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Determination of volatile compounds in Txakoli wine from Biscay by gas chromatography–mass spectrometry

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Abstract

An analytical method combining a Soxhlet extraction and gas chromatography with mass spectrometric detection is proposed for the determination of volatile compounds in samples of Txakoli from Biscay (region located in the north of Spain). The use of direct injection was compared with the results obtained from the organic extract. The extraction of the compounds from the matrix was performed with dichloromethane–hexane (1:1, v/v). Both procedures were applied to the study of Txakoli varieties of wine. © 1997 Elsevier Science B.V.

Keywords: Wine; Food analysis; Volatile organic compounds

1. Introduction

The Txakoli from Biscay is a kind of wine produced in the Basque country, a region located in the north of Spain with an Atlantic climate. This wine is undergoing a strong increase in production. The aim of this work is to determine the volatile organic compounds in white Txakoli wine and to establish the major differences between the two grape varieties used to produce it: Hondarrabi Zuri and Folle Blanche. This work is part of a project whose object is the chemical characterization of Txakoli from Biscay [1–4]. We have determined the minor volatile components of the Txakoli resulting from the grapes fermentation. Up to the present, Txakoli is a wine meant to be consumed within a year of its production and not to be reserved. This wine is a young one and the compounds present are to be found in the undamaged plant cells of the grape

or formed during the processing of the grapes, by chemical, enzymatic and thermal reactions in grape must and during the alcoholic fermentation. This wine does not present a bouquet caused by chemical reactions during maturation process.

Usually, capillary gas chromatography (GC) with EM detection is the preferred separation technique for the determination of volatile compounds in wine [5–7]. Capillary GC is a technique with high selectivity and sensitivity, suitable for the determination of multiple components, at residual level, in wines [8–10].

Because of the low levels of these compounds allowed in Txakoli wine, the use of a preconcentration system is always required [11,12]. Several preconcentration methods based on different physicochemical principles are commonly used such as liquid–liquid extraction and solid-phase extraction [13–15]. We have used Soxhlet extraction in this work. The samples were extracted with dichloromethane–hexane (1:1, v/v). We compared the results

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obtained from the organic extraction with the direct injection of the Txakoli samples.

The proposed methods are based on the determination by GC with EM detection, after Soxhlet extraction.

2. Experimental

2.1. Reagents and samples

Volatile organic compounds were obtained from commercial sources. All solvents (dichloromethane and hexane) used were analytical-reagent grade from Merck.

The samples of Txakoli wine from Biscay were

supplied by the Experimental Center of the Agriculture Department of the Diputación Foral de Bizkaia. (Local Government of Biscay). Various samples produced from Hondarrabi Zuri and Folle Blanche grape varieties were studied. All samples were stored at 2–4°C.

2.2. Equipment

A Hewlett–Packard 5890 series II gas chromatograph equipped with a mass spectrometer detector (HP 5989) was used. A HP-5 capillary column (5% of methylsilicone, 30 m×0.25 mm I.D.) with 0.25- μ m film thickness from Hewlett–Packard was employed.

