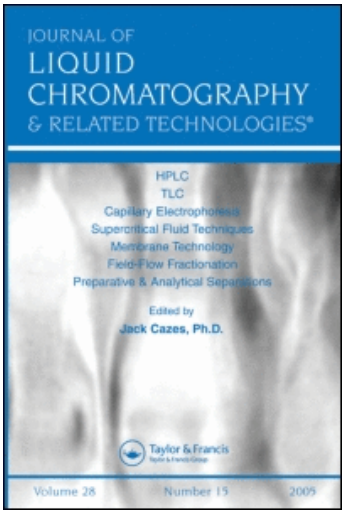

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HPLC Analysis of Aldehydes and Ketones in Sherry

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HPLC ANALYSIS OF ALDEHYDES AND KETONES IN SHERRY

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ABSTRACT

A method was developed to obtain comparative data on aldehydes and ketones which contain 3-10 carbon atoms in various types of sherry. The method consists of continuously extracting sherry samples with Freon 11 and then treating the concentrated extracts with 2,4-dinitrophenylhydrazine in a hexane-water solvent system. The hexane solution is separated and then extracted with acetonitrile; the acetonitrile solution is submitted to HPLC analysis. Overall recoveries (starting from model sherry solutions) of representative compounds (not containing hydroxy groups) were between 37 and 100 percent.

The method intentionally gives poor recoveries for acetaldehyde which, because of its high concentration in sherry, would otherwise mask less concentrated aldehydes and ketones in the chromatogram.

Comparison of the chromatograms of Fino, Amontillado, and Oloroso sherries reveal that aldehyde/ketone production is clearly associ ated with time in sherry production. Differences between gas chromatograms of the Freon extracts of these sherries are less pronounced.

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INTRODUCTION

A number of sensitive methods are available for identification and analysis of aldehydes and ketones present in wine (1), beer (2) and whiskey (3) etc. Most of these analyses have made use of High Performance Liquid Chromatography (HPLC) of derivatives of these compounds. While fluorescent derivatives are well suited for trace analysis, the 2,4-dinitrophenylhydrazone derivatives are easier to form and are less subject to artefact formation.

Continuous extraction of wine followed by capillary gas chromatography has provided a great deal of useful data on aroma compounds which are obtained in good yields from wine (4). It is probable, however, that many influencial compounds are masked or covered over by less influencial, but more abundant ones in the chromatograms. GC-sniff techniques have been successful in aiding the isolation and identification of highly aromatic compounds (eg 5). Other compounds, however, which are less aromatic but nevertheless are accurate indicators of a wine's age, quality, etc. may also be in low enough concentration to be hidden in chromatograms produced with nonspecific detection devices.

It occurred to us that aldehydes and ketones may be indicative of a sherry's history and that their identification and measurement, free from interference from other compound types, would show differences between sherries that are otherwise difficult to distinguish. In this paper we report a method of analysis of sherry for aldehydes and ketones containing between 3 and 11 carbon atoms and not containing hydroxy groups.

MATERIALS AND METHODS

Chemicals.

Solvents used were: deionized and filtered ("Milli-Q") water; HPLC grade acetonitrile (Scharlau) which was passed through a 0.45 μm filter; spectrofluorometric grade hexane (Merck);

Freon-11 which was distilled through 30 cm of glass collars shortly before use. Authentic samples of aldehydes and ketones were obtained commercially.

Instrumentation.

HPLC separations were performed on a Waters Associates assembly consisting of two Model 6000A pumps, a Model 660 solvent programmer, a U6K injector and a Model 481 Spectrophotometer whose wavelength was set at 336 nm and whose sensitivity was set at 0.1 AUFS. A 250 x 4.6 mm CP^{tm} Spher C_{18} (Chrompack) column was used.

Gas chromatographic analyses were made with a Shimadzu Model R1A instrument using a 10 x 0.8 mm glass column which contained 80% IGEPAL and 20% Carbowax 20M on Volaspher. Hydrogen (flow rate 20 ml/min) was used as carrier gas. The initial temperature, heating rate per minute and final temperature were 60, 3, and 180 degrees respectively.

Extraction, Concentration, and Derivatization.

Sherry samples or model solutions (250 ml) were treated with 0.083 mg of undecanal and then continuously extracted for 8 hr with approximately 100 ml of Freon. A fritted disk was used to distribute droplets evenly over the aqueous phase.

The extract was then concentrated at 30^{o} C using a 30 cm Vigreaux column for approximately 5 hr to a volume of 0.5 ml. The derivatization step followed immediately.

