HPLC Determination of Volatile Phenols in Wines

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Key Words
Column liquid chromatography
Wine
Volatile phenols

Summary
An alternative to the traditional solvent extraction method used to extract and rapidly quantify ethyl- and vinylphenol and ethyl- and vinylgaiacol from wine is presented. The method is based on retention of volatile phenols on adsorbants. Among the tested resins, the most efficient, AG 2-X8 (anion exchange resin), worked as well with a synthetic solution as with wines. The percolation of clarified wine adjusted to pH 9 on this resin permits, in particular, the elimination of organic acids. Phenols are not eluted after rinsing the column with 1N HCl, but are eluted with methanol after this treatment. Good recovery (91%) and good repeatability are observed. The eluate is directly analysed by HPLC on an RP18 column after two-fold dilution in water. The four volatile phenols were completely separated and detected by UV at 280 nm with high sensitivity (20-40 ppb). No interference with other compounds were noted in the different wines analysed.

Introduction
The olfactory importance of volatile phenols in wines was first mentioned by Dubois [1]. More recently, the importance of 4-ethylphenol and ethylgaiacol in red wines [2, 3] and the importance of 4-vinylgaiacol in white traminer wines [4] has been demonstrated. The possible olfactory impact of other volatile phenols arising from the contact of wine with oak is also strongly suspected [2, 5, 6].

The hedonic tone of phenols in wines changes from positive to negative with increasing concentrations [2, 3]. It is therefore important to be able to quantify them accurately and quickly in order to settle the exact relationship existing between their concentrations and their acceptability in different types of wines. The availability of such a technique is also necessary in order to clarify the different and sometimes contradictory hypotheses about the biochemical origin of some of these phenols [2, 3, 5].

Since the amount of phenols to be determined may be as low as few µg/l, most authors have selectively extracted the acidic volatile constituents from wines, or from wine distillates prior to analysis [1,3-6,8-13]. The same has been the case for the study of phenols in cured meat [14, 15], beer [16] and fermented grains [17]. A direct analysis is only possible when the concentration of these compounds is unusually high, for example, as found in contaminate wines or wines made from contaminated harvests [18, 19].

From such selective extractions, samples containing mainly fatty acids and phenols can be directly analysed by high resolution GC using FFAP columns [3]. However, since bonded FFAP phases have only recently become available for HRGC, most authors were obliged to eliminate fatty acids from the extract before analysis of the phenols using mainly three methods. Method 1: acids can be eliminated from the wine at pH 9 before phenols which are then extracted at a higher pH [1, 5, 6, 8, 13]. Method 2: neutral components, acids and phenols can be separated by liquid chromatography of the total extract or of the acidic extract on silica gel, alumina or DEAE cellulose columns [8-10, 12]. Method 3: neutral components from wine adjusted to pH 12 can be removed and then the phenols selectively extracted from acids at pH 9.5 [12].

Although the different papers cited above do not specify the time required for complete quantification of the phenols, from our experience, we estimate that at least 4 hours work for one sample, or for a limited number of samples is necessary. As the time required for these analyses is not compatible with the study of a large number of samples, our aim was to develop a faster method for the quantification of phenols at or over their supraliminal level, that is, at concentrations for which these components are likely to contribute to the aroma of wines.
Experimental

Adsorbents and Reagents

The different adsorbents tested were as follows: Amberlite XAD 4 and XAD 16, 20–50 mesh from Bio-Rad Laboratories (Richmond, CA, USA); anion exchange resin Amberlite IRA-400 (chloride form), 20–50 mesh from Prolabo (Paris, France) and AG 2-X8, 50–100 and 200–400 mesh from Bio-Rad Laboratories. Diethyl ether, ethyl acetate, dichloromethane and acetone (purex quality from Solvant Documentation Synthèse, Peyrin, France) were freshly distilled before use. Water was purified on a Milli-Q system (Millipore Corporation, Bedford, MA, USA). Methanol and acetonitrile (HPLC gradient grade from Farmitalia Carlo-Erba, Milan, Italy) were used without further purification. Acetonitrile was vacuum filtered on a Millipore membrane (polyvinylidene fluoride, 0.45 μm) and sonicated for 5 min before use.