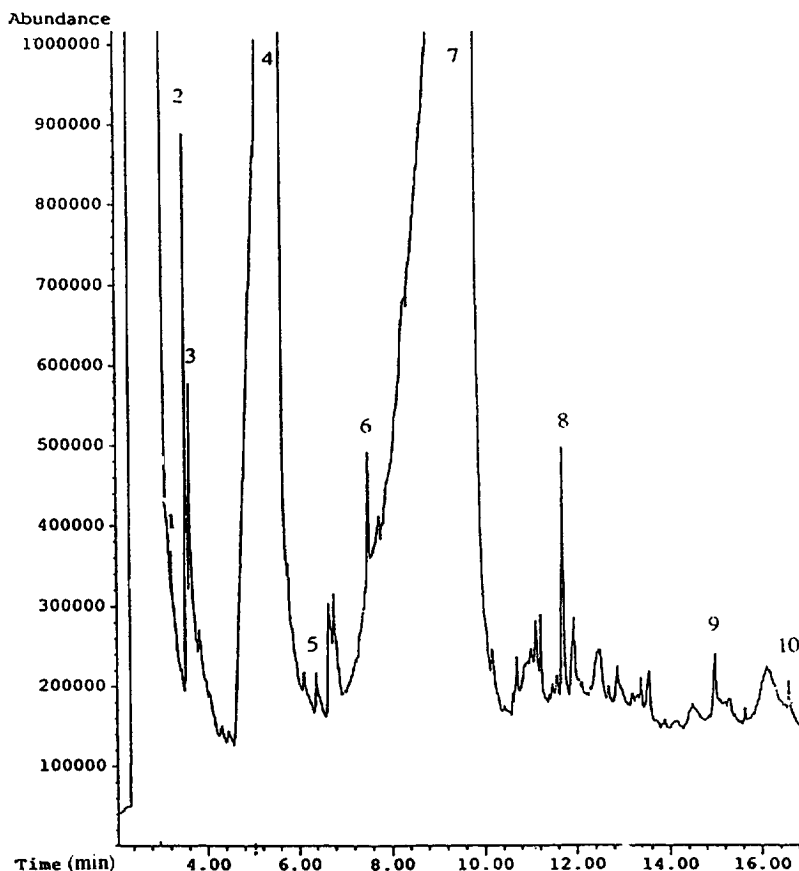


Fig. 1. Chromatogram obtained by method 1. Peaks: 1=acetic acid; 2=2,3-buthanediol; 3=2-propanol; 4=lactic acid; 5=ethyl lactate; 6=succinic acid; 7=glycerine; 8=malic acid; 9=vanillinic acid; 10=3,4-dimethoxyphenol.

2.3. Procedure

Two analytical methods were proposed and applied in the study of Txakoli wine:

2.3.1. Method 1

The capillary column and injection port temperature was maintained at 200°C. The oven temperature was programmed to increase from 60–150°C at a

Table 1
Results obtained by method 1

Compounds	t_R (min)	m/z (%)	Fragmentation
Acetic acid ($M_r=60$)	3.20	60 (65)	M^+
		45 (95)	$M-CH_3$
		43 (100)	$M-OH$
		15 (10)	$M-COOH$
2,3-Butanediol ($M_r=90$)	3.70	90 (1)	M^+
		75 (5)	$M-CH_3$
		57 (10)	$M-CH_3, -H_2O$
		45 (100)	CH_3-CHOH^+
2-Propanol ($M_r=60$)	3.80	60 (1)	M^+
		45 (100)	$M-CH_3$
		42 (20)	$M-H_2O$
		27 (18)	$M-H_2O-CH_3$
Lactic acid ($M_r=90$)	5.40	75 (2)	$M-CH_3$
		55 (4)	$CH_2=CH-CO^+$
		45 (100)	$M-COOH$
		27 (18)	$CH_2=CH^+$
Ethyl lactate ($M_r=118$)	6.40	103 (3)	$M-CH_3$
		85 (1)	$M-H_2O-CH_3$
		75 (10)	$M-CH_2=CH_2-CH_3$
		45 (100)	$M-COOEt$
Succinic acid ($M_r=118$)	7.60	101 (3)	$M-OH$
		100 (5)	$M-H_2O$
		74 (5)	$M-CO_2$
		73 (2)	$M-COOH$
		56 (100)	$M-CO_2-H_2O$
Glycerine ($M_r=92$)	9.60	74 (3)	$M-H_2O$
		61 (100)	$M-CH_2-OH$
		43 (95)	$M-CH_2OH, -H_2O$
		31 (50)	CH_2-OH^+
Malic acid ($M_r=134$)	11.80	116 (10)	$M-H_2O$
		98 (28)	$M-2 H_2O$
		89 (80)	$M-COOH$
		71 (100)	$M-COOH-H_2O$
		45 (55)	$M-COOH-CO_2$
		43 (75)	$M-COOH-CO_2-H_2$
Vanillinic acid	15.10	168 (100)	M^+
		153 (64)	$[M-CH_3]^+$
		125 (25)	$[M-CH_3-CO]^+$
		97	$[M-CH_3-2CO]^+$
3,4-Dimetoxifenol	16.60	154 (100)	M^+
		139 (70)	$[M-CH_3]^+$
		111 (35)	$[M-CH_3, CO]^+$
		93 (10)	$[M-CH_3-CO-H_2O]^+$