To the concentrated mixture (or representative compound) was added 20 ml of hexane, 10 ml of water and 18 ml of 2.4M aqueous HCl containing 54 mg of 2,4-dinitrophenylhydrazine. The mixture was stirred at room temperature for 16 hr and then transferred to a separatory funnel. The aqueous layer was discarded and the hexane solution was washed twice with 20 ml of water, then extracted 10 times with 2 ml of acetonitrile. The latter solution was passed through a 0.45 μm filter before HPLC analysis.

HPLC Analysis.

Samples of ca. 10 μl were coinjected with 0.02 μg of nonanal 2,4-dinitrophenylhydrazone. The solvent, with a flow rate of

2 ml/min, was programmed linearly from 40% to 99% acetonitrile in water over 14 min. The program was begun at sample introduction and the analysis time was 20 min. Peak areas for the recovery tests were calculated from peak height x width at half height; areas for the representative compounds were compared to that for the nonanal. Recoveries for sherry samples were estimated by comparing the peak areas of uncecanal and nonanal.

Model Solutions.

The model solutions containing one or more of the representative compounds (Table 1) consisted of 250 ml of 15% ethanol in water to which 1 g of tartaric acid, 0.12 ml of acetic acid and the initial amount (0.28 mg) of aldehyde or ketone. The extraction, concentration, derivatization and analysis were conducted as above.

RESULTS AND DISCUSSION

Recovery of Compounds from the Model Solutions.

Recovery data are presented in Table 1. Two tests were made for each representative compound. The first column ("From Derivatization") lists the recovery of each compound when it was introduced at the derivatization step and continuing to HPLC analysis. The mixed phase method which was reported by Selim (6) gives reasonble recoveries for the compounds tried; shorter reaction periods gave reduced yields.

The recovery data for the individual compounds when they were introduced to a model solution at the initial extraction stage are presented under the second column and reflect the efficiency for the entire method. Lower recoveries indicate incomplete extraction and/or losses during concentration, particularly for acetaldehyde whose relatively high polarity prevented complete extraction and low boiling point which caused further losses during concentration; low recovery of propionaldehyde was similar but not as severe. Carbonyl compounds

TABLE 1
Recovery Data for Representative Compounds

Compound	Percent Recovery	
	From Derivatization	From Initial Extraction
Acetaldehyde	-	12
Propionaldehyde	83	22
Benzaldehyde	71	45
Cyclohexanone	79	37
Cinnamaldehyde	90	90
Heptaldehyde	74	50

having 4 or more carbon atoms are extracted and concentrated with reasonable recovery.

The low recovery of the acetaldehyde is beneficial for the method because it is at least 10 times more concentrated than any of the other aldehydes or ketones (7); since methods are available for quantitatively determining acetaldehyde (8), efforts were made to reduce its amount before derivatization.

Chromatograms of the Sherry Samples

The aldehyde/ketone chromatograms of two Fino sherries are shown in Figure 1 and those for the Oloroso and Amontillado types are shown in Figure 2. A solvent program similar to that reported by Demko (9) was used. Numbered peaks were identified by peak enhancement when the sherry sample was coinjected with authentic samples of the aldehyde/ketone derivatives.

A comparison of the four chromatograms shows clear differences between sherry types. The longer time needed for barrel and yeast contact for the Oloroso and Amontillado sherries is clearly indicated by aldehyde/ketone formation. The chromatograms for the Fino types show differences between brands.

Gas chromatograms of the Freon extracts of the sherry samples studied are presented in Figure 3. While there are clear differences between them, they are far less pronounced than for the HPLC analyses in Figures 1 and 2.

