

Determination of Phenolic Compounds from Wine Samples by GC/MS System

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Wine and especially the red wine is known to have beneficial health effect, due to its content of phenolic compounds. Their concentration is influenced by many factors, therefore the determination of the amount of individual phenolic compounds is an important issue. The investigation of unstable phenolic compounds, from the flavonoid family, was possible using a sensitive and selective coupled gas chromatography-mass spectrometer system. The analysis was done on derivatized (silylated) wine extracts from two different types of wine - red and white wine - belonging to the same region from Romania. Using this method it was possible to identify two major flavonoid compounds as well as five minor compounds belonging also to the class of polyphenols. In the present paper the concentration of the free polyphenolic compounds detected is presented and the main ion fragments appearing in the mass spectrum of catechin are discussed, too. A comparative study of the results on red wine and white wine samples was made.

Keywords: flavonoids, polyphenols, wine extract, TMS derivatives, GC/MS

In the last time there is a large interest for natural antioxidants contained in food-stuff [1-4]. The antioxidant activity is mainly attributed to the occurrence of phenolic compounds in foods and beverages [1, 3]. The phenolic compounds are known to be beneficial for human health, due to their radical scavenging activities, which may contribute to preventing atherosclerosis, inflammatory diseases [5]. They also can manifest antimicrobial and immune-modulatory effects, as well as stimulate DNA repair in prostate cancer cells [6-8].

Beverages, especially red wines are a rich source of phenolic compounds, which may act to prevent lipid oxidation as dietary antioxidants [8]. The last decade's studies show that phenolic compounds constitute of red wine, have a beneficial health effect [4]. These compounds also contribute to the color and sensory properties of wine.

Most phenolic compounds belong to the class of polyphenols, having several hydroxyl groups attached to aromatic benzene rings. They appear as secondary metabolites in plants [9] having a protecting role against pathogens and contributing to the chemical defence of the plants. The amount of polyphenols in wines is influenced by many factors, like type of grapes, reaping moment and processing technology, even preservation conditions [10-13]. It is known that their level may decrease during maturation and aging processes. Due to the above considerations, the abundance of polyphenolic compounds varied largely among the different wines, generally speaking, the red wines contained higher amounts than white wines [12]. Wines contain a large variety of polyphenolic compounds, mostly from the flavonoids and benzoic acids family [11, 14], but also hydroxybenzoic and hydroxycinnamic acids, tannins and anthocyanidines [9, 15]. Polyphenols appear both as free compounds as well as in the form of oligomeric adducts with sugars mainly. For the quality differentiation of wines it is important to determine the nature and the level of these compounds.

The analysis of polyphenols in wine is a complex issue,

starting with the Folin-Ciocalteu method based on a mixture of sodium tungstate and sodium molybdate and permitting the colorimetric assay of total phenols in terms of an equivalent standard phenol such as gallic acid or catechin. For the analysis of individual free or oligomeric polyphenol compounds several different instrumental methods as thin layer chromatography (TLC), gas chromatography (GC), high-performance liquid chromatography (HPLC), gas chromatography - mass spectrometry (GC/MS), high-performance liquid chromatography - electrospray ionization tandem mass spectrometry (HPLC/ESI-MS) have been worked out [15-19] depending upon the complexity of the investigation. Due of the fact that most of phenolic compounds are polar, polyphenols cannot be detected in good conditions by a GC/MS system. With the purpose to reduce polarity and to increase volatility of the compounds, a derivatization procedure has to be used [10, 17, 18], leading to symmetric peaks and an increase in sensitivity. In the present paper the derivatization technique, using N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA), for the determination of phenolic compounds as trimethylsilyl (TMS) derivatives was applied.

The GC/MS system in the selected ion-monitoring mode (SIM), was shown by Minuti and coworker, to be suitable for sensitive analyses of phenolic compounds, resulting from the complex wine matrix after a solid phase extraction (SPE) and a derivatization process [10]. Nevertheless by using the GC/MS system in the SIM mode the interferences can't be controlled. For this consideration in the present study we used an IT-MS (Ion Trap - Mass Spectrometer) - in full scan mode- as part of the GC/MS system, which equals in full scan mode the sensitivity of common quadrupole MS under SIM mode.

The aim of the research was the identification and quantification of free polyphenols and mainly flavonoid compounds using GC/MS system in full scan mode, after a proper extraction and derivatization process. The study was performed on a red wine (dry Pinot Noir, 2006) and a white

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wine (dry Italian Riesling, 2006) samples obtained from the same geographical region of Romania, purchased from a local supermarket. The results obtained from both samples were compared and discussed.