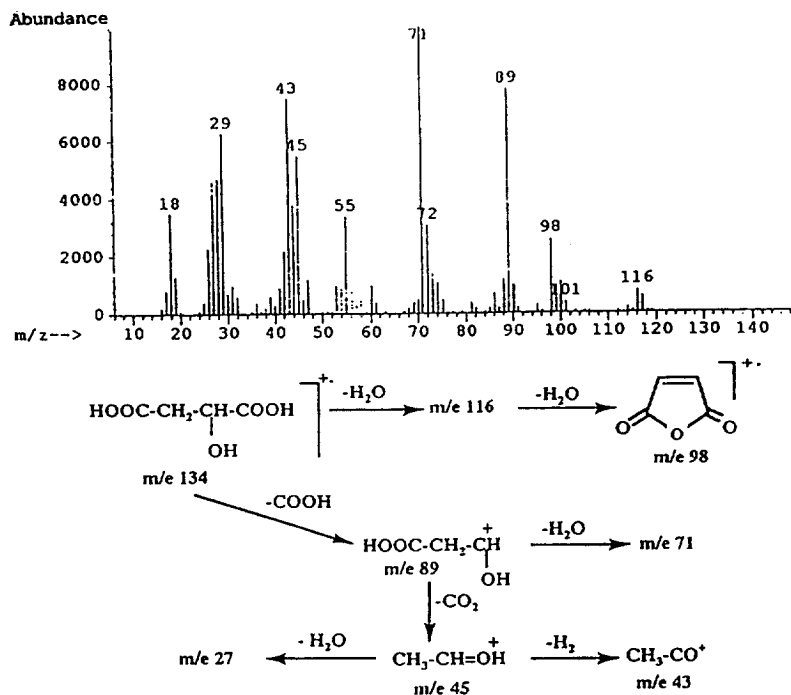


Fig. 2. Mass spectrum of malic acid and major fragments.

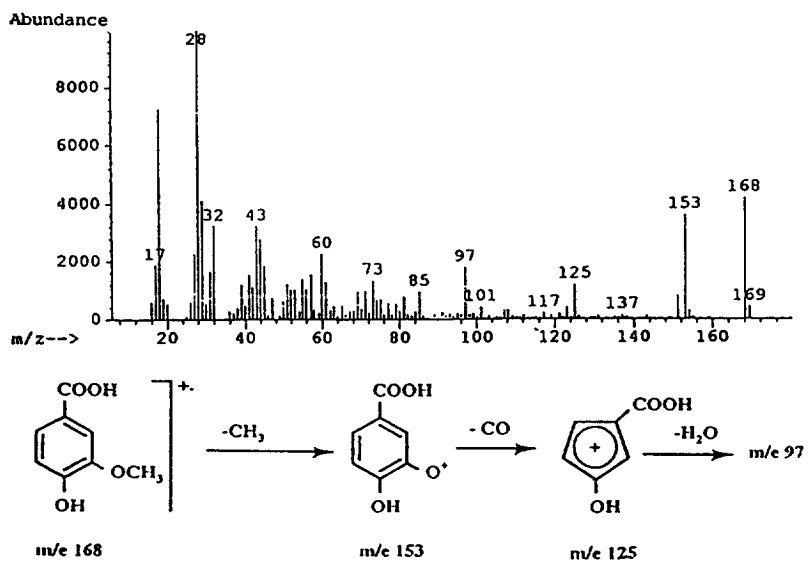


Fig. 3. Mass spectrum of vanillic acid and its major fragments.

rate of 5°C/min and held at the final temperature for 1 min. A 1- μ l volume of Txakoli wine was directly injected into the GC–MS system.

