a process control valve in the beer feed line, which is fed by a constantly running feed pump. More recent technology has made it possible to control the proof more directly by using the temperature-corrected density function of a mass flow meter, which can be correlated to the proof of the discharge from the still. The only downside to the use of a mass flow meter is the process lag time that results from having to measure the proof of the distillate after condensation. Additionally, the control valve can be eliminated from this loop by using a frequency inverter, as described above. Some distillers employ a more sophisticated means of controlling the beer feed to the still by use of a cascaded control loop. Typically, a magnetic flow meter is used to measure the flow of beer to the still and control the operation of the control valve. The still top temperature transmitter provides a signal which is used to manipulate the control settings for this flow control loop.

In addition to the above controls, one or more condensers must also be controlled. Generally, a control valve on the inlet cooling water line is used to control this process. The control signal typically comes from a temperature transmitter which can either be located on the discharge water line or the discharge product line. Due to the relatively quick flow rate of the cooling water with respect to the product flow rate, process control response is generally much better if the temperature transmitter is located on the cooling water discharge line.

Finally, if the product is double-distilled in a true doubler, one additional control loop is required. The steam flow to the steam coils inside the doubler must be regulated. Almost without exception, this loop consists of a steam control valve and a temperature transmitter on the vapour discharge from the doubler. In a manner similar to the still top control, new technology has made it possible to control the discharge proof more directly using a mass flow meter.

Conclusion

A sign at the Stitzel-Weller distillery in Louisville, Kentucky sums up the traditional view of the impact of science on the beverage alcohol industry:

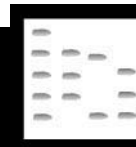
> **No Chemist Allowed**
> Nature and the oldtime know-how of the master distiller get the job done here. Because traditional Kentucky whisky is a natural product, we disdain synthetics, scientist, and their accompanying apparatus. This is a distillery, not a whisky factory.
>
> Pappy Van Winkle

Tradition handed down through the generations is the predominant means of whisky production. There are numerous stories of a distiller who had to replace his still because it had worn out. When the new still was being installed, the distiller would make sure that it was identical to the one it was replacing, right down to the dent in the side of the still, which was generally reapplied by the master distiller himself. As a result, technological change is slow to be adopted in an industry where any change in the process may result in a changed taste. Technology is gaining a foothold in the area of process control, where new and better final control elements, transmitters and control systems are always being applied. This traditional approach has also resulted in an almost complete lack of published literature on the topic of whisky distillation, which at best is viewed by the industry as only part science and part art.

See Colour Plate 127.

See also: **III/Wine: Gas and Liquid Chromatography.**

WINE: GAS AND LIQUID CHROMATOGRAPHY



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Introduction

From the chemical point of view, wines are aqueous alcoholic solutions containing more than 1000 components that can be present at high concentrations ($g\,L^{-1}$), but also at trace levels ($ng\,L^{-1}$). Some of these components determine the organoleptic properties of wines, while others are significant for classifying their origin and/or for checking whether some adulteration has taken place. Concentration levels of these compounds vary according to the variety of vine, the climatic conditions under which the grapes were grown, and the conditions under which vinification and ageing processes have been developed.

The quality of wines is established by sensory analysis, which is clearly correlated to their chemical

composition. To assure and control this quality, some essential parameters and characteristic compounds are determined by physical and chemical analyses, which are established principally by the *Office International de la Vigne et du Vin* (OIV). Other constituents whose determination is included in these methods are those associated with the toxicity of wines or which are allowed to be present to some maximum permissible levels.

Until recently, the techniques used in the analysis of this broad variety of properties and compounds have been based on classical methods (mainly gravimetric, titrimetric and colorimetric), which allow an adequate control of the vinification in the wineries. Although chromatographic techniques are not widely used in the OIV methods, the complexity of wine composition has pointed to the use of chromatography in many oenological laboratories, and the improvement in the analysis of wines is undeniably bound up with the development of chromatography.

Almost all the chemical compounds present in wines can be analysed by chromatography, either by direct injection or by prior derivatization. For volatile, thermally stable compounds, gas chromatography (GC) is the most used technique, while for the analysis of nonvolatile and thermally unstable compounds, high performance liquid chromatography (HPLC) is preferred. These techniques are considered in more detail below.

Gas Chromatography

Gas chromatography has been responsible for the most important advances in the knowledge of the volatile fraction of wines. Although the nonvolatile fraction can also be analysed by GC after derivatization of the analytes, HPLC methods are simpler and therefore they are preferred for these compounds.

