polyphenols and results in the formation of theaflavins and thearubigens. The formation of these condensation products is dependent on the duration of oxidation during fermentation. Analytical methods for the characterization of aroma components remain to be established and the relationship between individual components and product quality has yet to be elucidated.

See also: **Derivatization of Analytes**. **Extraction**: Solvent Extraction Principles. **Food and Nutritional Analysis**: Contaminants; Oils and Fats. **Gas Chromatography**: Detectors. **Lipids**: Fatty Acids. **Liquid Chromatography**: Food Applications. **Sample Handling**: Comminution of Samples. **Sensory Evaluation**. **Spectrophotometry**: Organic Compounds.

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Alcoholic Beverages

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Introduction

Alcoholic beverages comprise a large group of beverages that contain varying amounts of alcohol (ethanol). Alcoholic beverages produced on an industrial scale include beer, wine, and China rice wine, and distilled spirits such as brandy, whisky, rum, gin, cognac, vodka, tequila, pisco, and China distilled spirit. The components of alcoholic beverages are highly complex and over 1300 compounds have been identified in various beverages.

The constituents of each alcoholic beverage can be divided into major, minor, or trace components. The major constituents usually consist of ethanol and water. The minor or trace constituents are fusel alcohols, organic acids, carbonyl compounds, esters, aldehydes, lactones, sulfur compounds, sugars, preservatives, and colorants. There are two general approaches to analyzing the components of alcoholic beverages. The first, and most usual, is by using chemical and physiochemical analysis. The other is to use sensory evaluation. In recent years, instrumental analytical methods have become an important tool for analyzing minor and trace constituents of alcoholic beverages. The most widely used methods are ultraviolet (UV)–visible spectrophotometry, gas chromatography (GC), liquid chromatography (LC), mass spectrometry (MS), and paper chromatography, and thin-layer chromatography.

This article will review the methods applied for the determination of major and minor components in alcoholic beverages.

Sensory Assessment

Sensory assessment is a scientific discipline used to evoke, measure, analyze, and interpret responses to those properties of a substance (food or beverage) that are perceived by the five human senses: sight, smell, taste, touch, and hearing. Even though individual judgments are subjective, the techniques of sensory evaluation use objective scientific testing methods principally based on working with a panel of assessors. Two types of analytical objective tests can be observed: (1) The discriminative tests: is there a difference between several products? (2) The

descriptive tests: what is the difference and how big is it?

The sensory assessment of alcoholic beverage, such as wine or beer, has, in general, three basic steps (except the decision on rating method):

1. Visual inspection: Checking if the container is bottle full and examination for transparency or sediment. After pouring the liquid into the glass (for wine there exist ISO standardized glasses), an additional appearance inspection follows. The sample is oriented against a bright background. In the case of wine, the sample is viewed at an angle of 30–45°; in the case of beer it is agitated to get a generous head. The appearance is scored later. One examines absence of haze, color shade and depth, viscosity, effervescence, or head retention. Visual inspection may seriously affect the subsequent evaluation steps, so one needs to be careful not to prejudge the beverage based on a visual inspection of the bottle.
2. Smell: There are various modes of odor evaluation. In the case of wine, it is recommended not to swirl first, as the most volatile compounds are perceived in this way. The nature and intensity of odors is recorded according to the rating system. Successive swirling should promote release of the heavier polar compounds that are retained in the hydroalcoholic solution more than in the apolar ones. Some tasters hold the glass between the hands when the beverage is too cold. The impressions are recorded yet again.
3. In-mouth sensations: The most complex task is the evaluation of simultaneously appearing sensations while tasting the beverage in the mouth. After coating the inside of the mouth with the liquid, one should score: (1) Taste and mouth-feel (body) – presence of taste sensations (sweet, acid, and bitter taste: their duration and changes in quality and intensity) and tactile sensations (astringency, prickling, body, temperature, and heat). (2) Odor – rating of beverage odors at the warmer temperatures of mouth, which increases volatility of odorants, before swallowing. Mainly the differences between the in-mouth and in-glass odor features are observed. (3) Aftersmell – rating of odor sensations after swallowing the liquid and expiring through the nose. The final stage of wine tasting is termed finish. It is the final impression from the wine (good or bad), may include odor-related, hedonic (e.g., flourish), or time-related terms (e.g., short finish). Typically, the longer the finish the more highly rated the wine.

