

# Analysis of Wine Distillates Made from Muscat Grapes (Pisco) by Multidimensional Gas Chromatography and Mass Spectrometry

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A multidimensional system consisting of a 45-m polar capillary column linked to a 50-m nonpolar capillary column is evaluated with regard to its practical usefulness in Pisco aroma analysis. Fifty-four components can be separated and identified from the information resulting from use of such a multidimensional procedure in addition to the gas chromatography-mass spectrometry technique. Eighteen of these components including esters, alcohols, terpenes, acids, aldehydes, and miscellaneous compounds are detected for the first time in Pisco.

## INTRODUCTION

Qualitative gas chromatographic analysis of difficult mixtures containing many components covering a wide range of volatilities, polarities, and concentrations demands very efficient separations. However, even the most efficient capillary columns do not provide in some cases total separation of the significant components from the other species of the matrix since optimization of the chromatographic parameters yields new overlappings after the previous ones have been removed.

Multidimensional gas chromatography (MDGC) is often required to achieve a noticeable improvement of the resolution of the components of interest by transferring selected and very narrow cuts from one chromatographic column (precolumn) into another (main column) of very different characteristics, i.e., polarity, phase ratio, thick film, etc. Also, a significant reduction of the analysis time by backflushing of uninteresting compounds and an improvement of the detection limit have been previously reported as further advantages of multidimensional systems (Bertsch, 1978a,b,c; Deans, 1981; Schomburg et al., 1975, 1982, 1984; Oreans et al., 1983).

On the other hand, the analysis of complex mixtures requires an optimized sample preparation procedure and the use of different techniques allowing for simplification of the analytical problem and simultaneously for obtention of the most representative components. Trichlorofluoromethane (Freon 11) has been previously suggested as a suitable solvent for liquid-liquid extraction and subsequent concentration of volatiles from food flavors, particularly those containing high concentrations of alcohol such as alcoholic beverages (Hardy, 1969; Rapp et al., 1976).

Multidimensional gas chromatography has not been extensively used to date for the flavor analysis of alcoholic beverages, although several papers have been already published in this field (Schomburg et al., 1984; Phillips et al., 1982; Van Ingen et al., 1987). However, no significant research has been done to date on the usefulness of multidimensional gas chromatography for evaluating flavor quality of distillates.

Pisco is a common drink, originating in some South American countries, which is obtained by distillation of

wine made from Muscat grapes. It is considered a high-quality product and a factor of important economical repercussion. However, no definitive rules have been established concerning its elaboration, and consequently, the alcohol content of Pisco as well as its volatile composition may vary broadly (Ureta et al., 1986; Loyola et al., 1990). As a result, the introduction of this beverage into the international market has been quite hindered.

It is widely accepted that the specific flavor of wines made from Muscat varieties is mainly due to terpene alcohols and their derivatives. However, further investigation of other constituents, which probably explain all the nuances of Muscat aroma, is still required.

The purpose of this work was to contribute to the identification of Pisco aroma components, even those which are present in very low concentrations since they may have a significative sensorial relevance.

