Quantitative determination of heavy metals in red wine using TXRF and ICP-OES

Script for the experiments TXRF and ICP-OES as part of the basic module Analytical Chemistry

The first submission of both protocols should take place no later than the third working day after the ICP-OES experiment has been carried out. Corrections also on the third working day after receipt of the correction notes. Each group prepares a protocol for the TXRF and ICP-OES experiment.

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1 Introduction

After beer, wine is the most popular alcoholic beverage in Germany. While beer consumption has been steadily declining for years, wine consumption has remained constant. In 2020, every German drank an average of 20.7 liters of wine. The Rheinhessen region, where Johannes Gutenberg University is located, is the largest wine-growing area in Germany with 26,800 hectares of vineyards.

The content of various metals is one of the many quality characteristics of wine. Excessive levels of copper and iron, for example, are responsible for clouding of the wine (French: casse), which is one of the so-called wine defects. Together with manganese, these metals are also held responsible for a faster oxidation of the polyphenolic wine components, which can impair the sensory quality and shelf life as well as cause precipitation of condensed phenolic components. Zinc is also an essential trace element for humans, but can also have a toxic effect if excessive doses are ingested. Metals can enter the wine in many different ways. On the one hand, before the harvest through contaminated soils or metal-containing fertilizers and pesticides, and on the other hand during the processing stages. Here, for example, machines and their lubricating oils as well as storage containers are discussed as sources of contamination.

Depending on the application, inductively coupled plasma emission spectrometry (ICP-OES) is often used for elemental analysis. This technique is sufficiently powerful for most applications, robust, easy to handle, relatively inexpensive and easy to automate, which enables a high sample throughput. In the case of complex matrices, including wine, these are usually separated to avoid strong matrix defects. This is achieved in the practical test by a so-called digestion. Total reflection X-ray fluorescence analysis (TXRF) is also used as a complementary method. As a direct method, this can offer a decisive advantage over ICP-OES and the time-consuming digestion, which also involves a non-negligible risk of contamination. In contrast, however, the measuring time per sample is exceptionally long (several hours depending on the requirements).

In this experiment, the element concentrations of the metals Cu, Fe, Mn and Zn are to be determined in a red wine sample (approx. 50 mL**) provided by the participants** directly and after prior digestion. The two methods will then be compared in terms of accuracy, precision, detection capacity, risk of contamination, workload and sample throughput. The results from the ICP-OES test section with separated matrix are to be used as "reference values".

2 Basics

Inductively coupled plasmas are widely used in elemental analysis. They provide a particularly high energy density with which an introduced sample can not only be vaporized and atomized, but also elements can be excited and even ionized. In contrast to flames, which are used in AAS/AES, for example, and have a lower temperature than the ICP, the high temperatures prevailing in the ICP can be used to excite a large number of elements to emit radiation. Further information can be found in the lecture "Vertiefende Atomspektrometrie" as well as in relevant primary and secondary literature.

The basics of total reflection X-ray fluorescence analysis can be found in the lecture "Vertiefende Atomspektrometrie" as well as in relevant primary and secondary literature.

The aim of **quantitative digestion** is initially to convert the sample with the elements relevant for the analysis into a soluble form. Digestion is necessary, for example, for solid samples such as rocks and glasses in conjunction with techniques that require a liquid sample (e.g. ICP-OES with pneumatic nebulization). Digestion is also necessary if the sample matrix is liquid but causes strong matrix effects and must therefore be separated before analysis.