2.3.2. Method 2

The capillary column and injection port temperature were as before. The oven temperature was kept at 100°C for 1 min, then programmed to increase from 100–280°C at a rate of 5°C/min and held at the final temperature for 1 min. Two simple procedures depending on the compounds to be determined were followed before injection into the chromatograph:

Method 2A: all samples were filtered with a 45- μ m nylon membrane.

Method 2B: the samples were extracted by a Soxhlet extractor (see Section 2.4)

A 1- μ l sample of organic extract was injected splitless (method 2B), with the valve closed for 1 min. The results obtained were compared with the direct injection (method 2A).

The MS detector was operated in the electron-impact ionization (EI, 70 eV) mode. The full scan acquisition mode (70–400 u) was used as this allowed identification of the compounds present in Txakoli wine.

2.4. Soxhlet extraction

The sample (150 ml, Txakoli from Biscay) was

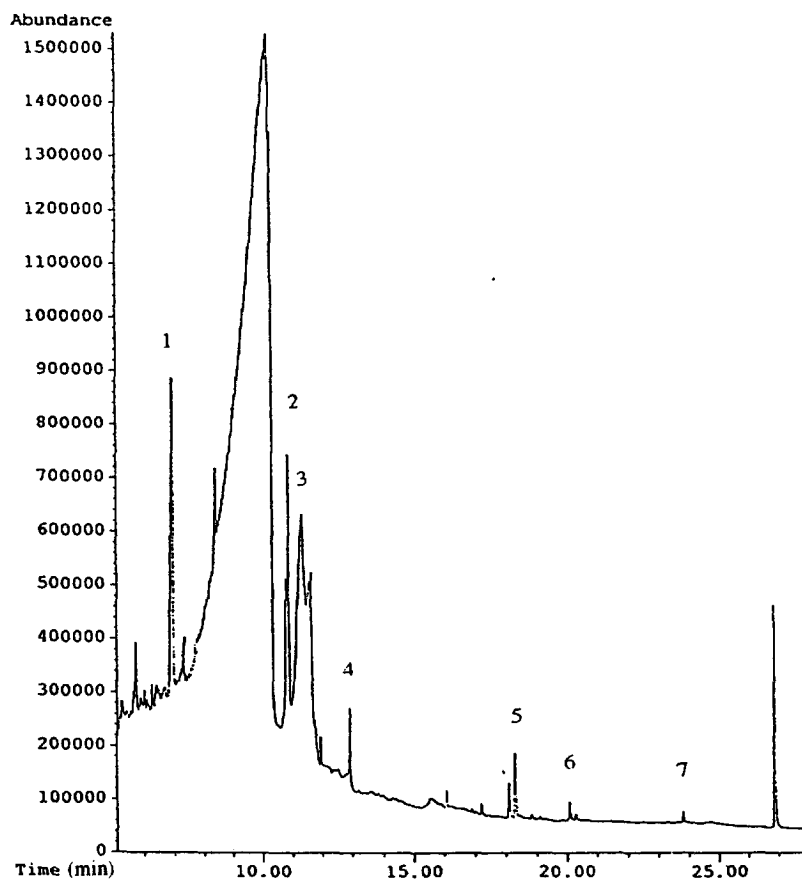


Fig. 4. Chromatogram obtained by method 2A. Peaks: 1=hexyl acetate; 2=3-pentanol; 3=1-phenyl-1-propanol; 4=1-phenyl-1,2-ethanediol; 5=dodecanoic acid; 6=4,5-dimethoxy-2-methylphenol; 7=farnesol.

extracted by a Soxhlet extractor with 100 ml of dichloromethane–hexane (1:1, v/v) for 24 h. The extract was evaporated at 60°C in a rotary evaporator under low pressure to reduce the volume to 25 ml.