Experimental part

All chemicals and standards were of high purity, suitable for GC analysis. The derivatization reagent N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA), pyridine and the internal standard, hexylsalicylate were purchased from Sigma-Aldrich (Taufkirchen, Germany).

Sample preparation

To 1 mL wine sample 1 mL NaCl (36%) was added, and then the solid phase extraction procedure was applied. The C18 cartridge (Isolute C18, 100 mg, 6 mL) column was preconditioned with 8 mL of ethyl acetate, 5 mL of acetone and 10 mL of HPLC water. The wine diluted sample was then percolated by gravity flow through the cartridge. After 1 h of drying the compounds were eluted with 3 mL of ethyl acetate and then dried over Na_2SO_4 during 12 h. The sample was evaporated to dryness using a gentle flow of nitrogen and 50 μL BSTFA were added. The mixture was kept at room temperature for 1 h and completed with pyridine to 1 mL. A volume of 2 μL of sample was used for GC/MS analysis.

Instrumentation

Analysis was performed on a GC/MS system (Trace GC Ultra-Polaris Q MS, Thermo Finnigan). The GC was equipped with a HP-5MS of 30 m length and 0.25 mm internal diameter capillary column, with 0.25 μm film thickness. The carrier gas was helium set to flow at 1.5 ml/min. The injector was operated in splitless mode at the 280 °C temperature. The chromatographic working conditions were optimized for complete separation of the target compounds. The oven was programmed from 120 °C (3.0 min) to 315 °C with 5 °C/min and maintained for 5.0

min. The mass spectrometer was set at the following conditions: ion source temperature at 280°C, Electron Impact (EI) ionization at 70 eV, current emission 250 μA . Contrary to the earlier reported method, where SIM mode was used [8] the mass spectrometer was operated in the present work in full scan mode, taking advantage of the high sensitivity of the ion trap analyser in full scan mode. This way possible chemical interferences were eliminated.

Results and discussions

The GC/MS analysis was performed on the wine samples, subjected prior to analysis to the SPE extraction and derivatization (silylation) procedures, described in the experimental section. The chromatogram obtained for the derivatized red wine extract is presented in figure 1 for the time range 4 – 47 min. The main compounds determined are indicated with numbers from 1 to 7. The compounds identification was made based on their mass spectra registered in the mass range of 50 – 650 Daltons, by comparison with mass spectra from the database and also based on rules of ion fragmentation in the mass spectrometer ion source.

Using the GC/MS system in full scan mode, with the experimental conditions described before, were determined in the wine extract, the presence of two derivatives of flavonoid compounds, with similar mass spectra: 368 (100%), 355 (79%), 73 (63%), 369 (36%), 383 (24%), 650 (6%). These two compounds were identified as being stereo isomers, namely (-)-epicatechin (**6**) and catechin (**7**).

The pattern of characteristic ions of the trimethylsilyl derivatives of identified compounds – (-)-epicatechin and catechin - arise by two fragmentation mechanisms:

a) Simple fission process of the molecular ion, $[\text{M}]^+ = 650$. The ion at $m/z = 635$ is produced by the elimination of a methyl group $[\text{M}-\text{CH}_3]^+$ and the ion at $m/z = 577$ is produced by elimination of the TMS group $[\text{M}-\text{Si}(\text{CH}_3)_3]^+$, both being common fragments for