The main application of GC to wine analysis is the study, characterization and determination of the aroma of wines, which originates from their volatile components. Aroma compounds are usually classified according to their chemical functionality, the most important being esters, alcohols, acids, lactones, carbonyl compounds, volatile phenols and sulfur- and nitrogen-containing volatiles. With the exception of ethanol and glycerol, the concentrations of the individual aroma compounds range from 100 mg L^{-1} to 0.1 ng L^{-1}. The human sensory organs are extremely sensitive to certain aroma substances, which can show sensory thresholds much lower than their concentrations in wine. The analysis of wine aroma must, therefore, be optimized in order to determine all these compounds. To perform this kind of analysis, two different steps must be considered: sample preparation and gas chromatographic separation.

Sample Preparation

The sample pretreatments are conditioned by the wine matrix and the character and the concentration of the analytes to be determined. Wines can be directly injected, but it is more common to inject the extract obtained after the application of pretreatment techniques.

Direct injection is applied to the analysis of the volatiles whose concentration is close to the mg L^{-1} level, so they are easily detected by GC detectors. When injecting wine in this way, the nonvolatile fraction remains in the injector and may be thermally degraded, giving rise to potential interfering substances (artefacts) and unstable baselines. Distillation of the volatile fraction or filtration, after addition of a water-miscible solvent to reduce the polarity of the wine matrix, helps to minimize this problem.

Injection after clean-up and concentration treatments is usually applied to the analysis of trace compounds (μg L^{-1} to ng L^{-1}) to enhance their detectability. At the same time, interfering substances are removed during the isolation procedure. The main problems with these techniques are the occasional quantitative and qualitative changes of the analytes, the formation of artefacts by chemical reactions or thermal decomposition, and the introduction of impurities. For these reasons, the suitability of the isolation and concentration methods for a particular analyte has to be carefully evaluated.

Distillation is normally used to isolate the wine volatiles from the nonvolatiles. It can be carried out at atmospheric or reduced pressure in different distillation modes (direct, steam and fractional). By working at reduced pressure and low temperature chemical reactions or thermal decomposition can be minimized. The most important disadvantage is that the isolates obtained are diluted and it is necessary to combine the distillation with other methods that concentrate the volatile fraction.

Solvent extraction is used to simultaneously isolate and concentrate the volatiles. It is carried out in batch mode (simple or multiple) or continuous mode (by using continuous liquid–liquid extractors). The choice of the solvent is conditioned by the high concentration of ethanol (10–15%) in wines. Owing to their low boiling points and very low polarities, pentane, dichloromethane and their azeotropic mixtures are commonly used because they discriminate against ethanol. Other solvents used are diethyl ether and ethyl acetate. Fluorocarbons were widely used because of their extraction efficiency and very low boiling points, but nowadays they are environmentally

unacceptable. One of the disadvantages of solvent extraction is the use of large volumes of solvents (normally not free of contaminants) and the large amount of time spent in the extraction. Whenever possible, the use of minimum solvent/sample volume ratios enhances the concentration and minimizes contamination problems. At the same time, the efficiency of the extraction can be raised appreciably by salting-out the solution with sodium chloride.

The low boiling points of these solvents allow the concentration of the extracted volatiles by distilling off the solvent. By using a Kuderna–Danish concentrator the loss of volatiles is minimized. Furthermore, a gas stream is used to remove the solvent excess from the extract. This procedure is very effective, but may lead to the introduction of contaminants from the gas and to losses of the most volatile compounds.

Simultaneous distillation–extraction, using the apparatus originally described by Likens and Nikerson, has not been commonly applied to wine analysis. Supercritical fluid extraction is not common, but it is becoming increasingly accepted.

Solid-phase extraction (SPE) has also been used for the isolation of wine aroma compounds. The most common adsorbents are charcoal, silica gel and porous polymers (Chromosorb™, Porapak™, Amberlite XAD™ and Tenax™). The volatile compounds retained are usually eluted and/or fractionated by pentane, diethyl ether, dichlorometane, ethyl acetate or their mixtures. This technique is preferred for the analysis of a specific group of volatiles, since the adsorbent used is normally selective. Large volumes of adsorbents and solvents are used in order to assure the whole recovery of analytes, so dilute solutions are obtained. A final step including solvent evaporation is therefore needed.

Headspace techniques are widely used in wine aroma determinations because they enable the direct analysis of the headspace gas above the heated samples, where the compounds responsible for the aroma detected by the human nose are transferred. The static headspace technique is suitable for the analysis of the aromatic compounds of highest concentration, but for the analysis of trace levels it is necessary to use dynamic headspace (purge and trap) techniques. The retention of the volatiles is usually achieved by using either cryo or sorbent traps. Sorbent traps are normally preferred because the retention of water and ethanol is minimized, using the same adsorbents mentioned for SPE. The trapped volatiles are recovered by extraction with small volumes of solvent or by thermal desorption, which can be carried out in the chromatographic injector, enabling the overall sample to be analysed in a single step. On the other hand, the volatiles obtained by solvent extraction are more dilute, but the sample can be fractioned and therefore injected in several chromatographic runs.