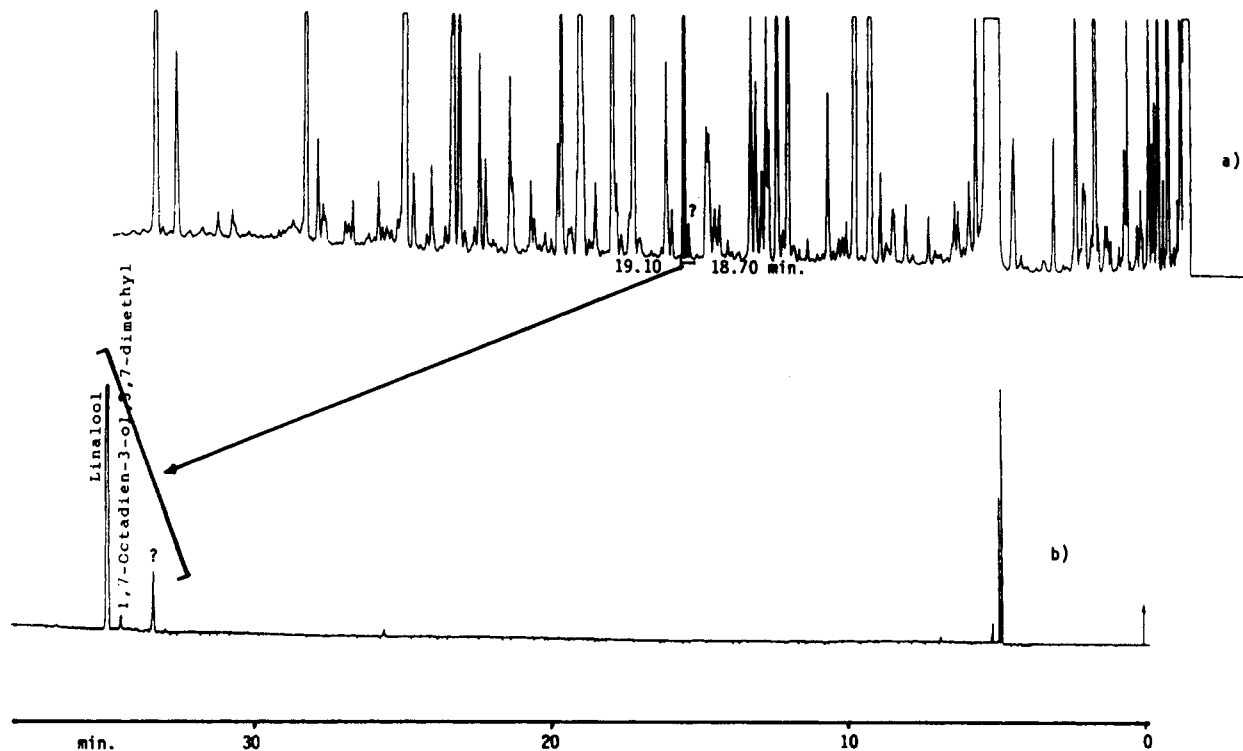
## EXPERIMENTAL PROCEDURES

**Extraction.** Chilean Pisco (250 mL) was extracted continuously in downward displacement extractors with 150 mL of Freon 11 which had been supplied by Atochem. The solvent was redistilled twice prior to use. All the extractions were carried out after a 7- $\mu$ L volume of methyl caprylate (2% v/v in ethanol) was added as internal standard. According to Hardy, (1969), the extractors worked most efficiently by using a sintered disk to split up the solvent stream into fine droplets which descend through the sample and are finally collected in a suitable flask. The extractors were kept cool by circulating water at 1 °C from a cryogenic unit. After 24 h of extraction, the Freon 11 remaining in the body of the extractor was discarded and a 45-mL volume of the Freon extract was then concentrated in a water bath kept at 32 °C, by fitting the flask with a 50-cm length of Vigreux column, to a volume of 300  $\mu$ L. A 1- $\mu$ L volume of the concentrate was then analyzed by multidimensional gas chromatography.

