



Fast-SEC coupled to ICP-MS for selected metal determination and its stability over time in polish wine

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Abstract

Metal ions contribute to the wine browning (as reaction catalysts), turbidity (in complexes), and astringency (due to interaction with tannins). Being aware that bioavailability and metabolism of metal ions in the human body are strictly related with their concentration and the form they occur it is important to monitor not only the total content of metals but also their speciation. Thus, we can obtain more information about their potential toxicity to human health. Present research shows the application of fast size exclusion chromatography (Fast-SEC) coupled to ICP-MS for the evaluation of the distribution of elements in different sized complexes in Polish wine. The study was focused on the selected ten elements (Al, B, Ba, Cu, Fe, Mn, Ni, Pb, Sr, Zn). Additional information of this work was the evaluation of the metal complex stability over time. Based on the obtained results, it can be seen that metal complexes present in the studied wine samples are stable even 10 months after the wine bottle opening.

Graphical abstract



Keywords Polish wine · Metal complexes · Mass spectroscopy

Introduction

Analysis of Polish wines are rarely addressed topic in the literature. This is probably due to the fact that wine production in Poland is still a niche, aimed mainly at enriching the ecotourism offer of a given agritourism farm. Polish wine is a product coming from small wineries, established due to strong motivation and passion of founders. Unfavorable conditions for vines cultivation forced winemakers to look for more resistant hybrid species of grapes, these are mostly: Regent, Rondo (red wines) and Solaris (white wines) [1].

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Climate condition, winemaking technology and the grape cultivars used for its production cause that each wine has its own specific characteristic which is related with the wine composition and thus its organoleptic and aesthetic properties [1].

Metals stand as an important constituent of wines influencing their organoleptic properties and conservation [2, 3]. They influence the pH and ionic balance which in turn supports the cellular metabolism of yeast. Since specific metallic ions have important role in redox reactions, their presence influences wine browning, turbidity, and astringency [3]. Metals in wine come from primary (endogenous) sources of natural origin like soil, in which vines are grown and secondary sources (exogenous). Exogenous sources result from external contamination like: environmental pollution of a specific region where the vineyard is located, viticulture practice—the use of fertilizers rich in Ca, Cu, or K, the use of pesticides or fungicides being a potential source of Cd, Cu, Mn, or Pb; or due to wine making technology and long contact of acidic wine with aluminum, brass, and stainless steel parts of the equipment used for wine production [4, 5]. The amount of metal varies during the vinification process. Concentration of most of metals present in wine is decreasing with each step of vinification process due to yeast consumption or precipitation with the use of tartrate [5]. However, if the maceration process is carried out and is extended too much or the wine has prolonged contact with the metal parts of the equipment the concentration of some metal ions such as Cr, Cu, Fe, and Zn may increase [5].

Monitoring of the content and forms of metals present in wines is important for both wine production and human health. Due to stable wine consumption after a significant boom in early 2000s, it is becoming one of the source of metal ions in a human diet; it contributes not only to the intake of metals that are essential for human health (i.e., Cu, Fe, and Zn) but also those which can cause adverse effects (i.e., Al, Pb) [3, 6]. However, their toxicity, bioavailability, and metabolism of metals in the human body is related to their chemical form and the concentration level at which they occur. Knowledge about metals pathways and their transport is crucial. Thus, in addition to monitoring the total content of metals in wine, metal-binding ligands and complexes tracking their speciation is of high interest [2].

Metals in wine can potentially occur as dissolved free ions and as complexes with various organic substances constituting wine matrix: low molecular weight compounds such as organic acids, amino acids and high molecular weight ones such as polysaccharides and polyphenols [7].

Analytical methods used for the metal complex analysis in wines and beverages usually involves extraction techniques like solid phase extraction or liquid–liquid extraction [8]. Herein, we describe the application of a fast size exclusion chromatography (Fast-SEC) ICP-MS methodology for

evaluation of elements (Al, B, Ba, Cu, Fe, Mn, Ni, Pb, Sr, Zn) distribution in different sized complexes in wine originating from Polish wineries (located in different parts of Poland). An important part of the study was the assessment of the evaluation of distribution of different sized metal complexes over time.