Solid-phase microextraction (SPME) is a single-step solvent-free extraction technique that combines the advantages of both SPE and headspace techniques. It has been increasingly applied to the isolation of flavour compounds. The adsorbent (usually polydimethylsiloxane-, divinylbenzene- or polyacrylate-coated fused silica fibres) is fixed in the needle of a specially designed chromatographic syringe and exposed either to the liquid sample or to the headspace above it. After exposure of the fibre to the sample, absorbed analytes are recovered from the fibre by thermal desorption in a conventional GC injection port.

Chromatographic Separation

The chromatographic separation is normally carried out in a gas chromatograph equipped with a split/splitless injector and a flame ionization detector (FID). On-column injectors with retention gaps are very useful for the analysis of traces because they enable the injection of large volumes of wine extracts and the concentration of the volatile fraction at the head of the chromatographic column. Programmed temperature vaporizer (PTV) injection is also suitable for the direct desorption of volatile compounds trapped on injector glass liners filled with adsorbents.

The detection of the analytes is usually carried out with a FID or a mass spectrometer detector (MSD). Other detectors are used only to detect more specific compounds. The flame photometric detector (FPD) and, more recently, the sulfur chemiluminiscence detector (SCD), are widely used for detection of sulfur-containing compounds, mainly thiols, sulfides, disulfides and heterocyclic compounds. The electron-capture detector (ECD) is used to detect halogenated substances, such as chlorophenols and chloroanisoles, which are associated with cork taint off-flavours. The ECD and the nitrogen phosphorus detector (NPD) are widely used for the analysis of pesticide residues and some specific additives.

To characterize the wine flavour, gas chromatography–olfactometry (GCO) has been coupled with different methods that determine the relative aroma intensity. The smell of the different components of the wine aroma is assessed by sniffing the effluent of the chromatographic column in parallel with FID detection.

All kinds of chromatographic columns can be used to separate the volatile analytes of wines, but fused silica capillary columns with different stationary phases are the most common. Polyethylene glycol

phases are preferred for the evaluation of the global wine aroma, while less polar phases (such as methylsiloxane and methylphenylsiloxane phases) are needed for assessing the identification of individual compounds. Chiral phases are used for the separation of the enantiomers of volatile compounds, which exhibit very different sensory properties. Multidimensional gas chromatography is used for the analysis of volatiles that are not well separated with a single column.

Selected Applications

Many of the sources listed in the Further Reading section deal with the analysis of wines by GC. The following methods are usually performed in oenological laboratories for routine control and research studies.

According to the OIV method, the determination of methanol and ethyl acetate in wines is carried out by GC-FID. Wine distillates are injected in the split injection mode on a polyethylene glycol column under isothermal conditions. This method enables the simultaneous determination of other compounds present in the distillate, such as acetaldehyde, 1-propanol, 2-methylpropanol, 1-butanol, 2- and 3-methylbutanol, 1-pentanol, 1-hexanol, 2-phenylethanol, ethyl lactate, ethyl succinate, 3-methylbutyl acetate, acetic acid, some polyalcohols and so on. In routine analysis, this procedure is usually simplified when wines are directly injected. **Figure 1** shows an example of the chromatogram obtained under these conditions.

The main aroma compounds of wine distillates are analysed by direct split injection of samples with a temperature programme that optimizes the separation of the different substances. The compounds determined in wine distillates by this OIV method are methanol, acetaldehyde, acetals, higher alcohols, ethyl esters of fatty acids, acetates of the main alcohols and volatile fatty acids. There are other aroma compounds that can be detected by splitless injection of the extract obtained with a batch extraction using ether/hexane (1 : 1) and magnetic stirring. **Figure 2** shows an example of the chromatogram obtained when a methylene chloride wine extract is injected.

In research work, the isolation of the global aroma of wine is usually performed by continuous solvent extraction using different ratios of pentane–dichloromethane and different times of extraction. The extract is dried over anhydrous sodium sulfate and concentrated either in a Kuderna–Danish device or with a gas stream. The concentrate is injected into the GC-FID, working in splitless mode and with a programmed column temperature. Purge and trap methods are suitable alternatives to this procedure and, more recently, SPME has also found some applications in the determination of the ethyl esters of spirit beverages and in the analysis of the main aroma of fruit juices.