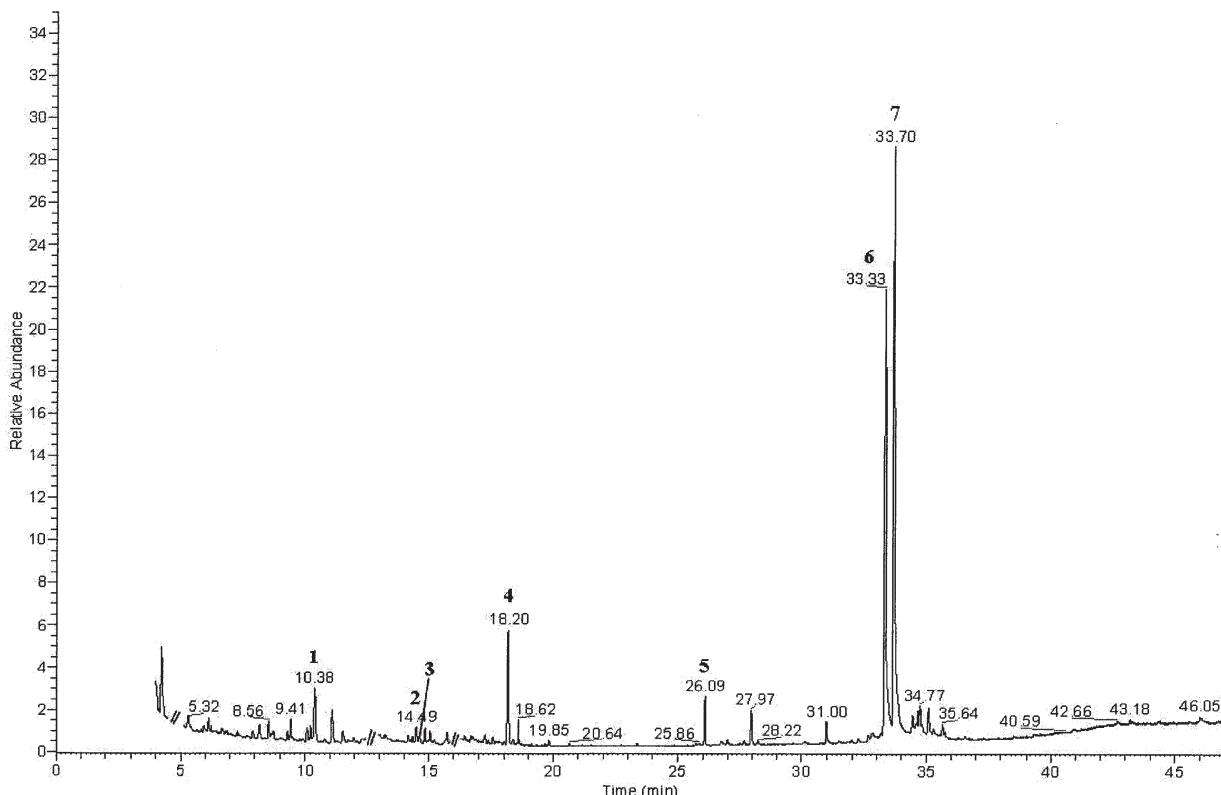


Fig.1. The GC/MS chromatogram of silylated red wine extract. The experimental conditions as in Experimental. The peak identification is in table 1

Nr.	Compound name	Chemical structure	Molecular mass	Retention time [min]	Concentration [mg/l]	
					Red wine	White wine
1	Tyrosol - 2TMS		282	10.38	13.65	10.94
2	Hydroxy-anisic acid - 2TMS		312	14.51	0.66	0.02
3	Hydroxy-tyrosol - 3TMS		370	14.63	1.10	0.14
4	Ethyl-ester-gallic acid - 3TMS		414	18.20	25.32	-
5	Gallic acid - 4TMS		458	26.09	6.34	0.8
6	(-)-Epicatechin - 5TMS		650	33.33	74.71	2.01
7	Catechin - 5TMS			33.70	110.04	3.90