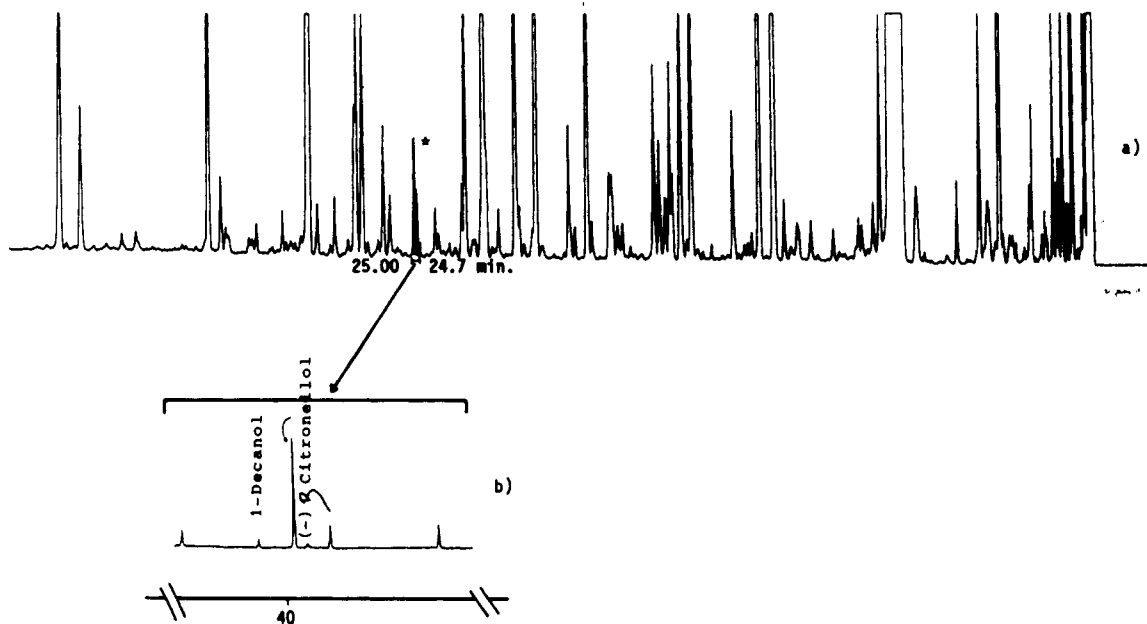
**Multidimensional Gas Chromatography (MDGC).** A Siemens Sichromat 2 double-oven gas chromatograph equipped with two FIDs and the so-called "live column switching system" was used. This system enables the transference of selected components between two different capillary columns, with several advantages resulting from its design features: use of a coupling device with a very small volume, avoidance of any dead volume, no valves in the separation passage, high switching speed, monitor-detector directly at the coupling of the columns, and the possibility of fixing the switching time exactly (Oreans et al., 1983).

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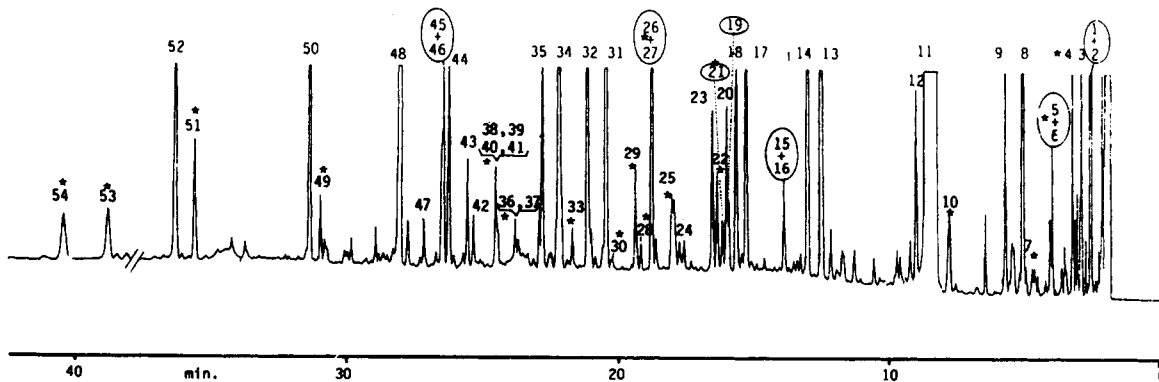
<sup>§</sup> Universidad de Chile.



**Figure 1.** Multidimensional separation of a Pisco aroma extract using a system of coupled columns with different polarities. Volume injected: 1.5  $\mu$ L. Columns a: 45 m Carbowax 20 M, i.d. = 0.32 mm,  $d_f$  = 0.20  $\mu$ m. Carrier gas: helium, 2.4 bar. Column b: 50 m OV-1, i.d. = 0.32 mm,  $d_f$  = 1.80  $\mu$ m. Carrier gas: helium, 1.2 bar. See text for further details.



**Figure 2.** MDGC separation of Pisco. Sample: 2  $\mu$ L of Pisco aroma extract. (a) Chromatogram obtained in the precolumn; (b) chromatogram obtained in the main column. Chromatographic conditions are as stated in Figure 1.



**Figure 3.** Chromatogram obtained in a 45 m  $\times$  0.32 mm i.d. fused silica capillary column coated with a 0.20- $\mu$ m layer of cross-linked Carowax 20 M from a Pisco aroma extract. Temperature program: 60  $^{\circ}$ C for 5 min, raised to 200  $^{\circ}$ C at 5  $^{\circ}$ C/min. Identification of the peaks as stated in Table I. Asterisks indicate compounds identified for the first time in this work.

