pfeiffer and F Radler, *Z Lebensm -Unters Forsch* , 181 (1985) 24
14 R F Frayne, *Am J Enol Vitic* , 37 (1986) 281
15 P Beaud, V Marie and J D Aubort, *Mitt Geb Lebensmittel unters Hyg* , 80 (1989) 420
16 K Tanaka and J S Fritz, *J Chromatogr* , 409 (1987) 271
17 N Kiba, H Maruyama and M Furusawa, *J Chromatogr* , 456 (1988) 398
18 J Haginaka and T Nomura, *J Chromatogr* , 447 (1988) 268
19 A Rapp, A Markowetz, M Spraul and E Humpfer, *Dtsch Lebensm -Rundsch* , 83 (1987) 375
20 W J Dixon, *Biomedical Computer Programs*, University of California Press, Los Angeles, CA, 1983
21 G Sanchez Muñoz, E Garcia Romero, M S Perez Coello and P J Martin Alvarez, *Aliment Equipos Tecnol* , 10 (1991) 79