Results and discussion

All ten measured analytes were determined in the studied wine samples. Detailed results together with the standard deviation as well as RSD are given in Table 1. The most abundant elements were: B (2.9–5.2 mg/dm³ for the white wine, 3.6–5.6 mg/dm³ for the red wine); Mn (0.8–2.9 mg/dm³ for the white wine, 0.6–2.3 mg/dm³ for the red wine); Zn (0.4–1.1 mg/dm³ for the white wine, 0.07–1.4 mg/dm³ for the red wine); and Fe (0.5–4.2 mg/dm³ for the white wine, 0.6–2.5 mg/dm³ for the red wine). The least abundant was Ni (0.003–0.1 mg/dm³ for the white wine, 0.03–0.4 mg/dm³ for the red wine) and Pb (0.008–0.1 mg/dm³ for the white wine, 0.3–1.7 mg/dm³ for the red wine). It needs to be mentioned that Pb content in examined red wines is higher than the acceptance limits established by The International Organization of Vine and Wine, which is 0.15 mg/dm³ [9]. Higher content of the selected elements observed in red wines than white wines fits in the trends described in the literature [10]. It is difficult to observe any uniform tendency among the studied wines.

In the next stage, we assessed metal complexes size distribution in wine with the use of Fast-SEC ICP-MS. SEC with element selective detection by ICP-MS has been extensively used for similar applications including Pb speciation in wine [11], metal carbohydrate complexes in fruit and vegetable [12], Ni speciation in *Sebertia acuminata* [13], and metal complexes in cocoa [14]. However, due to the poor separation efficiency of classical SEC columns, this chromatographic technique has been considered unable to provide speciation data and used solely for the estimation of the molecular weight of the species present [15]. The recent introduction of SEC materials, having the solvent stability and mechanical strength which allow their use in Ultra-High-Performance Liquid Chromatography (UPLC) mode, provided significant improvements in separation efficiency and 3–fourfold shortening of the analysis time [16, 17]. In a given study, the species were separated within 15 min producing sharp peaks (2 peaks for B, Ba, Pb, and Sr; 1 peak for the rest of compounds). The separation efficiency was compared to HILIC which is considered to have much better resolving power [18]. A care has been taken to assure that the eluent is compatible with both detections techniques which required. Fast-SEC ICP-MS allowed the observation of relatively high molecular weight (10 kDa) species which

Table 1 The average concentration of metals in studied wine samples ($n=3$) (W -white wine; R-red wine)

| Wine samples | Concentration of metals in wine samples / $\mu\text{g dm}^{-3}$ | | | | | | | | | |
|--------------|---|----------|-----------|-----------|-----------|------------|-----------|----------|-----------|------------|
| | Al | B | Ba | Cu | Fe | Mn | Ni | Pb | Sr | Zn |
| 1 W | 162.4±6.4 | 4620±110 | 37.1±2.3 | 39.1±1.8 | 690±20 | 1610±50 | 6.3±0.5 | 7.8±0.2 | 220±10 | 780±30 |
| 2 W | 336.3±4.4 | 3760±30 | 93.6±7.7 | 140±20 | 490±20 | 1870±20 | 9.1±0.7 | 3.0±0.1 | 182.5±8.1 | 388.0±3.2 |
| 3 W* | 700±20 | 4630±80 | 99.9±4.7 | 92.8±4.3 | 4210±230 | 930±20 | 2.9±0.4 | 28.4±0.3 | 210±10 | 310.0±5.5 |
| 4 W | 770±50 | 3300±10 | 140±10 | 138.4±0.3 | 710±70 | 3000±190 | 4.2±0.5 | 9.6±0.1 | 370±30 | 1080±70 |
| 5 W | 1710±10 | 3370±90 | 133.6±6.5 | 12.1±1.3 | 711.1±6.8 | 900.7±5.3 | 3.9±0.3 | 16.6±0.1 | 430±20 | 1509.6±9.1 |
| 6 W | 565.7±6.3 | 3250±30 | 92.6±4.4 | 25.5±0.6 | 283.8±6.5 | 1071.6±3.6 | 5.1±0.5 | 6.5±0.1 | 260±10 | 566.6±1.5 |
| 7 W | 959.2±8.2 | 5210±30 | 113.6±5.2 | 387.8±3.1 | 3930±110 | 840±10 | 5.2±0.7 | 30.7±0.3 | 210±10 | 451.3±5.9 |
| 1R* | 242.3±6.1 | 3950±170 | 14.2±0.9 | 10.7±0.1 | 2490±190 | 852.8±4.2 | 211.2±5.3 | 990±40 | 279.3±5.5 | 360±90 |
| 2R | 246.4±7.0 | 3600±160 | 4.7±0.3 | 2.2±0.1 | 670±280 | 570±30 | 41.2±0.4 | 340±10 | 883.0±8.2 | 72.6±2.1 |
| 3R | 490±30 | 4750±130 | 20.0±0.3 | 8.5±0.6 | 2600±200 | 780±30 | 192.4±3.7 | 1730±60 | 368.5±2.7 | 630±40 |
| 4R* | 151.8±4.2 | 3830±100 | 11.9±0.8 | 7.1±0.1 | 1050±240 | 1510±30 | 401.5±5.2 | 1100±40 | 257.2±3.1 | 940±20 |
| 5R | 94.3±9.1 | 4830±30 | 5.9±0.4 | 2.8±0.2 | 604±191 | 850±40 | 53.6±2.5 | 260±10 | 287.6±4.4 | 191.5±2.1 |
| 8R* | 562.3±7.4 | 4390±140 | 15.6±1.0 | 20.5±0.1 | 1423±238 | 958.1±8.2 | 119.1±0.5 | 670±20 | 385.7±5.5 | 549.4±6.5 |