Synthetic Solution

Synthetic solutions resembling wine were made from an ethanolic solution (12 % V/V) containing tartaric acid (1 g · l⁻¹), glycerol (10 g · l⁻¹) and the four volatile phenols at different individual concentrations (0.002 to 10 mg · l⁻¹), adjusted to pH 9 with 1N NaOH.

Preparation of the Amberlite Resins

XAD Amberlite resins (35 g) were washed to remove organic impurities in a Soxhlet extractor using successively 300 ml methanol (24h) and 300 ml diethyl ether (8h). They were dried at 30 ºC and stored at 4 ºC before use.

AG 2-X8 resin was used directly without any particular pretreatment except activation by agitation with 1N NaOH in a beaker for 5 min. After activation, the resin (2g) was poured into a glass column (20 cm x 1 cm i.d.). Excess of alkali was then removed with 30 ml purified water.

Isolation of the Phenolic Extract

100 ml of wine, or of the synthetic solutions, previously adjusted to pH 9 with 1N NaOH, were passed slowly through the resin. After washes with 100 ml 1 N HCl and 30 ml purified water, phenols were eluted using an appropriate volume of organic solvent (see below).

HPLC

HPLC separation of the extracts was carried out with a Waters 6000 pump modified with two 510 heads connected to a 5 μm LiChrochroom 18 precolumn (4 mm x 4 mm i.d.) and a 5 μm LiChroChrom 100 RP-18 column (25 cm x 4 mm i.d.) from Merck (Darmstadt, Germany). Detection was with a UV spectrophotometer at 280 nm (Shimadzu SPD-6A, Kyoto, Japan; cell vol. 6 μl; cell length 1 cm).

Quantification

HPLC: The recoveries of the different phenols were estimated from a triplicate extraction of the synthetic solution and from external calibrations.

HRGC: Volatile phenols eluted from the resin were extracted three times with dichloromethane. After concentration down to 1 ml using a Snyder distillation column, the constituents were separated on a Girdel 3000 gas chromatograph (Delsi Instruments, France) equipped with a splitless injector, a fused silica column (J. & W. Scientific, Folsom, CA, USA; 30 m; 0.32 mm i.d.; DB-FFAP; 1 μm), and a FID. Oven temperature was programmed from 60 to 220 ºC at 3 ºC · min⁻¹. Peak areas were estimated using a Coconut acquisition system (INRA, Dijon, France) and compared to peak areas of standards injected under the same conditions (external calibration).

Results and Discussion

Extraction

Compared to solvent extraction, a quicker extraction can be obtained by trapping volatiles on polymers by either head-space or solid phase adsorption. Since phenols are relatively polar and have high boiling points, head-space analysis is not suitable for their quantification at low concentrations in wines (< ppm). On the other hand, adsorption on solids has been successfully used for the quantification of various volatile compounds in beer and wine [21, 22]. Although volatile phenols were not mentioned in these papers, the high recoveries given for polar (fatty acids) and for nonpolar volatiles led us to conclude that the technique is probably suitable for these particular substances. Amberlite IRA 400, XAD 4, XAD 16, and AG 2-X8 were tested to optimize the method. Resin, AG 2-X8, showed the best adsorption properties and recovery of phenols.

Further experiments were therefore conducted using AG 2-X8 resin. In order to avoid saturation of the resin with organic acids (mainly tartaric, malic or lactic) and with fatty acids, both the wine and the synthetic solution was previously adjusted to pH 9 prior to extraction. At concentration higher than 10 ppm (maximum concentration ever reported in wine) volatile phenols were completely adsorbed onto the resin as verified by GC analysis of the eluate.