Sensory richness of alcoholic beverages, especially of wine, promoted an intense effort to unveil the chemical background behind these percepts. As the impression derived from sensory evaluation is mainly governed by olfaction, the area of volatile compounds was the most investigated. Especially the performance and wide accessibility of GC and MS allowed identifying many volatiles, which could potentially contribute to the sensations experienced during wine or beer tasting. However, it was the introduction of GC–olfactometry in the 1970s that radically advanced the discovery of the real key compounds.

The establishment of odor units (a ratio of actual concentration of odorant to its threshold concentration) was also beneficial, even if in reality more emphasis is given to intensity ratings.

The most useful flavor descriptive procedure has so far turned out to be the development of flavor vocabulary precisely defined for each product. It is important to find sufficiently refined terms for the characteristic sensory properties of the specific alcoholic beverage. There are various intensity rating strategies in the sensory assessment practice. Perhaps the most widely used are the aroma wheels. The first was M.C. Mailgaard's 'Beer Flavor Wheel', jointly adopted in the 1970s by the European Brewery Convention, the American Society of Brewing Chemists, and the Master Brewers Association of America. A similar attempt was made in the 1980s by A. Nobel's 'Wine Aroma Wheel'. These systems provide standardized flavor descriptors and recipes for their reconstruction. Various methods were proposed for flavor profiling, among which the Quantitative Descriptive Analysis (QDA^{TM}) and Free Choice Profiling are prominent. The reliability of the results must be tested statistically even when using threshold values or relative values like odor unit values. Statistics are also needed in the different profile techniques, both to examine how judges employ the methods and to develop the terminology.

Endeavors have been made to find a link between two data sets (sensory versus instrumental data). The common goal of these tools is to discover the components or parameters whose variation explains the variation of sensory characteristics. The most useful statistical methods used for such purpose are partial least squares regression and generalized procrustes analysis. From a practical point of view, the models can be used to complement sensory assessment in routine quality control or in product and process development work. Regression-based statistical techniques are often used in conjunction with GC to distinguish well-known brands of alcoholic beverages from less expensive ones to detect counterfeit products.

Proof and Extract

The proof of an alcoholic beverage is a measurement of its ethanol content. The proof of spirit and wine is defined as the percentage by volume of ethanol. The traditional method for determination of proof uses a pycnometer or a small, accurately graduated hydrometer. Recently, the European Community has adopted a reference method for ethanol determination in spirits, based on two steps. The first step is a prescribed distillation stage and the second step allows a choice from three different methods of measuring the density of the distillate: (1) pycnometry: time consuming and susceptible to error; (2) electronic densimetry: measurement of the oscillations of a vibrating U-tube, this method is simple and fast; (3) densimetry using hydrostatic balance: this method is also fast with the new generation of instrumentation.

The extract of an alcoholic beverage is a measurement of its nonvolatile compounds. The extract of a wine is the most important among the alcoholic beverages. Wine extract is composed of sugars, fixed acids, organic salts, and other substances. Its value is a measure of wine quality. The traditional method of determining wine extract is drying at 100°C. The method has the disadvantages of being time consuming and limited to wines having an extract not greater than 6 g per 100 ml. It is being used less frequently today.

Of the two AOAC methods (Official Methods of Analysis of the Association of Official Analytical Chemists) to determine wine extract, the more convenient one relates the extract of wine to the specific gravity (relative density) of the dealcoholized wine. In spirits, the reference method adopted by EU legislation is gravimetry, involving weighing the residue left by evaporation of the spirit on a boiling water bath and drying in a drying oven. The method is not suitable for liqueurs because of their high sugar content.

Some recent and convenient procedures have been used to determine both ethanol content and extract. Regarding ethanol, an AOAC-approved method exists for the determination of ethanol in wine based on GC and the technique is very precise and accurate. An increasingly popular technique is the use of near-infrared spectroscopy. Instruments are commercially available allowing the determination of ethanol and other common parameters in wine.

Determination of Alcohols

Alcohols in alcoholic beverages mainly consist of aliphatic monohydric alcohols and di- and trihydric alcohols. Methanol and fusel oil in alcohols are determined but ethanol is determined as a proof in a routine analysis of alcoholic beverages.

Methanol is ubiquitous and is usually a minor component in alcoholic beverages, but some distilled alcoholic beverages produced from potato and stone fruits, such as cherries and plums, are exceptions and may contain rather large amounts. Large amounts of methanol have also been determined in some fortified wine, China distilled spirit, distilled wine, and brandy.