*Wines on which the evaluation of the speciation of metals over time was studied

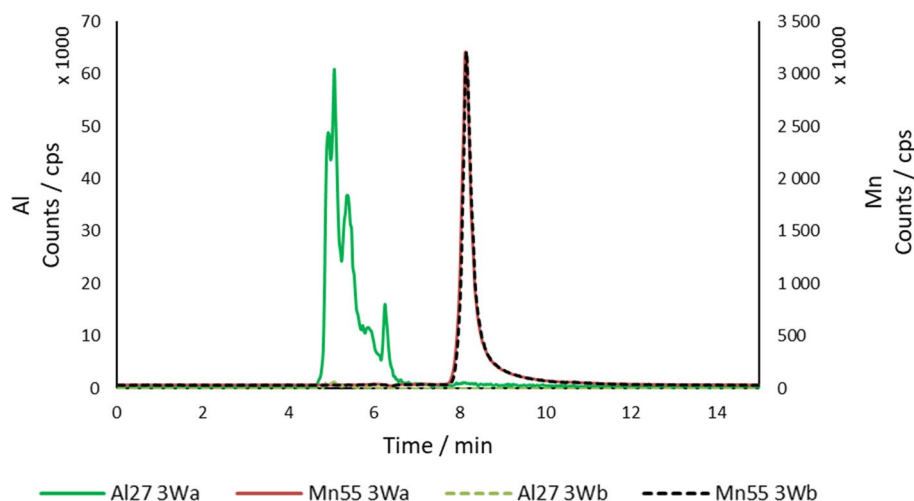
cannot be analyzed by HILIC ICP-MS. Peak widths and peak shapes are repeatable ~3% RSD. Moreover, in HILIC gradient elution is commonly used which results in shifting ionization conditions in ICP-MS potentially affecting the dataset acquired (species more or less readily ionized) [16].

Wine is known for the evolution of its color, aroma, and taste following the aging. These features, which are responsible for the exceptional character and prestige of this drink, make the task of an analytical chemist very difficult. Under the oxygen exposure, wine can change its aroma, color, and taste. Due to the oxidation reaction taking place in wine beverage the quality of wine can be adversely affected. Polyphenols having in their structure catechol ring can undergo nonenzymatic oxidation in wine [19]. Oxygen may not react directly with the phenolic compounds. In the process of chemical oxidation, the superoxide anion is formed resulting from the addition of single electron transition of metal ions which is further converted into hydroperoxyl radical form at the pH of wine. Given compound causes oxidation of phenols into quinones. What is more, quinones may bind to phenols, amines, or thiols thanks to high electrophilicity or may be reduced by hydrogen peroxide to phenols again or to the product with sulfone group. Iron ions in the reaction of hydrogen peroxide can produce hydroxyl radical which is highly reactive and may oxidize most of the organic compounds as well as inorganic molecules [19]. It needs to be mentioned that the amount of inorganic ions and metal ions, such like Fe(III), in wine is of great interest, because of their influence on wine technology as well as their toxic effects. In fact, it has been shown that the content of the total selected metal ions (Mn, Sr, and Zn) differs significantly for young and aged wines [20]. Evaluation of the metal complex size distribution over time shows that metal complexes formed in wine do not change even 10 months after the wine bottle opening. There are only few exceptions, presented on Figs. 1, 2, and 3: one peak corresponding to Al(III) disappeared in the white wine sample 3 W (Fig. 1); changes in the Sr(II) peak intensity was observed in all four wines examined; changes in the Cu(II) and Zn(II) peaks intensity were observed in all red wines examined (Fig. 2). However, retention times for all the peaks of all the metal elements analyzed did not change.