Currently, many other volatile compounds are investigated by GC for their sensory contribution. The most important are terpenes, lactones (solerone, sotolone and oak lactones), carbonyl compounds (hexenals, β-damascenone and α- and β-ionone), volatile phenols (alkylphenols and alkylguaïacols), thiols, sulfides, disulfides, pyrazines and vitispiranes. The particular methods of analysis for these compounds are fully described in publications listed in Further

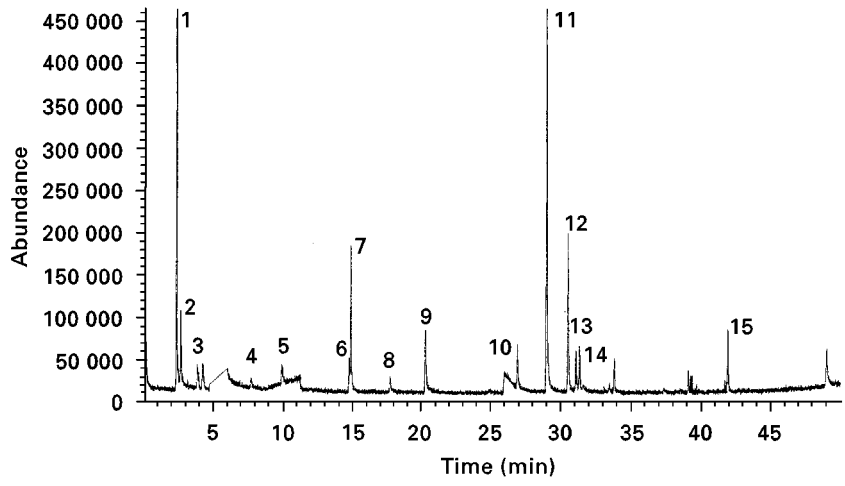

Figure 1 Chromatogram from direct injection of a wine sample (GC-MS). Key: 1, carbon dioxide; 2, acetaldehyde; 3, ethyl acetate; 4, 1-propanol; 5, 2-methyl-1-propanol; 6, 2-methyl-1-butanol; 7, 3-methyl-1-butanol; 8, acetone; 9, ethyl lactate; 10, acetic acid; 11, (D)-2,3-butanediol; 12, *meso*-2,3-butanediol; 13, 1,2-propanediol; 14, 3-ethoxy-1-propanol; 15, 2-phenylethanol.

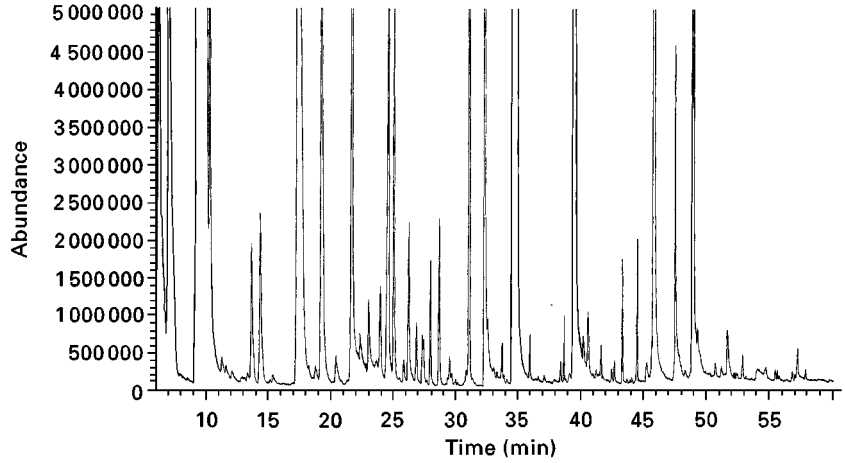

Figure 2 Chromatogram of the injection of a concentrated wine extract obtained by methylene chloride extraction (GC-MS).

Reading. Two examples of chromatograms obtained from the analysis of sulfur compounds (**Figure 3**) and pyrazines (**Figure 4**) are shown.

High Performance Liquid Chromatography (HPLC)

The first studies on the application of HPLC to the analysis of wines appeared at the end of the 1970s for the analysis of polyphenols. Since then, HPLC has been applied to the separation, characterization and determination of a large number of wine compounds or groups of compounds. The literature concerning the application of HPLC in wine and must analysis is very extensive, but it is important to emphasize the particular interest of this technique in the study of polyphenols, amino acids, biogenic amines, organic acids and sugars.

The use of HPLC is rarely recommended in the official methods. However, carboxylic acids, saccharose, hydroxymethylfurfural and some additives such as sweeteners can be determined by official HPLC methods.

As mentioned above, one of the main problems when dealing with wines is the complexity of the matrix. Although HPLC offers the possibility to choose columns, solvents, detectors and derivatizing reagents, many of the chromatographic procedures developed for HPLC determinations in wines involve some kind of sample pretreatment. These procedures generally make use of either ion exchange