Table 1
COMPOUNDS IDENTIFICATION AND CONCENTRATION IN RED AND WHITE WINE

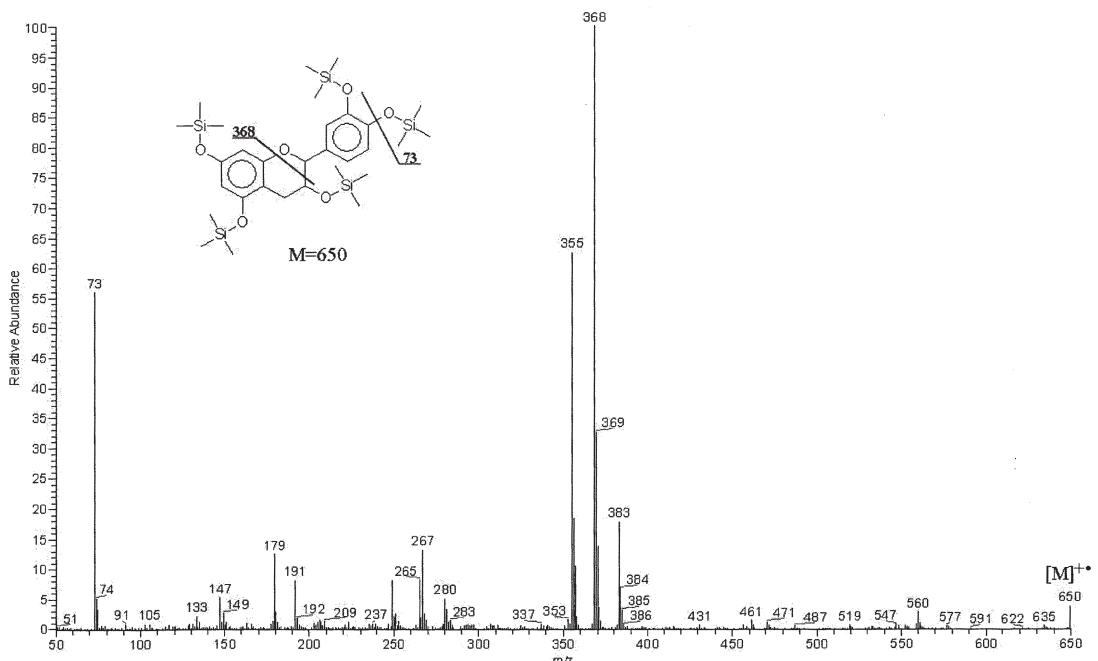


Fig.2. The mass spectrum of compound at elution time 33.70 min, identified as catechin-5TMS

trimethylsilyl derivatives. The abundant ion at $m/z=73$ is produced also by simple fission and has the structure $[\text{Si}(\text{CH}_3)_3]^+$. It is complementary to the ion fragment at $m/z=57^3$ described before.

b) Double fission process or rearrangement involving H or CH_3 group transfer, common fragmentation pathway reported for heteroatom molecules [16, 17]. The ion at $m/z=560$, $[\text{M}-\text{HOSi}(\text{CH}_3)_3]^+$ is produced by the elimination of the neutral molecule HO-TMS after H transfer. The formation of the ion at $m/z=368$ (base peak) is initialized by localization of the positive charge on oxygen, bonded to the oxane ring, followed by two bond fissions from oxane. The ion at $m/z=383$ is produced by a similar mechanism.

involving the transfer of a CH_3 group from $\text{Si}(\text{CH}_3)_3$ to the phenyl ring at radical place, formed after C-O bond fission. The ion at $m/z=355$ is produced from ion $m/z=383$ by elimination of the neutral CO molecule, also a common fragmentation present in EI mass spectra of heteroatom molecules [20, 21].

The quantitative determination of the detected compounds was performed by comparison of their peak areas of characteristic ion fragment with the area of the base peak of hexylsalicylate, used as internal standard. The limit of quantification (LOQ) for hexylsalicylate was 5 $\mu\text{g/l}$ (having the intensity of peaks six times higher than noise signal) and the calibration curve was linear in the

range of 0.01 - 150 mg/L. The quantitative determination includes the influence of sensitivity of studied compounds with respect to hexylsalicilate. In the comparison of red and white wine (as peak ratio relative to internal standard) the relative quantities of compounds are correct.

The results (see Table 1) show a significant quantitative difference between the concentrations in red and white wine of free (-)-epicatechin and catechin compounds. In the red wine was found (-)-epicatechin in the concentration of 74.71 mg/L and catechin in the concentration of 110.04 mg/L, nevertheless in the white wine the concentrations were much lower, being 2.01 mg/L for (-)-epicatechin and 3.9 mg/L for catechin. The obtained concentrations for red wine are significantly higher relative to those reported early, for similar samples-but from other geographical region (compound concentration of ~25 mg/L for catechin and ~10 mg/L for (-)-epicatechin) [10].

The other five compounds were detected as being all polyphenols. Four of them, tyrosol, hydroxy-anisic acid, hydroxy-tyrosol and gallic acid, appear among the secondary plant metabolites while the ethyl-ester of gallic acid is normal to appear after fermentation. The structures of tyrosol, hydroxy-anisic acid, hydroxy-tyrosol, ethyl-ester of gallic acid and gallic acid are shown together with their concentrations in table 1. The data show also a higher relative abundance of free polyphenols in red wine as compared to white wine.

Conclusions

The simplified GC/MS method is suitable for the simultaneously determination of the concentration of individual free phenolic compounds from derivatized wine extract and also for routine laboratory analysis of these dietary antioxidants in different winemaking processes. It was possible to indentify two important flavonoids as well as other five free polyphenols in wine. The results sustain the statement that red wines have higher amount of phenolic compounds than the white wines. Moreover, the relative ratio of (-)-epicatechin and catechin could be used as a diagnostic parameter related to the wine geographical provenance, and the characterization of processing technology and aging processes.

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