Conclusion

This is a preliminary study with the use of Fast-SEC ICP-MS technique as a promising tool for feasible and effective way to evaluate metal complex size distribution in wine samples. Short time of analysis, easiness in sample preparation (filtration and dilution is only needed) are crucial advantages of a given technique, which encourages for further development that needs to be done to use it in a speciation studies.

Fig. 1 Al (III) and Mn (II) complex size distribution over time in wine 3 W



Thus, size calibration and complete validation should be done to create robust and reliable method for speciation analysis in wine.

The additional information obtained in this research was the evaluation of the distribution of elements in different sized complexes over time. Hence, the stability of metal–organic matter complexes formed in wine was checked on wine from freshly opened bottle and then 10 months after the wine bottle opening. The retention time, number of peaks, and in most cases even intensities of peaks responsible for the compounds associated with the monitored metal ions did not change. Based on the mentioned observation, it can be assumed that metal complexes formed in wine are stable regardless the chemical changes occurring in wine during its ageing. However, different analytical techniques like ESI–MS, which allows metal complex identification should be used to be sure that the metal complexes analyzed are not a different complex of the same species.

Materials and methods

Reagents

The reagents used for digestions, dilutions, and the preparation of HPLC mobile phases were: ammonium acetate ($\geq 98\%$ for molecular biology, Sigma-Aldrich St. Louis, USA), nitric acid (V) (70%, Fisher Chemical, Loughborough, UK), acetonitrile ($\geq 99.9\%$, Sigma-Aldrich St. Louis, USA), hydrogen peroxide (30%, Sigma-Aldrich St. Louis, USA), hydrochloric acid (37%, Fluka, Steinheim, Germany). Standard solutions (1000 ppm, SPC SCIENCE) of Al, B, Ba, Cu, Fe, Mn, Ni, Pb, Sr, Zn were used for the preparation of calibration curves and as internal standards (Sc and Rh). Methanol (Sigma-Aldrich St. Louis, USA) was used for column cleaning. Milli-Q® Type 1 Ultrapure Water

Systems (Millipore, Bedford, MA) deionized water was used throughout.

Instrumentation

MiniSpin centrifuge (Eppendorf AG, German) and DigiPrep MS system (SCP Science, Quebec, Canada) were used. Two chromatographic systems: Agilent 1200 Series (Tokyo, Japan) and Dionex Ultimate 3000 RS (Thermo Scientific, UHPLC, Germany) were used. The ICP-MS spectrometer was ICP-MS 7500 (Agilent Technologies, Tokyo, Japan) equipped with an integrated autosampler (I-AS).

Wine samples

For the analysis, 13 bottles of wines from different regions of Poland were purchased in the local market. The wine bottles were protected from light and were kept in consistent temperature (20 °C) and (4 °C) after opening. The bottles of wine were opened directly before the analysis. Red wine (six bottles), white wine (seven bottles) were analyzed. Specific characteristic of all the wine samples is gathered in Table 2.

Total metal analysis

The method used for the elemental analysis was based on the method described in the previous studies [21–23]. In brief, for the total metal analysis 25 cm³ of wine was evaporated to dryness (80 °C through 7 h) and then digested with a mixture of nitric acid and hydrogen peroxide in ratio 3:1 in DigiPREP MS system for 2 h at 65 °C. The digests were cooled down to the room temperature. After an appropriate dilution and the addition of internal standards (Sc and Rh), the total content of metals of interest, including: Al, B, Ba, Cu, Fe, Mn, Ni, Sr, Pb, Zn was measured by inductively coupled plasma mass spectrometry. The experimental