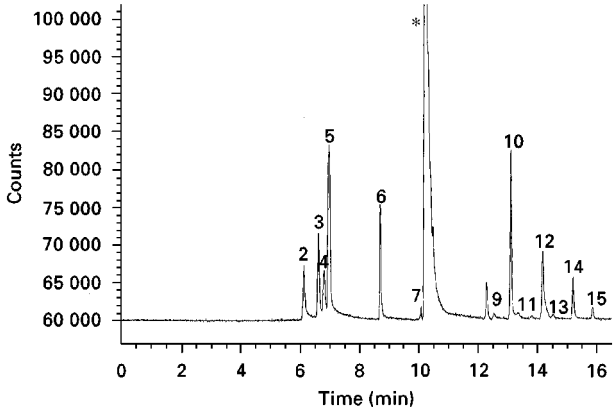

Figure 3 Sulfur compounds found in the headspace of a cryogenically trapped wine (GC-FPD). Key: 1, hydrogen sulfide; 2, methanethiol; 3, carbon disulfide; 4, ethanethiol; 5, dimethyl sulfide; 6, methyl ethyl sulfide (internal standard); 7, diethyl sulfide; 8, methyl propyl sulfide; 9, ethanol; 10, tiophene (internal standard); 11, methyl thioacetate; 12, dimethyl disulfide; 13, ethyl thioacetate; 14, ethyl methyl disulfide; 15, diethyl disulfide. (Reproduced with permission from Mestres M, Busto O and Guasch J (1997) Chromatographic analysis of volatile sulfur compounds in wines using the static headspace technique with flame photometric detection. *Journal of Chromatography A* 773: 261–269. Copyright 1997, Elsevier Science.)

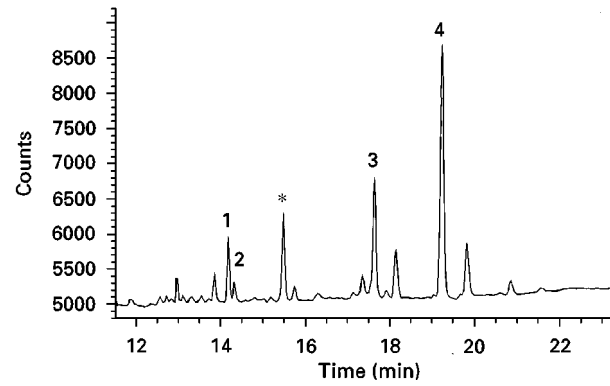

Figure 4 Pyrazines found in the headspace of a wine after SPME (GC-NPD). Key: 1, 3-isopropyl-2-methoxypyrazine; 2, 3-ethyl-2-methoxypyrazine; 3, 3-*sec*-butyl-2-methoxypyrazine; 4, 3-isobutyl-2-methoxypyrazine; *, 3-isopropyl-2-ethoxy-pyrazine (internal standard).

chromatography and/or solvent of solid-phase extraction.

Sample Preparation

Filtration of samples through a 0.45 μm membrane is always recommended before injecting wines into the HPLC system. This process can be before or at the same time as more complex pretreatments.

One of the methods for avoiding the presence of interfering substances is eluting wine through a low pressure liquid chromatographic column. Polyvinylpyrrolidone (PVP), polyvinylpolypyrrolidone (PVPP) and polyamide adsorbents are used to eliminate polyphenolic substances and silica is used to retain proteins. Ion exchangers are commonly used either to clean up samples or to isolate ionized amines and organic acids from wines.

The most common method used to pretreat samples is solvent extraction with ether or ethyl acetate, although many researchers use more selective solvents.

Solvent extraction has generally been replaced by SPE and SPME. Although there are some applications of carbon and ion exchange cartridges, octadecylsilane (C_{18}) is the most commonly used adsorbent. Many of the analytes whose determination in wines is of interest (such as polyphenols, amino and aroma compounds) can be selectively retained or eluted with slight modifications of matrix conditions (such as pH or addition of ion pair reagents) or by transforming the analytes by derivatization.

Chromatographic Separation

Reversed stationary phase (RP) are the most popular in the HPLC analysis of wines, although it is fully recognized that they are not capable of separating all kinds of analytes in wine. Apart from some special applications, silica is utilized almost exclusively as the support material and C_{18} as the bonded phase.

Since wines are constituted of analytes spanning a wide range of polarities, linear solvent strength gradients are preferred for analysis. Mobile phases normally consist of binary mixtures of either methanol or acetonitrile and slightly acidified water.

The variable UV-visible (UV-vis) wavelength detector is the most popular, although fluorescence and refractive index detectors are also common. Photodiode array detection has also found some application.

Selected Applications

In contrast to GC, the analysis of wines by HPLC has focused on determining compounds with similar chemical functionality.

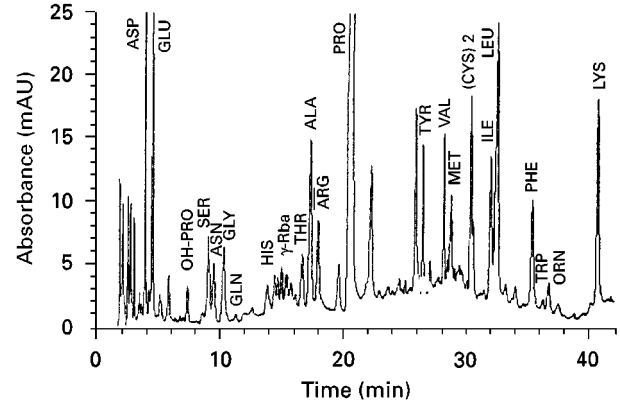

Figure 5 Chromatogram of free amino acids in white wine after derivatization with PITC and DAD detection. (Reproduced with permission from Calull M, Fábregas J, Marcé RM and Borrull F (1991) Determination of free amino acids by precolumn derivatization with phenylisothiocynate. Application to wine samples. *Chromatographia* 31: 272–276.)