**Table I. Identified Compounds in the Volatile Concentrate from Pisco Aroma**

compound	peak <sup>a</sup>	concn <sup>b</sup>	identification <sup>c</sup>
<b>esters</b>			
ethyl acetate	1	100–1000	GC, MS <sup>d</sup>
isopentyl acetate	8	0.1–5	GC, MS <sup>d,e</sup>
ethyl hexanoate	12	0.1–5	GC, MS <sup>d,e</sup>
ethyl lactate	13	1000–2000	GC, MS <sup>d,e</sup>
methyl caprylate	15		(internal standard)
ethyl octanoate	17	0.1–5	MDGC, MS <sup>d,e</sup>
ethyl decanoate	32	0.1–5	GC, MS <sup>d,e</sup>
diethyl succinate	34	0.1–50	GC, MS <sup>d,e</sup>
2-phenylethyl acetate	43	0.1–5	GC, MS <sup>d,e</sup>
ethyl dodecanoate	44	0.1–1	GC, MS <sup>d,e</sup>
ethyl tetradecanoate <sup>f</sup>	49	0.1–10	GC, MS <sup>d,e</sup>
ethyl hexadecanoate <sup>f</sup>	51	0.1–20	GC, MS <sup>d,e</sup>
ethyl linoleate <sup>f</sup>	54	0.1–10	GC, MS <sup>d</sup>
<b>alcohols</b>			
ethanol	3		GC, MS <sup>d,e</sup>
2-methyl-1-propanol	5	100–1000	GC, MS <sup>d,e</sup>
1-butanol	9	5–20	GC, MS <sup>d</sup>
isoamyl alcohol	11	1000–5000	GC, MS <sup>d,e</sup>
1-hexanol	14	1–10	MDGC, MS <sup>d,e</sup>
<i>cis</i> -3-hexen-1-ol	16	0.1–5	MDGC, MS <sup>d,e</sup>
1-heptanol <sup>f</sup>	21	0.1–5	MDGC, MS <sup>d</sup>
1-octanol <sup>f</sup>	28	0.1–1	MDGC, MS <sup>d,e</sup>
1-nonanol <sup>f</sup>	33	0.1–1	MDGC
1-decanol <sup>f</sup>	40	0.1–1	MDGC
benzyl alcohol	47	0.1–1	MDGC, MS <sup>e</sup>
2-phenylethanol	48	10–100	MDGC, MS <sup>d,e</sup>
<b>terpenes</b>			
(+)-limonene <sup>f</sup>	10	0.1–1	MDGC
<i>cis</i> -furan-linalool oxide	18	0.1–10	MS <sup>d,e</sup>
nerol oxide <sup>f</sup>	22	0.1–5	MS <sup>d</sup>
<i>trans</i> -furan-linalool oxide	23	0.1–5	MS <sup>d,e</sup>
$\beta$ -ionone <sup>f</sup>	25	0.1–5	MS <sup>e</sup>
3,7-dimethyl-1,7-octadien-3-ol <sup>f</sup>	26	0.1–1	MDGC
linalool	27	0.1–20	MDGC, MS <sup>d,e</sup>
terpinen-4-ol <sup>f</sup>	30	0.1–5	MDGC, MS <sup>d</sup>
hotrienol	31	1–50	GC, MS <sup>d</sup>
$\alpha$ -terpineol	35	0.1–10	MDGC, MS <sup>d,e</sup>
<i>cis</i> -pyran-linalool oxide	37	0.1–1	MS <sup>d,e</sup>
(-)- $\beta$ -citronellol	38	0.1–5	MDGC, MS <sup>d,e</sup>
<i>trans</i> -nerol	39	0.1–1	MS <sup>d,e</sup>
<i>trans</i> -pyran-linalool oxide	41	0.1–1	MS <sup>d,e</sup>
<i>cis</i> -nerol	42	0.1–1	MDGC, MS <sup>d,e</sup>
geraniol	46	0.1–5	MDGC, MS <sup>d,e</sup>
<b>acids</b>			
acetic acid	19	100	GC, MS <sup>d</sup>
hexanoic acid	45	1–20	GC, MS <sup>d,e</sup>
octanoic acid	50	5–50	GC, MS <sup>d,e</sup>
decanoic acid	52	5–50	GC, MS <sup>d</sup>
dodecanoic acid <sup>f</sup>	53	0.1–5	GC, MS <sup>d</sup>
<b>carbonyls</b>			
furfuraldehyde	20	1–10	MDGC, MS <sup>d,e</sup>
benzaldehyde	24	0.1–1	MDGC, MS <sup>e</sup>
5-methylfurfuraldehyde <sup>f</sup>	29	0.1–5	GC, MS <sup>d,e</sup>
<b>miscellaneous</b>			
1,1-diethoxyethane	2	10–200	MS <sup>d,e</sup>
1,1-diethoxy-2-methylpropane <sup>f</sup>	4	0.1–5	MS <sup>e</sup>
2,4,6-trimethyl-1,3,5-trioxane <sup>f</sup>	6	0.1–1	MS <sup>e</sup>
2-(2,4,6-ethenyltetrahydro)-2 <i>H</i> -pyran <sup>f</sup>	7	0.1	MS <sup>e</sup>
1,5,8-trimethyl-1,2-dihydronaphthalene <sup>f</sup>	36	0.1–1	MS <sup>e</sup>