Fig. 2 Cu (II) and Zn (II) complex size distribution over time in red wines

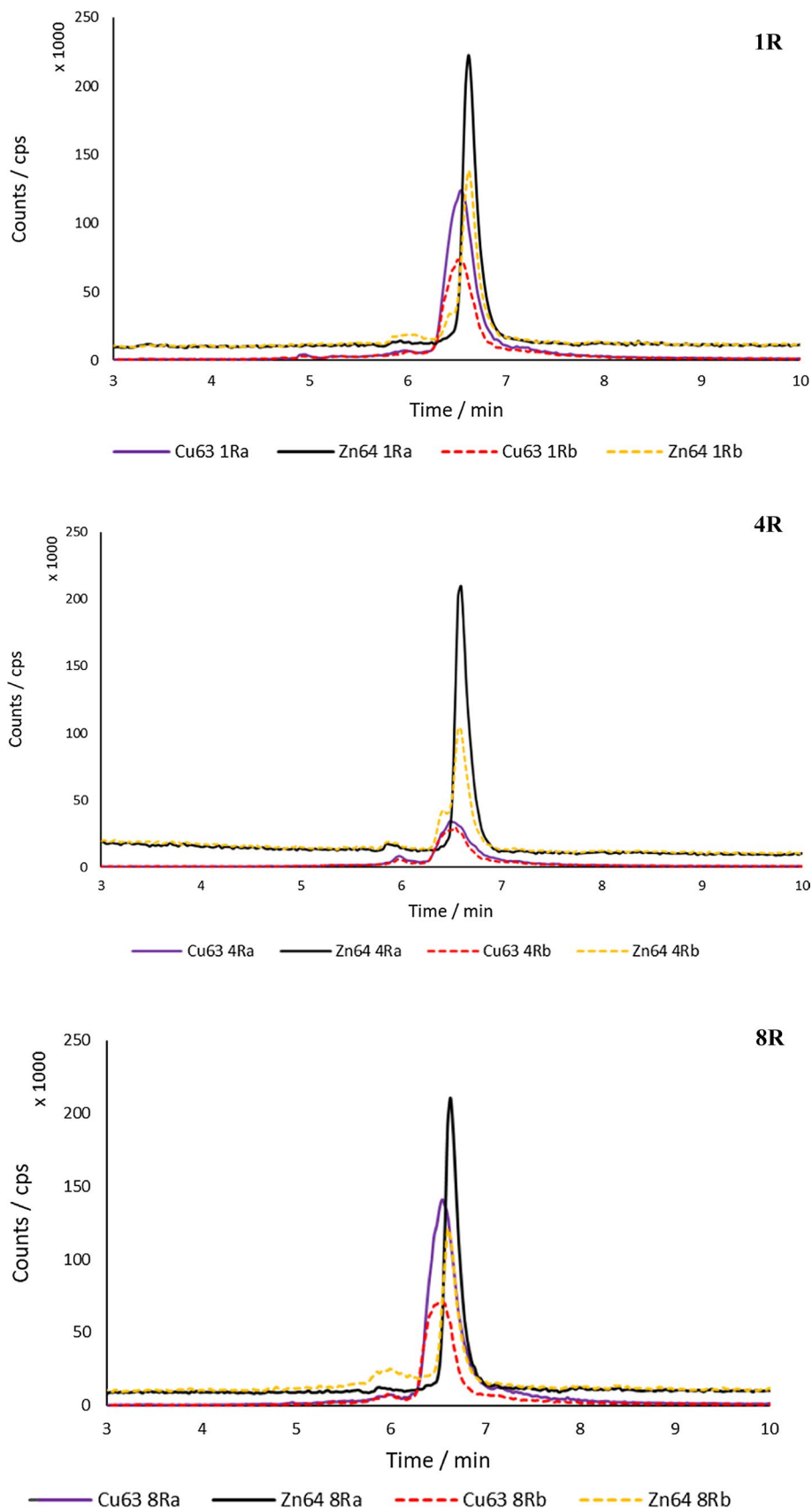
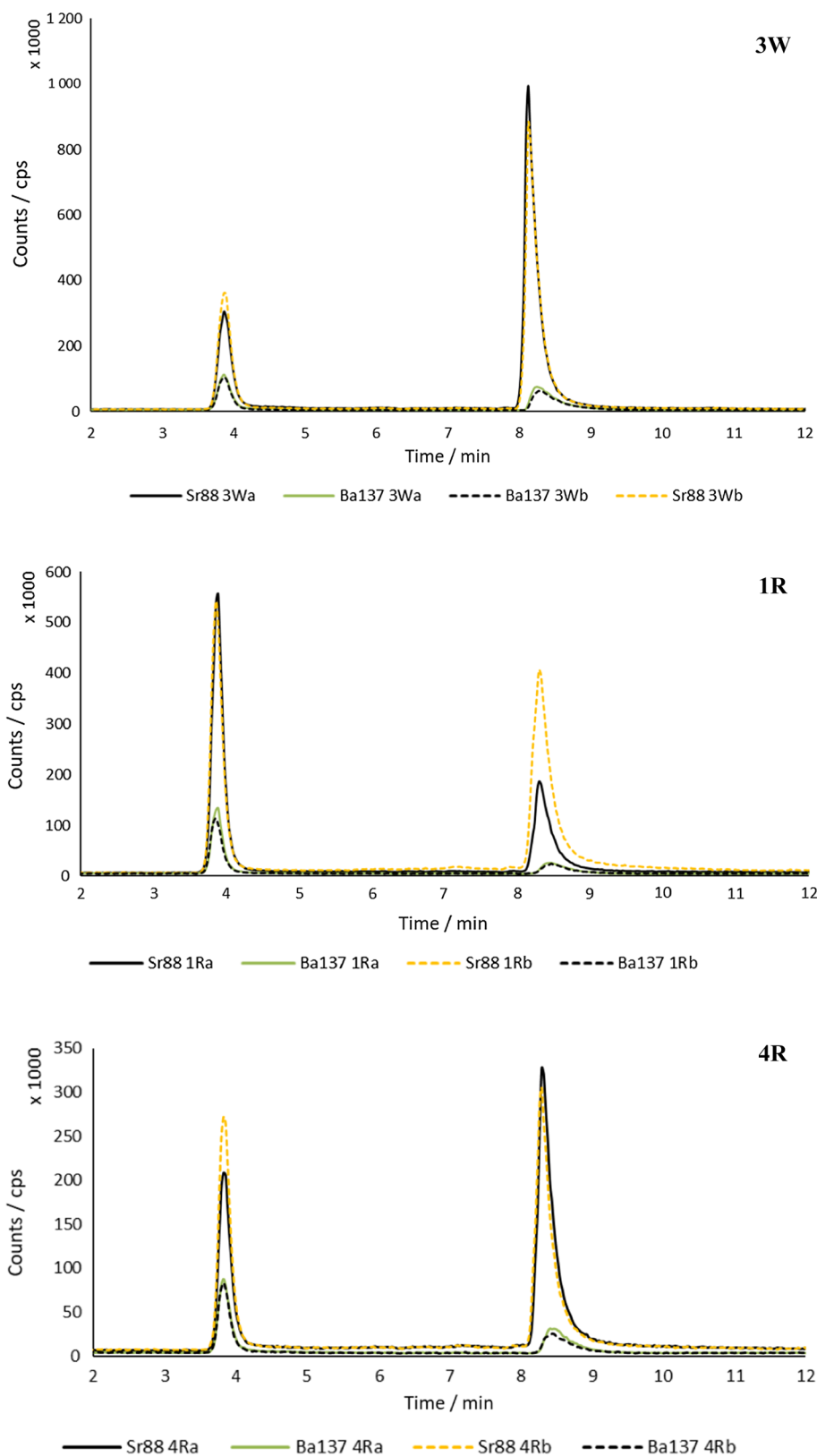