3. Results and discussion

Volatile organic compounds were extracted and analysed following the previously reported methods and good results were obtained with these procedures.

Fig. 1 shows the total ion chromatogram (obtained by method 1) acquired in the EI mode. Their retention times together with the main ions of their mass spectra are shown in Table 1. Several organic acids were identified by this method. Figs. 2 and 3

show the mass spectra of malic and vanillic acid respectively with their major fragments.

Fig. 4 shows the total ion chromatogram obtained by method 2A (direct injection) and Fig. 5 shows the chromatogram obtained by the same method with prior extraction (method 2B). Tables 2 and 3 show the retention times and the main ions of their mass spectra for methods 2A and 2B respectively.

Several alcohols were identified by method 2A. Figs. 6 and 7 show the mass spectra and the major fragments of 1-phenyl-1,2-ethanediol and 4,5-dimethoxy-2-methylphenol respectively.

The application of method 2B allowed the identification of esters and carbonylic compounds. Mass spectra of 2-nonanone and citral with their most important fragments are shown in Figs. 8 and 9.

The extraction process was carried out using

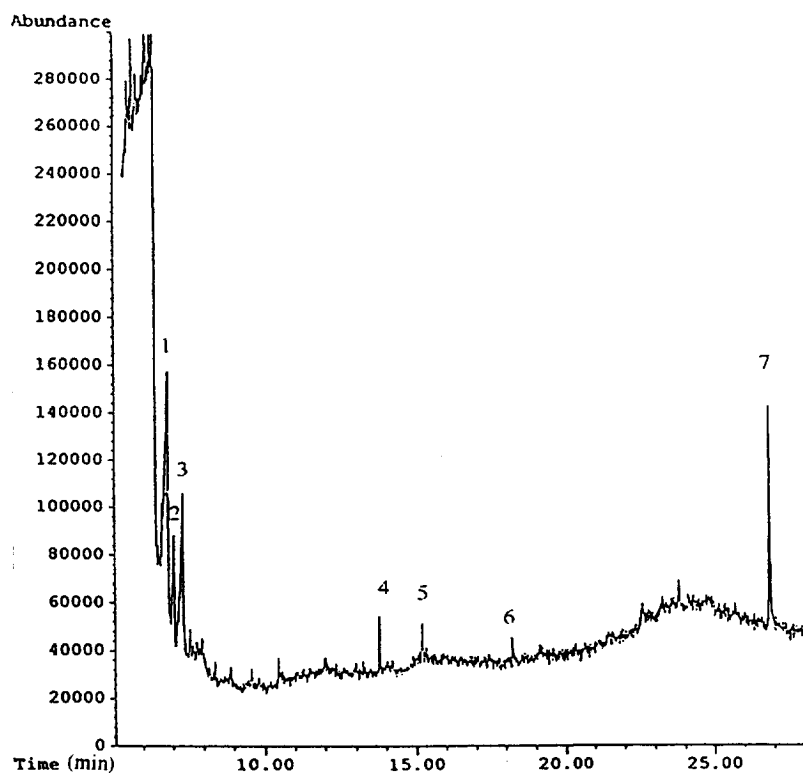


Fig. 5. Chromatogram obtained by method 2B. Peaks: 1=isoamyl acetate; 2= diethyl acetate; 3=3-pentanone, 4=3-nonanone; 5=propyl pentanoate; 7=geranyl acetate.