<sup>a</sup> Peak numbers correspond to those in Figure 3. <sup>b</sup> Milligrams per liter of absolute ethanol. <sup>c</sup> GC, compounds identified by their relative retention time in GC; MS, compounds identified by gas chromatography-mass spectrometry, MDGC, compounds identified by the retention times of reference compounds in the multidimensional system. <sup>d</sup> Finnigan MS. <sup>e</sup> Perkin-Elmer ITD. <sup>f</sup> Compounds identified for the first time in Pisco.

A 45 m  $\times$  0.32 mm i.d. fused silica capillary column coated with a 0.20- $\mu$ m layer of cross-linked Carbowax 20 M was employed as precolumn of the multidimensional system. The second column was a nonpolar 50-m modified OV-1 (99%

methylsilicone, 1% vinylsilicone) with i.d. of 0.32 mm. The thick film ( $d_f = 1.80 \mu\text{m}$ ) was selected to achieve sufficiently high  $k$  values for improving the resolution of the less retained solutes and also because of its suitable coverage of potential adsorptive areas and its adequate sample capacity for analyzing trace components in complex aroma extracts. Both columns were made in the Max-Planck Institut für Kohlenforschung, Mülheim/Ruhr, Germany.

The double oven allowed for independent yet simultaneous operation of both columns at different temperature programs to optimize the required selectivity for the chromatographic separation. The precolumn was maintained at 60 °C for 5 min and then it was raised to 200 °C at 5 °C/min. The main column was operated isothermally at 60 °C for 10 min and then programmed at 4 °C/min to 250 °C. The coupled piece used for the valveless flow switching was located within the first oven at the outlet of the precolumn. In all cases, chromatographic separations were accomplished with helium as carrier gas. Injector and detector temperatures were maintained at 200 and 250 °C, respectively, in all the analyses.

**GC-MS.** To dispose of supplementary information on component identities when the spectroscopic techniques were applied, two different instruments having columns of unequal selectivity were used: (a) A Perkin-Elmer F-22 gas chromatograph linked to a Finnigan MAT CH-7A magnetic sector mass spectrometer (electronic impact, 70 eV) was used. In this case, the first column of the above-mentioned multidimensional system was also used for the chromatographic separation. (b) A Perkin-Elmer 8400 gas chromatograph coupled to a Perkin-Elmer ITD-50 ion trap detector (electronic impact, 70 eV) was also used. A 50 m  $\times$  0.32 mm i.d. fused silica column coated with a 0.27- $\mu$ m layer of cross-linked Carbowax 20 M (Chrompack) was used.

The chromatographic separations were performed by starting from an initial temperature of 60 °C (5 min) and then programming at 2 °C/min to 200 °C (15 min). In all cases helium (30 psig) was used as carrier gas and a volume of 1  $\mu$ L was injected into the gas chromatograph (split ratio 1/50).