Ion exchange chromatography has become the most popular technique for the determination of amino acids due to the use of autoanalysers. After chromatographic separation, the analytes are derivatized with ninhydrin, fluorescamine or *o*-phthaldialdehyde and detected by spectrophotometry. Amino acids can also be determined by RP-HPLC, which is faster than ion exchange chromatography. The stationary phases are based on amino and, especially, C_{18} chemical groups. Although isocratic elution is used in some applications, gradient elution is preferred because it enables the simultaneous determination of amino acids of different polarities. Mobile phases are normally of binary composition (methanol or acetonitrile and an aqueous buffer solution). As in ion chromatography methods, the amino acids are derivatized, but this time dansyl chloride or phenylisothiocyanate (**Figure 5**) are used for UV-vis detection and *o*-phthalaldehyde for fluorimetric detection.

Amines are also determined by HPLC, either by direct injection or, more commonly, by derivatization. When they are directly injected, they are separated by ion pair chromatography on a C_{18} column and detected by conductimetry or spectrophotometry. The main limitation of these procedures is that mobile phases shorten the life of the column, so procedures involving the separation of derivatized amines are preferred. Ninhydrin is one of the reagents commonly used in post-column derivatization. The separation is done either by RP-HPLC or by ion exchange chromatography. However, these methods are very time-consuming and so pre-column derivatizations are preferred. The derivatizing reagents used in this case are the same as when dealing with

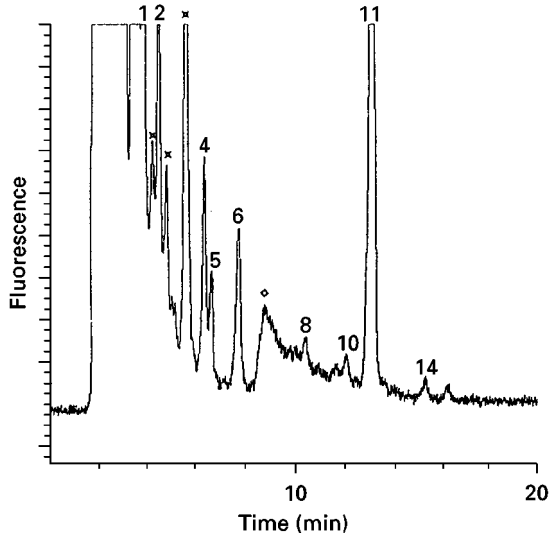

Figure 6 OPA-derivatives of biogenic amines in red wine after SPE and fluorescence detection. Key: 1, ethanolamine; 2, histamine; 4, ethylamine; 5, tyramine; 6, isopropylamine; 8, tryptamine; 10, phenethylamine; 11, putrescine; 14, cadaverine; ◇, peak corresponding to the excess of OPA; x, unknown. (Reproduced with permission from Busto O, Guasch J and Borrull F (1995) Improvement of a solid-phase extraction method for determining biogenic amines in wines. *Journal of Chromatography A* 718: 309–317. Copyright 1995, Elsevier Science.)

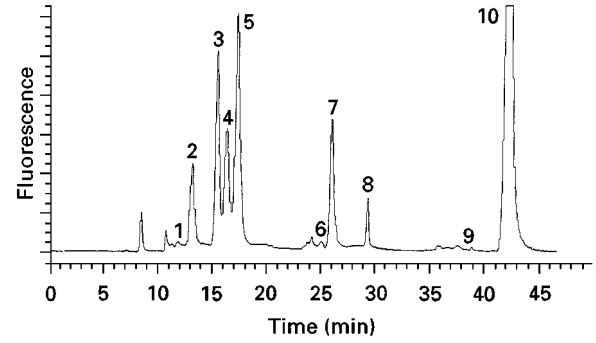

Figure 7 Chromatogram of a wine obtained by high resolution ion exclusion chromatography and refractometric detection. Key: 1, citric acid; 2, tartaric acid; 3, glucose; 4, malic acid; 5, fructose; 6, acetic acid; 7, glycerol; 8, lactic acid; 9, methanol; 10, ethanol.

amino acids, *o*-phthalaldehyde being the most used (**Figure 6**). The derivatives are separated by RP-HPLC and detected by spectrophotometry or fluorimetry.