Table 2
Results obtained by method 2A

Compounds	t_R (min)	m/z (%)	Fragments
Hexyl acetate ($M_r=144$)	6.70	144 (23)	M^+
		115 (4)	$[M-Et]^+$
		101 (20)	$[M-Pr]^+$
		73 (14)	$CH_3-COO-CH_2^+$
		61 (50)	$[Acid+1]^+$
		43 (100)	CH_3CO^+
3-Pentanol ($M_r=88$)	10.36	73 (22)	$[M-Me]^+$
		59 (100)	$[M-Et]^+$
		41 (25)	$[M-Et-H_2O]^+$
		31 (60)	$CH_3=OH^+$
1-Phenyl-1-propanol ($M_r=136$)	11.50	136 (5)	M^+
		107 (10)	$[M-Et]^+$
		77 (8)	Ph^+
1-Phenyl-1,2-ethanediol ($M_r=138$)	13.00	138 (8)	M^+
		107 (100)	$Ph-COH^+$
		77 (28)	Ph^+
		61 (14)	CH_2-OH^+
Dodecanoic acid ($M_r=200$)	18.18	199 (1)	$[M-1]^+$
		155 (5)	$[M-COOH]^+$
		129 (10)	$[M-C_5H_{11}]^+$
		73 (30)	$[M-C_9H_{19}]^+$
4,5-Dimethoxy-2-methylphenol ($M_r=168$)	20.10	168 (100)	M^+
		153 (95)	$[M-Me]^+$
		125 (90)	$[M-CH_3, -CO]^+$
Farnesol ($M_r=222$)	23.85	191 (1)	$[M-CH_2OH]^+$
		137 (10)	$C_{10}H_{17}^+$
		95 (16)	$C_7H_{11}^+$
		81 (58)	$C_6H_9^+$
		69 (100)	$C_5H_9^+$

continuous extraction. The blank of the organic solvents was initially checked and no peaks were found. The samples were subjected to two experiments involving the same amount of volatile compounds; in one of them, the sample was extracted.

A comparison of methods 2A and 2B illustrates the different results obtained. With the organic extraction it was possible to identify esters and ketones which could not be done with direct injection. This may be due to their low levels in the sample.

As result of this study, organic extraction was selected for the study of minor volatile compounds. To show the applicability of the method to the

routine analysis of real samples, different Txakoli varieties were used. The results obtained for two Txakoli varieties (Hondarrabi Zuri and Folle Blanche) are given in Fig. 10. Special attention was given to samples with the generic label. The compounds found in both samples were similar, although the amounts were higher for Hondarrabi Zuri. The peak areas corresponding to 1-phenyl-1,2-ethanediol, dodecanoic acid, 4,5-dimethoxy-2-methylphenol, farnesol and geranyl acetate were bigger for the samples produced from the Hondarrabi Zuri grape variety. There was a relationship between the concentrations of volatile compounds found in the Txakoli samples showed and the varieties used.

Table 3
Results obtained by method 2B

Compounds	t_R (min)	m/z (%)	Fragments
Isoamyl acetate ($M_r=130$)	6.84	87 (5) 70 (14) 61 (7) 60 (8) 43 (100)	$[M-iPr]^-$ $[M-acid]^+$ $[Acid+1]^+$ CH_3-COOH^+ CH_3-CO^-
Diethyl succinate ($M_r=174$)	7.03	128 (10) 101 (55) 73 (15)	$[M-EtOH]^+$ $[M-COOEt]^+$ $EtOOC^+$
2-Pentanone ($M_r=86$)	7.30	86 (18) 57 (20) 43 (100)	M^+ $[M-Et]^+$ CH_3-CO^+
3-Nonanone ($M_r=142$)	13.77	142 (3) 113 (18) 85 (12) 28 (100)	M^+ $[M-Et]^+$ $[M-Et-CO]^+$ $CH_2=CH_2^+$
Propyl pentanoate ($M_r=144$)	15.20	101 (10) 85 (100) 59 (40) 57 (25) 43 (32) 42 (38)	$C_4H_9-COO^+$ $C_4H_9-CO^+$ $C_3H_7-O^+$ $C_2H_5^+$ $C_3H_7^+$ $[M-acid]^+$
Citral ($M_r=152$)	18.00	152 (1) 151 (1) 123 (3) 69 (20)	M^+ $[M-1]^+$ $[M-C_5H_9]^+$ $C_5H_9^+$
Geranyl acetate ($M_r=196$)	26.90	137 (14) 136 (12) 81 (63) 69 (100)	$[M-CH_3-COO]^+$ $[M-CH_3-COOH]^+$ $C_6H_9^+$ $C_5H_9^+$