Higher alcohols from propanol up to 3-methyl-1-butanol (isoamyl alcohol), frequently encountered in so-called fusel oil fractions, are considered to be an important aroma factor, and for this reason they have been determined in a large number of beers, wines, and distilled alcoholic beverages. Because higher alcohols are believed to have a marked effect on the volatile flavor of distilled beverages, numerous papers have been published describing both the relative and absolute compositions of fusel oil fractions.

Generally, colorimetric methods and GC are used for the determination of methanol and fusel oil in distilled alcoholic beverages and spirits.

According to the procedure presented in the AOAC method, methanol is oxidized to formaldehyde by a potassium permanganate solution. The aldehyde formed is allowed to react with chromotropic acid in strongly acidic solution to form a colored product. Its absorbance is measured spectrophotometrically at 575 nm.

The colorimetric methods recommended for the determination of total fusel oil contents in beverages are based on a color reaction. In this reaction, 2-methyl-1-propanol, 2-methyl-1-butanol, and 3-methyl-1-butanol lose water during heating in a strongly acidic solution and the unsaturated hydrocarbons formed yield colored complexes with vanillin, salicylaldehyde, and 4-dimethyl-aminobenzaldehyde. The absorbance is measured spectrophotometrically at 445 and 560 nm.

GC provides a means of evaluating small amounts of methanol in beer, wine, and distilled alcoholic beverages. It is the most convenient method to determine single fusel oil and methanol in beverages simultaneously.

Although, direct injection with GC-flame ionization detection (FID) is sensitive enough to quantify methanol and fusel oils, it is advisable only for spirits because of the dirt accumulated in the injection port when beer, wine, or liqueurs are analyzed. Thus, liquid–liquid extraction has been widely applied to separate and concentrate fusel oil from beer, wine, and distilled alcoholic beverages, followed by GC determination of the compounds. Nowadays, many simpler and online extraction techniques are applied

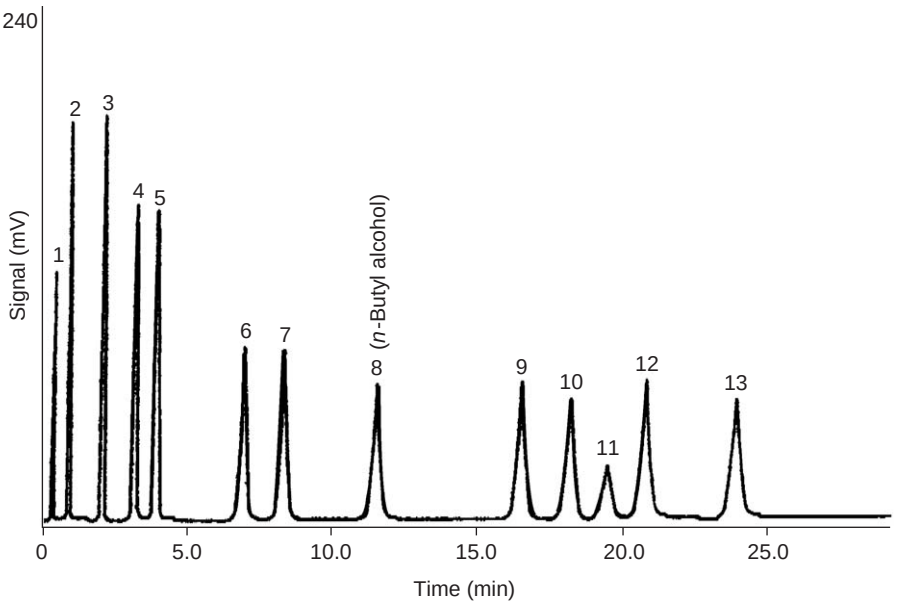

Figure 1 Chromatogram showing baseline resolution of the 13 alcohols in the order listed in **Table 1**. Chromatographic conditions: 6 ft (~180 cm) × 2 mm ID glass column packed with 0.2% Carbowax 1500 on Carbopack C (80–100 mesh); column temperature held at 55°C for 4 min, then programmed to 95°C at 2°C min^{-1}.

in alcohol determination such as liquid microextraction, solid-phase extraction (SPE), and solid-phase microextraction (SPME).