## RESULTS AND DISCUSSION

Figures 1 and 2 show some separations performed by using the multidimensional system mentioned under Experimental Procedures. In both cases the upper chromatogram was obtained by using the precolumn for the chromatographic separation, whereas the lower chromatogram comes from the transfer into the nonpolar OV-1 column of the cuts taken from the separation as marked in the figures. As can be seen, changes in polarity, phase ratio, and temperature within the system of coupled columns allow for a significant improvement of the resolution of the compounds contained in the relatively narrow cuts which have been selected and finally result in the separation of the isomers 3,7-dimethyl-1,6-octadien-3-ol (linalool), and 3,7-dimethyl-1,7-octadien-3-ol (Figure 1). The transfer of the cut marked in Figure 2 allows for the separation and identification of (-)- $\beta$ -citronellol and 1-decanol. From this chromatogram is also clear the possibility of analyzing compounds masked by major components when a single column is used, although in this case it was not possible to identify them. Consequently, lower detection limits are achieved and determination of trace compounds which may eventually exhibit undesirable sensorial tones could be finally achievable.

Likewise, different cuts taken from the eluate of the pre-separation were transferred into the main column, resulting lastly in the separation and identification of some volatiles of Pisco, many of them further confirmed by GC-MS (see Table I). It should be emphasized that MDGC and GC-MS on the whole allowed for the detection of the 54 compounds given in the chromatogram of Figure 3 and Table I. Eighteen of these components (those marked with an asterisk in Figure 3) were found for the first time in this work in the volatile concentrate of Pisco aroma. Moreover, some of these compounds, namely, 1,5,8-

trimethyl-1,2-dihydronaphthalene and 3,7-dimethyl-1,7-octadien-3-ol, had not been previously detected in other distillates from Muscat grapes. As was expected from previous research by several groups on Muscat aroma (Wenzel and De Vries, 1968; Rapp and Knisper, 1979; Williams, et al., 1981; Etievant et al., 1983; Wilson et al., 1984; Di Stefano, 1986), it was confirmed that Pisco contains large amounts of volatile terpenoids such as linalool, geraniol, hotrienol,  $\alpha$ -terpineol, *cis*-furan-linalool oxide, and *trans*-furan-linalool oxide.

## CONCLUSION

The use of a mass spectrometer and a ITD linked to chromatographic columns of different performances, in addition to the removal of significative peak overlappings in the entire volatility range of solute characteristics of Pisco aroma by means of a suitable multidimensional system consisting of a polar first column and a nonpolar second column, allows the reliable identification of 48 compounds and the tentative identification of 6 components of Pisco aroma, most of them present at low concentrations. Eighteen of these compounds were detected in Pisco for the first time in this work.

Multidimensional gas chromatography appears to be a valuable instrumental analytical technique for the rapid and reliable evaluation of the flavor quality of Pisco and for the control of its production, since eventual changes resulting from microbial, enzymic, or chemical deterioration might be monitored by such a technique, although further research in this respect remains to be done. On the other hand, the analysis of volatile terpenoids could be of interest in the establishment of parameters characteristic of distillates from Muscat grapes.

The identification of the Pisco aroma components may also contribute to significantly extend those studies dealing with the correlation between chemical composition and organoleptic analysis. The knowledge of the sensorial significance of Pisco aroma components could be finally of great importance for standardizing Pisco elaboration.

## ACKNOWLEDGMENT

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**Registry No.** Ethyl tetradecanoate, 124-06-1; ethyl hexadecanoate, 628-97-7; ethyl linoleate, 544-35-4; 1-octanol, 111-87-5; 1-nonanol, 143-08-8; 1-decanol, 112-30-1; (+)-limonene, 5989-27-5; nerol oxide, 1786-08-9; 3,7-dimethyl-1,7-octadien-3-ol, 598-07-2; terpinen-4-ol, 562-74-3; dodecanoic acid, 143-07-7; 5-methylfurfuraldehyde, 620-02-0; 1,1-diethoxy-2-methylpropane, 1741-41-9; 2,4,6-trimethyl-1,3,5-trioxane, 123-63-7; 1,5,8-trimethyl-1,2-dihydronaphthalene, 4506-36-9; 1-heptanol, 111-70-6.