Fig. 3 Ba (II) and Sr (II) complex size distribution over time in analyzed wines



parameters, such as inductively coupled plasma power, torch position, and voltage applied to extraction and focusing lenses, were optimized daily. Hydrogen was used as

a reaction gas to reduce spectral interferences. Analytical blanks were run in parallel. External calibration using 12-point calibration curves for metals of interest was used.

Fig. 3 (continued)

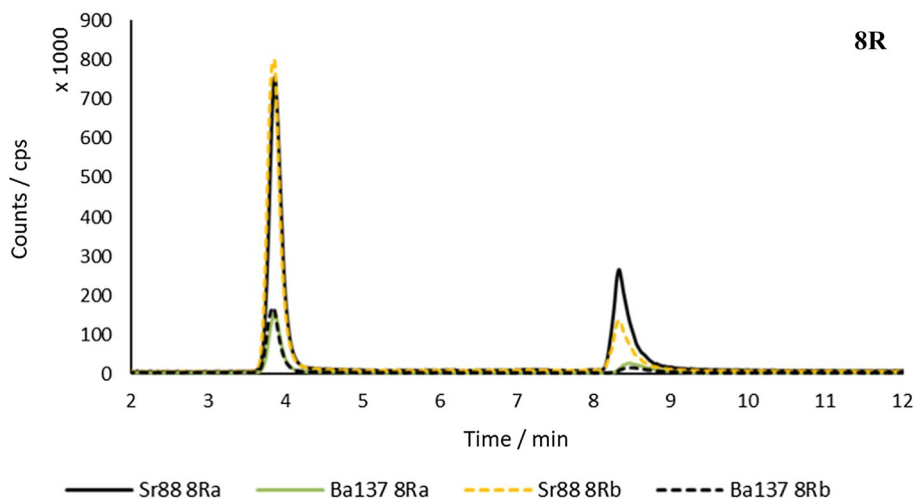


Table 2 Characteristic of polish wine samples

| Label | Voivodeship | Year of production | Type | Alcohol/% | Grape cultivar | Sugar content | pH |
|-------|---------------------|--------------------|-------|-----------|--|------------------------------|------|
| 1R* | Opolskie | 2015 | Red | 11 | Regent | Dry | 3.98 |
| 2R | Lubelskie | 2016 | Red | 11.7 | Zweigelt | Dry (2,3 g/dm ³) | 3.22 |
| 3R | Opolskie | 2015 | Red | 11.5 | Rondo | Dry | 3.55 |
| 4R* | Małopolska | 2013 | Red | 12.5 | Regent | Dry | 3.63 |
| 5R | Małopolskie | 2018 | Red | 12 | Regent | Dry | 3.75 |
| 8R* | Zachodnio-pomorskie | 2018 | Red | 13 | Rondo/Regent | Dry | 3.91 |
| 1W | Dolnośląskie | 2017 | White | 12.5 | Johanniter | Dry | 3.21 |
| 2W | Lubelskie | 2018 | White | 12 | Johanniter | Dry (1 g/dm ³) | 3.71 |
| 3W* | Lubuskie | 2016 | White | 12 | Pinot Gris, Riesling, Muscat Ottonel, Gewürztraminer | Semi-dry | 3.50 |
| 4W | Małopolskie | 2018 | White | 12 | Seyval Blanc, Hibernial, Johanniter, Solaris | Semi-dry | 3.41 |
| 5W | Małopolskie | 2017 | White | 12.5 | Seyval Blanc | Dry | 3.35 |
| 6W | Zachodnio-pomorskie | 2018 | White | 13.5 | Solaris | Dry | 3.31 |
| 7W | Lubuskie | 2016 | White | 13 | Pinot Gris | Semi-dry | 3.64 |

*Wines on which the evaluation of the speciation of metals in wine over time was studied

Speciation analysis

The selected wine samples were diluted (1:1 for the white wine and 1:2 for the red wine) with the mobile phase. For the separation of metal species with the SEC column (Acquity UPLC BEH 125 SEC 1.7 μ m, 4.6 mm \times 150 mm and guard column Acquity UPLC Protein BEH SEC guard column 125 \AA , 1.7 μ m, 4.6 mm \times 30 mm 1 K—80 K, 1/ pk), the mobile phase of 10 mM ammonium acetate (pH 6.5) at a flow rate 0.3 cm³/min with the isocratic gradient was applied. Injection volume was 5 mm³. The chromatographic conditions when HILIC column (TSKgel Amide-80,

2 μ m, 2.0 \times 150 mm and guard column TSKgel Amide-80 DC-guard 2 μ m, 2 \times 10 mm) were as follow: 10 mM ammonium acetate (pH 6.5) and ACN were used as mobile phases with a flow rate 0.1 cm³/min at gradient 0–2.5 min 80% B; 2.5–22.5 min 50% B; 22.5–25 min 50% B; 25–26 min 80% B; 26–35 min 80% B. Injection volume was 2 mm³.

The separated species were detected by ICP-MS on the basis of their metal-specific signal; the conditions were the same as those used for the total metal analysis.

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