The direct GC method is the simplest and fastest for simultaneous determination of methanol, acetaldehyde, and fusel oil. The efficiency and resolution of GC has improved with the development of more efficient supports and liquid phases. Liquid phases, such as Carbowax and combinations of two liquid phases, have been most useful for determining fusel oil. **Figure 1** shows the results obtained by using a single direct injection procedure for separating and quantifying methanol and fusel oil whisky. Baseline resolution was obtained for each of the 13 alcohols (**Table 1**) in 25 min. 1-Butanol was chosen as the internal standard with a relative retention time of 1.0.

Alcohols are also analyzed by LC; in most cases the choice of the column falls on an ionic-exchange resin of the styrene–divinyl benzene polymer type, to which has been linked a functional group of the sulfonic type in the form H^+ or with another suitable cation. Since the separation of alcohols is performed very often together with other analytes, such as organic acids and sugars, the choice of the type of detector also takes into account their chemical–physical properties. Usually, the choice is the UV detector, the refractive index detector, or the electrochemical detector (EC). The use of LC allows the determination of less volatile alcohols such as glycerol in wine and beer, which could be difficult to analyze by GC.

Table 1 Gas chromatographic retention times (RRTs) of alcohols (relative to 1-butanol)

IUPAC name	*Common name*	*RRT*
Methanol	Methyl alcohol	0.03
Ethanol	Ethyl alcohol	0.08
2-Propanol	Isopropyl alcohol	0.18
1-Propanol	*n*-Propyl alcohol	0.26
2-Methyl-2-propanol	*t*-Butyl alcohol	0.34
2-Butanol	*s*-Butyl alcohol	0.60
2-Methyl-1-propanol	Isobutyl alcohol	0.72
1-Butanol	*n*-Butyl alcohol	1.00
3-Pentanol		1.43
2-Pentanol	*s*-Amyl alcohol	1.58
2-Methyl-1-butanol	Active amyl alcohol	1.69
3-Methyl-1-butanol	Isoamyl alcohol	1.81
1-Pentanol	*n*-Amyl alcohol	2.08

(Reproduced with permission from Wilson LA, Ding JH, and Woods AE (1991) Gas-chromatographic determination and pattern-recognition analysis of methanol and fusel oil concentrations in whiskeys. *Journal of the Association of Official Analytical Chemists* 74: 248–256.)

Aliphatic Carbonyl Compounds

Aliphatic carbonyl compounds consist mainly of aldehydes and ketones. A large number of aliphatic aldehydes have been shown to be present in alcoholic beverages. Among the aldehydes determined quantitatively acetaldehyde is the major component, and generally constitutes more than 90% of the total aldehyde content of the beverage. For this reason it could be expected that acetaldehyde is of importance

to the aroma and is often associated with improved quality.

Ketones form a minor fraction in alcoholic beverages. Ketones can be said to be potential aroma compounds. In particular, diketones such as 2,3-butanodione and 2,3-pentanodione are of great importance to the aroma of alcoholic beverages because of their low sensory threshold values. Those aldehydes with 8–10 carbon atoms, such as (*E*)-2-nonenal, octanal, nonanal, decanal, or (*E*,*Z*)-nonadienal, are also strong odorants, related with important off-flavors.

Titmetric and colorimetric methods are available for the determination of aldehyde contents in alcoholic beverages. An iodimetric method has been used for the determination of the total aldehyde content of wines. A modified iodimetric titration method is included in the methods of AOAC. In order to avoid the reactions of interfering substances, aldehydes are separated by distilling an aliquot of wine into a neutral hydrogensulfite solution. After the excess of hydrogensulfite has been titrated with an iodine solution at pH 2, hydrogensulfite is released from its aldehyde addition compound at pH 9 and titrated with iodine solution.

Different colorimetric methods have been developed for the determination of aldehyde and diketone contents in alcoholic beverages. Fuchsin, piperidine, 3-methylbenzothiazole-2-one hydrazone, 2-naphtol, and creatine have generally been used as reagents to produce colored compounds, the absorbances of which are measured at a wavelength between 630 and 660 nm or 500 and 550 nm.

As has been previously said, 2,3-butanodione (diacetyl) is an important aroma of alcoholic beverages, it has not been studied and measured extensively in the past because of analytical difficulties in the quantitation caused by its highly volatile nature, chemical instability, and interference of other compounds. Colorimetric methods to measure diacetyl have been widely used in the past. These methods involve steam distillation to isolate diacetyl from the matrix. However, distillation has the disadvantage of incomplete isolation of diacetyl from other closely related compounds that will result in an overestimation of its concentration. A fluorometric method was developed to improve upon the lengthy distillation methods that involve derivatization. Although acetaldehyde and its acetal can be determined by direct injection GC-FID in spirit drinks (EU reference method for spirits), most chromatographic methods for minor aldehydes implicate also derivatization. While a very sensitive and accurate method based on SMPE without derivatization and MS detection has been developed, it requires the use of deuterated diacetyl-d_6 as an internal standard, not easily available.