Although carboxylic acids can be determined by GC, after suitable derivatization, the OIV proposes the use of HPLC for determining carboxylic acids in wines, as an alternative to the usual enzymatic procedures. RP-HPLC is used either by direct injection of the wine or by derivatization of the acids before separation. Direct injection of carboxylic acids is a simple method, but the use of mobile phases at low acidic pH (to avoid acid ionization) considerably reduces the life of the analytical column, hence derivatization is recommended. Furthermore, when acids are transformed into their corresponding esters, detection is more sensitive. The different derivatization methods reported so far for the determination of organic acids with spectrophotometric detection and which are worthy of special mention are those which use organic compounds containing the chromophore groups phenacyl, naphthacyl and *p*-nitrobenzyl. The organic groups containing courmarin and anthracene groups, on the other hand, are used for fluorimetric detection. Mobile phases are of binary solvents (normally methanol and water) and the elution is done isocratically or with a linear gradient.

Anion exchange chromatography is an alternative to RP-HPLC, using mobile phases made of organic solvents buffered at a pH close to 8 and with conductimetric or refractometry detection. Although good results are obtained by this method, ion exclusion chromatography using strong cation exchange phases has become the best technique. There are stationary phases specifically developed for determining carboxylic acids in fermented products, which permit the simultaneous determination of sugars, ethanol, methanol and glycerol in a single run. Mobile phases consist of slightly acidic water solutions and the detectors used are either UV or refractive index. **Figure 7** shows an example of the chromatogram that is obtained when wine is directly injected under these conditions.

Some papers have reported the separation of carboxylic acids by NP-HPLC and ion pair chromatography, but the results are not comparable to those obtained from the methods described above.

Although the official methods of analysis of sugars are based on enzymatic techniques, sugars can also be determined by HPLC. The preferred methods are based on the use of specific ion exclusion polymeric columns because they enable the simultaneous determination of carbohydrates and other analytes, as already mentioned. The mobile phase used is dilute sulfuric acid and the detection is carried out by spectrophotometry or refractometry. RP-HPLC of sugar benzoylated derivatives followed by spectrophotometric detection has also been used to determine carbohydrates in wines (Figure 7).

According to the OIV methods, saccharose is analysed by HPLC. In this case, the column used is based on 3-aminopropylsiloxane-bonded phases and the mobile phase is acetonitrile and water with refractive index detection.

There are classical methods for estimating the total phenol content of wines, but HPLC is necessary for the determination of individual polyphenolic