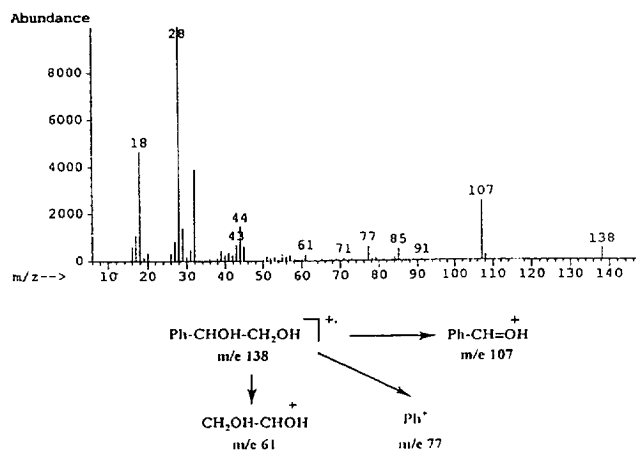


Fig. 6. Mass spectrum and major fragments of 1-phenyl-1,2-ethanediol.

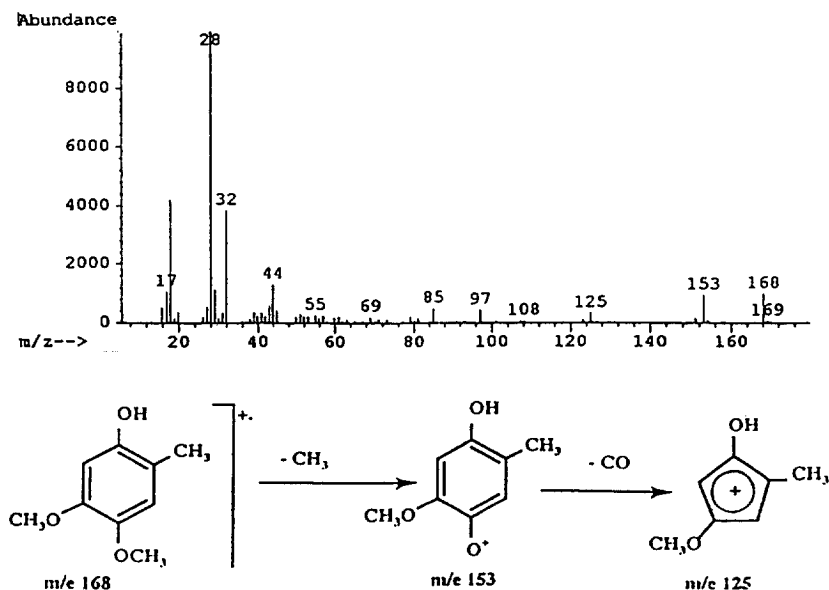


Fig. 7. Mass spectrum and major fragments of 4,5-dimethoxy-2-methylphenol.

4. Conclusions

The use of GC–EI–MS allowed the identification of a group of organic volatile compounds in Txakoli wine in the full scan mode. Soxhlet extraction gave

better results than direct injection for some minor compounds (esters and ketones).

The relative amounts of organic volatile compounds present in the Txakoli wine allowed to know the varieties employed in the production of the wine.