Due to the poor chromatographic and MS properties of higher aldehydes and ketones, most analytical methods are based on GC–MS or GC-ECD analysis of chemical derivatives. An added analytical problem is the high content of major carbonyl compounds, such as acetaldehyde, pyruvic acid, acetone, or diacetyl compared with C8-C10 aldehydes. Most of the methods previously developed for the analysis of carbonyl components in wine are based on the GC–MS or GC-ECD analysis of oximes formed by reaction with *O*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride, although other derivatization reagents have been explored such as 2,4-dinitrophenyl-hydrazine or cysteamine. On the other hand, the analysis of (*E*)-2-nonenal in wine, or of some higher methyl ketones in cognac, has been carried out by performing the chemical reaction over an organic extract obtained from the wine or cognac. All these methods use tedious liquid–liquid extractions. Nevertheless, several authors have shown the potential of direct derivatization of carbonyls on a solid phase, particularly in SPME- and SPE-based strategies.

Organic Acids

Organic acids in alcoholic beverages can be classified into volatile and fixed acids and may be of great importance. It has been found that some organic acids significantly influence the odor and taste of alcoholic beverages. Any substantial increase in the volatile acidity in wines seems to be a consequence of the activity of spoilage microorganisms. For this reason statutory restrictions exist in many countries for the maximum amounts of the volatile acids in wine.

Organic acids consist of aliphatic monobasic carboxylic acids from formic acid up to the C_{18} acids; aliphatic monobasic hydroxyl acids such as lactic acid; aldonic and uronic acids such as gluconic, glucuronic, and galacturonic acids; aliphatic monobasic oxo acids, mainly including succinic, malic, citric, and tartaric acids.

Distilled and undistilled beverages contain acetic acid in abundance. Frequently it amounts to 40–95% of the total volatile acids. Although the greatest part of the volatile acidity in wine consists of acetic acid, the remaining acids are nonetheless important aroma compounds. Many short-chain and long-chain acids, and in particular the branched-chain acids, have an appreciably strong odor.

Methods employed for the determination of organic acids in alcoholic beverages include

titrimetric, colorimetric, enzymatic, paper chromatographic, thin-layer chromatographic, gas chromatographic, and liquid chromatographic procedures, as well as GC combined with MS. Titrimetric methods are mainly employed to determine the total volatile acids from formic acid up to C_{18} acids. In the procedure, the volatile acids are isolated from alcoholic beverages by steam distillation followed by titration with a strong base. In order to concentrate the volatile acids methods such as microdiffusion, reduced pressure, or vacuum steam distillation can be used.

Titrimetric methods have also commonly been proposed for the determination of fixed and total acids in wines. After treating the wine with a cation-exchange resin, oxalic acid can be precipitated as its calcium salt and titrated with potassium permanganate. For determination of tartaric acid in wine the acid can be precipitated as calcium tartrate or potassium hydrogentartrate.

Colorimetric methods are used for the determination of lactic and tartaric acids in wines. In the determination of lactic acid the procedure consists of the conversion of lactic acid into acetaldehyde by heating in the presence of sulfuric acid or by oxidation with cerium(IV) sulfate and subsequent formation of a colored compound with *p*-hydroxydiphenyl or piperidine and sodium nitroprusside. The red color formed is measured at 560–570 nm.

In the determination of tartaric acid in wines, the tartaric acid produces a colored complex with ammonium metavanadate, which is measured at 530 nm.

Major acids in distilled alcohol beverages are usually analyzed by high-performance liquid chromatography (HPLC). Several methods based on LC have been developed allowing simultaneous determination in alcoholic beverages of citric, tartaric, malic, succinic, lactic, and acetic acids in a single run using cation-exchange or C18 columns and refractive index, UV, or electrochemical detectors. If a previous step for cleaning or concentration is required, SPE based on a strong anion-exchange is the most selective technique for the enrichment of the ionizable acid analytes. It is preferred instead of the conventional solvent extraction method, which is time consuming and uses relatively large volumes of solvents.