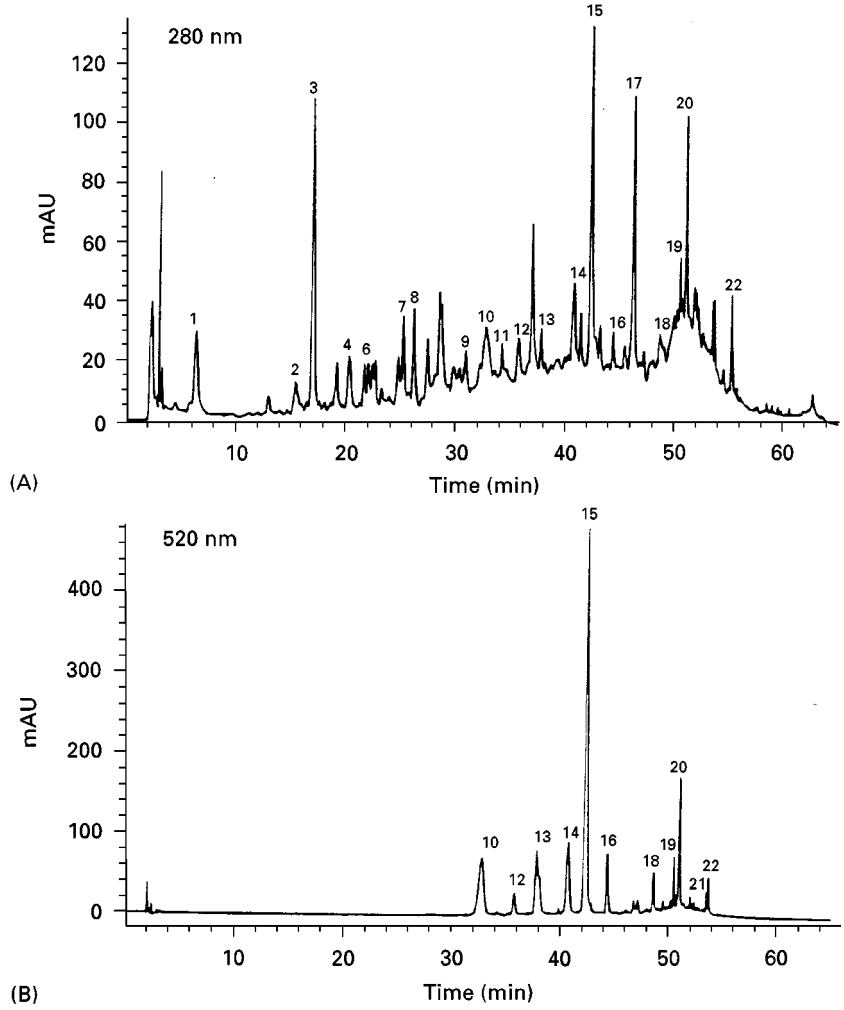

Figure 8 HPLC chromatograms of a must monitored (A) at 280 nm for all phenolic compounds, and (B) at 520 nm to selectively detect anthocyanins. Key: 1, gallic acid; 2, *cis*-caffeoyltartaric; 3, *trans*-caffeoyltartaric; 4, *S*-glutathionylcaftaric; 5, *cis*-coumaroyltartaric; 6, *trans*-coumaroyltartaric; 7, procyanidin B1; 8, catechin; 9, procyanidin B2; 10, delphinidin-3-glucoside; 11, epicatechin; 12, cyanidin-3-glucoside; 13, petunidin-3-glucoside; 14, peonidin-3-glucoside; 15, malvidin-3-glucoside; 16, cyanidin-3-glucoside acetate; 17, rutin; 18, petunidin-3-glucoside acetate; 19, peonidin-3-glucoside acetate; 20, malvidin-3-glucoside acetate; 21, peonidin-3-glucoside acetate (*p*-coumarate); 22, malvidin-3-glucoside acetate (*p*-coumarate). (Reproduced with permission from Lamuela RM and Waterhouse AL (1994) A direct HPLC separation of wine phenolics. *American Journal of Enology and Viticulture* 45: 1–5.)

compounds. Chromatographic procedures are conditioned by the lack of suitable standards and the complexity of chromatograms. Thus, the determination is tackled from the point of view of the two different families of phenolic compounds: flavonoids (anthocyanins, flavanols and procyanidins), and non-flavonoids (hydroxycinnamic and hydroxybenzoic derivatives). Nevertheless, only the pretreatment of samples is different depending on the fraction that has to be isolated. HPLC on reversed-phase columns is almost universally used for anthocyanin separation. The most common used support is C_{18}. Extremely acid solvents are required to suppress ionization of the analytes. Solvents such as methanol/water and acetonitrile/water (in varying proportions), acidified at low pH with phosphoric, perchloric or formic acid, have been used with different solvent programmes. When dealing with flavonols and procyanidins, extraction and purification of wines prior to HPLC is needed. HPLC analysis of flavonols is achieved on C_{18} columns with binary solvent systems consisting of acetonitrile and acetic acid in water and using gradient elution programmes. Procyanidins are chromatographed on C_{18}, C_8 or cyano columns and dilute acid is normally required as a component of the solvent to obtain satisfactory peak shapes. Hydroxycinnamic acids are also analysed by HPLC, by using C_{18} columns and methanol/water eluents slightly acidified with acetic acid. In all cases, detection is carried out spectrophotometrically (**Figure 8**).

Other substances which are present in wine but which are not determined as frequently as the ones discussed above can also be determined by HPLC. These include additives such as sorbic, salicylic, benzoic and ascorbic acids, which can be determined, according to OIV methods, by RP-HPLC coupled with either spectrophotometric or refractive index detectors.

Future Trends

Both GC and HPLC techniques are widely used in wine analysis. Although the methodologies are normally based on traditional separations, multidimensional chromatographic methods (with or without chiral phases) are increasingly being introduced, frequently coupled online with other analytical devices. More recently, capillary electrophoresis and supercritical fluid chromatography have also been used for wine determinations, but they are still in an early stage of application.

At present, most of the chemical compounds present in wine can be determined by means of a great variety of chromatographic methods described in the literature. Future trends, however, will focus more on internal method validation rather than on development of new methodologies. Future official methods of analysis will then include the minimum requirements (accuracy, precision, limit of detection, robustness, and so on) that an analytical method must fulfil in order to guarantee the validity of the results obtained.

See Colour Plate 128.

See also: **II / Chromatography. Extraction. II / Chromatography: Gas:** Headspace Gas Chromatography. **III / Amines:** Gas Chromatography. **Amino Acids:** Gas Chromatography; Liquid Chromatography. **Phenols:** Gas Chromatography; Liquid Chromatography; Solid-Phase Extraction.

Further Reading

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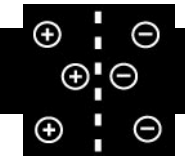
XENOBIOTICS: MAGNETIC AFFINITY SEPARATIONS

See **III / BIOLOGICALLY ACTIVE COMPOUNDS AND XENOBIOTICS: MAGNETIC AFFINITY SEPARATIONS**

ZEOLITES: ION EXCHANGERS



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The ion exchange properties of zeolites have been known since 1858, when Eichhorn studied the use of chabazite as an ion exchanger. In the 1920s and 1930s several ion exchange studies were reported.