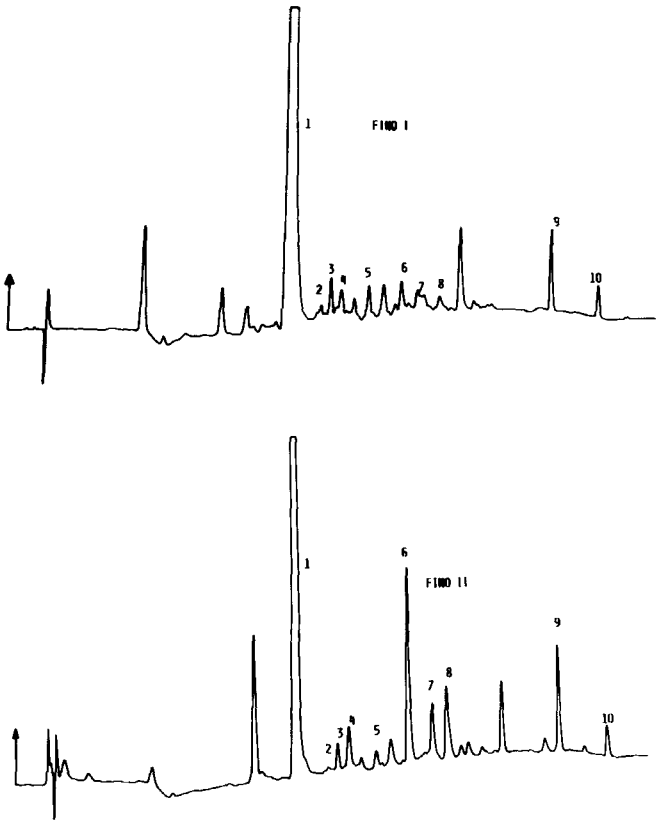

Figure 1. HPLC chromatograms of aldehyde/ketone derivatives from Fino type sherries. Injection at arrow. Peak identification: 1=acetaldehyde; 2=ethyl pyruvate; 3=furfuraldehyde; 4=propionaldehyde; 5=crotonaldehyde; 6=benzaldehyde; 7=isovaleraldehyde; 8=cinnamaldehyde and/or trans-2-hexenal; 9=nonanal; 10=undecanal (RT=18.5 min).

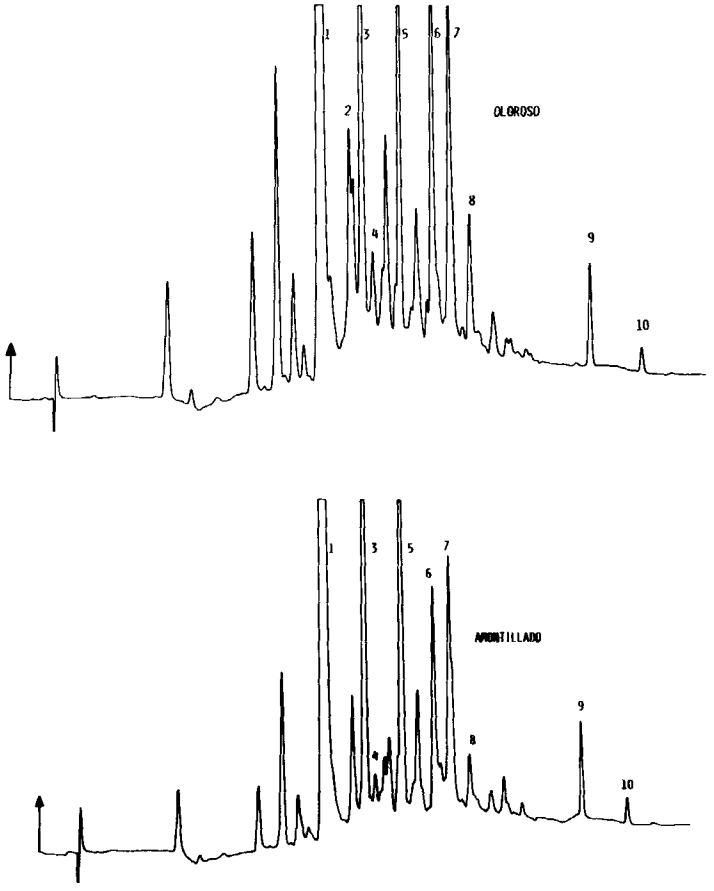

Figure 2. HPLC chromatograms of aldehyde/ketone derivatives. Peak identification as in Fig. 1.

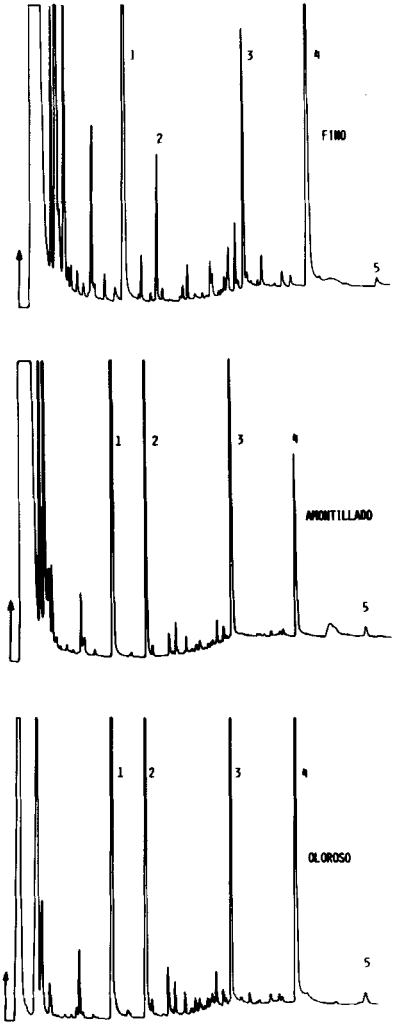

Figure 3. Gas chromatograms of Freon extracts. Peak identification: 1=isoamyl alcohol; 2=ethyl lactate; 3=diethyl succinate; 4=2-phenylethanol; 5=cinnamaldehyde (RT=73 min).

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