Methods for the determination of organic acids by GC consist of direct and derivative techniques. The methods of extraction can take advantage of the acid properties of the acids by using an anion-exchange resin, or they can use a more unspecific strategy carrying out the extraction with an apolar solvent or resin. In the latter case, the pH of the beverage must be adjusted to 7 to assure complete recovery of the acids from the aqueous phase.

With modern capillary columns derivatization of low molecular weight acids is not compulsory and peaks without tailing can be obtained. In this case, it is important to keep a permanent check on the inertness of the whole GC system, because acids are very sensitive to active sites on the column. However, to analyze heavier and more polar acids by GC, derivatization must be applied. Derivative methods involve the isolation of the acids from the matrix and afterward the conversion of the acids into the more volatile benzyl or trimethylsilyl esters.

A recent alternative to HPLC or GC in the analysis of organic acids is capillary zone electrophoresis. It provides completely different selectivities from chromatographic methods, shorter analysis times, and no derivatization is required. As a drawback, the lack of UV absorbance of aliphatic organic acids above 220 nm requires the addition of substances showing strong UV absorption for indirect UV detection.

Esters

The esters of aliphatic monocarboxylic acids are the most numerous of the neutral volatile aroma compounds in alcoholic beverages. Because of the relatively high volatility of the esters it has often been proposed that esters have a noticeable influence on the odor of a beverage.

Esters of aliphatic di- and tribasic carboxylic acids occur frequently as aroma components in alcoholic beverages. Because these compounds are mildly odored, they are apparently not very significant aroma factors. In addition to aliphatic fatty acid esters, several hydroxyl- and oxo-acid esters have been determined by GC/MS. Unlike the fatty acid esters, the esters of hydroxyl and oxo acids are slightly volatile compounds. However, they do not play an important role in the formation of the odor.

Titrimetric and colorimetric methods are two classical methods for determining the ester contents of alcoholic beverages. The titrimetric procedure involves the treatment of esters with an excess of base followed by determination of the quantity of base required to hydrolyze the esters present in the sample. The colorimetric procedure involves the esters reacting with hydroxylamine in an alkaline solution to form a hydroxamic acid, which forms a colored complex with iron(III).

It becomes clear that these chemical methods are too long and not specific, and are very rarely used today; instead, analysts prefer to use GC-based procedures. GC has many advantages over the classical

methods. The direct method of GC can give good reproducibility and enough sensitivity, especially when large volume injection can be performed. But regular direct injections of alcoholic beverages accumulate nonvolatile material in the injection port, leading to losses in chromatographic performance, appreciable after a few injections in the cases of wine and beer.

In most investigations, however, procedures for isolation and enrichment are required. Generally, liquid–liquid extraction with a high sample/solvent ratio is used prior to GC analysis. Among the solvents used are Freon 113, isooctane, ether, pentane, and dichloromethane. Enrichments of ~ 20 times of the original sample are enough to quantify a few milligrams per liter of the esters using splitless injection and FID detection. Many other procedures have been widely applied including distillation and headspace analysis but currently SPE and SPME are the techniques of choice for the possibility of automation and because of the low volumes of solvents used.

Summarizing, volatile compounds with concentrations $\sim 0.1\,\mathrm{mg\,l^{-1}}$, including major esters and higher alcohols acetates, can be determined after a single isolation step and GC-FID detection. Some minor esters, at concentrations between $0.1\,\mu\mathrm{g\,l^{-1}}$ and $0.1\,\mathrm{mg\,l^{-1}}$ will require a powerful isolation–preconcentration step and further GC–MS analysis.

Other Important Volatile Compounds

There is an important part of the aroma of beverages elicited by compounds that present very serious analytical problems. This is a heterogeneous group formed by compounds whose analysis is very difficult due to different reasons, such as bad chromatographic behavior and poor chemical stability, extremely low concentrations, or very poor analytical properties. Volatile sulfur compounds, alkyl methoxypyrazines, furaneol, sotolon, and some aromatic thiols are well-known examples of this group. In general, the analysis of these compounds requires the development of specific methods of isolation, or detection, or the use of chemical derivatives. Currently, a great effort is devoted to developing new analytical methods to characterize these flavor components, among them GC–olfactometry is a useful choice.

See also: **Ethanol**. **Food and Nutritional Analysis**: Overview; Wine. **Gas Chromatography**: Overview. **Indicators**: Complexometric, Adsorption, and Luminescence Indicators. **Liquid Chromatography**: Overview. **Sensory Evaluation**. **Spectrophotometry**: Organic Compounds.

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