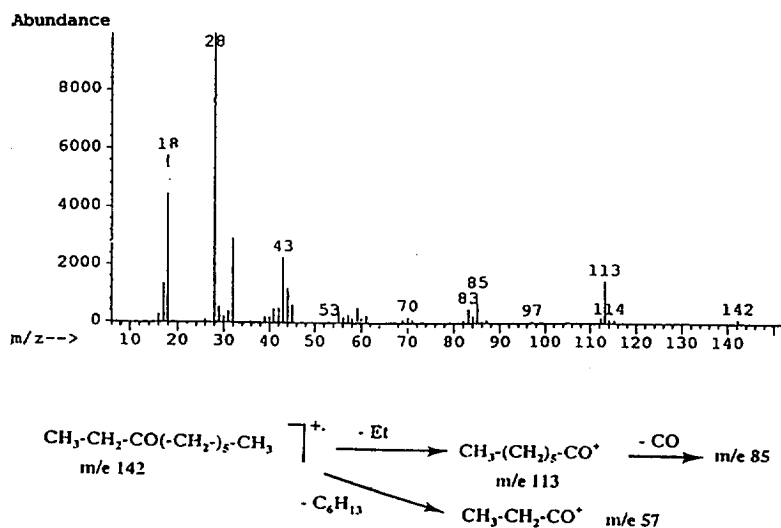


Fig. 8. Mass spectrum and major fragments of 3-nonanone.

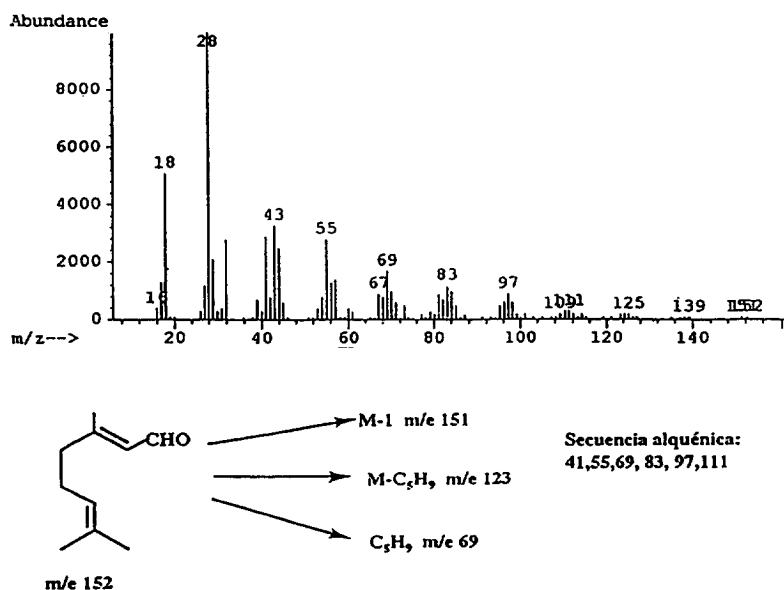


Fig. 9. Mass spectrum and major fragments of citral.

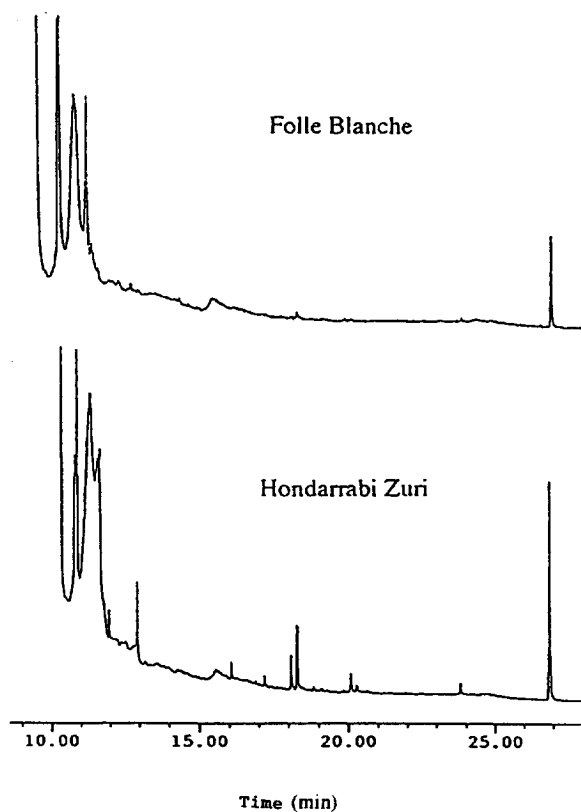


Fig. 10. Chromatograms obtained for Hondarrabi Zuri and Folle Blanche varieties (method 2A).

Acknowledgments

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