You have probably already encountered qualitative digestions in your Bachelor's degree course, including the soda potash and Freiberger digestions carried out in the Inorganic Chemistry 1 practical course. For quantitative trace analysis, however, different requirements are placed on the methods, reagents and cleanliness of the vessels. In particular, the high temperatures of the aforementioned **fusion digestions** result in the loss of numerous analytes. For this reason, digestions with liquids are preferred. Nitric acid is used in the practical course, which ideally oxidizes the organic sample matrix of the red wine completely to CO_2 and H_2O. In addition, H_2O_2 is added, which is itself a strong oxidizing agent and also decomposes easily at higher temperatures and releases O_2. The oxygen in turn reoxidizes the resulting nitrogen oxides to nitric acid and thus shifts the equilibrium to the product side (the same improvement in digestion quality is achieved if the digestion vessels are sealed under an overpressure oxygen atmosphere before digestion). To increase the reaction rate, the digestions can be carried out in pressure-sealed Teflon vessels placed in a microwave. The molecules involved absorb the microwave radiation due to their dipole moment, causing the contents of the vessel to heat up. The pressure also increases considerably due to the gases released. Temperatures of 260 °C and pressures of 50 bar are possible with the containers used in the Bings working group

without bursting. However, due to the low complexity of the red wine matrix, such a time-consuming **microwave-assisted pressure digestion** can be dispensed with in this practical course. An **open digestion** is sufficient to oxidize the matrix.

3 Procedure

The following is a brief description of the theoretical procedure of the experiments, which should serve as the basis for the experimental part of the respective protocol.

3.1 Procedure (TXRF)

For the open digestion, 5 mL of the red wine is first added three times to a centrifuge tube with 2 mL hydrogen peroxide (30 %) and then with 5 mL nitric acid (65 %). The samples are then heated together with a blank sample (blank sample = if possible all reagents in the same quantities, only without sample) for approx. 30 minutes with a watch glass in a water bath at 80 °C until the solution is colorless. After cooling to room temperature, the four digestion solutions are quantitatively transferred to a 25 mL volumetric flask and filled with ultrapure water. The solutions are then transferred to centrifuge tubes.

To prepare the sample solution for direct determination, first pipette 1 mL of the red wine into a snap cap glass using an Eppendorf pipette, noting the exact mass. Then add 10 µL of a gallium single element standard (concentration 100 mg/L) for internal standardization (note the exact mass) and homogenize the solution. In each case, 10 µL of the sample solution is then carefully pipetted into the middle of three quartz sample carriers (= triple determination), allowed to dry under an infrared lamp and analyzed using TXRF. Proceed in the same way with the four previously prepared digestion solutions (single determination in each case). A total of four snap lid vials and seven sample carriers are therefore required). The following measurement parameters are used: Acceleration voltage 600 kV, heating current 60 mA, measuring time 3000 s (direct) or 10000 s (digested, why is that?).

The centrifuge tubes must be labeled appropriately and stored tightly closed in the laboratory until the ICP-OES experiment is carried out!

Before using them, the sample carriers must be subjected to an extensive cleaning procedure, which has already been carried out by the assistants and is reproduced below for completeness:

1. mechanical cleaning (acetone + lint-free cloths).

2. cleaning with RBS solution + 10 % KOH in an ultrasonic bath at 40 °C (5 % RBS; 15 min).

3. rinse with ultrapure water.

4. cleaning with HNO_3 solution in an ultrasonic bath at 40 °C (10 % HNO_3; 2 hours).

5. rinsing with ultrapure water.

6. cleaning with ultrapure water solution in an ultrasonic bath at 40 °C (10 min).

7. drying in a drying oven in a beaker (covered with a watch glass) (110 °C; 1 hour).

8. siliconization with 10 µL Serva silicone solution.

9. drying in a drying oven in a beaker (covered with a watch glass) (110 °C; 30 min).

3.2 Procedure (ICP-OES)

To determine the heavy metal content in the digested sample (see experiment TXRF) by means of external calibration, five calibration solutions of the respective elements are prepared, starting from a stock solution of the concentration $\beta_{Mn,Cu,Fe,Zn}$ = 50 mg/L (= 50 ppm). To reduce the matrix effects due to the digestion reagents, the matrix of the calibration solutions is adjusted, so 5 mL nitric acid and 2 mL hydrogen peroxide are also added to each solution (Table 1).

For the direct determination of the heavy metal content, 5 mL of the red wine should be diluted to 25 mL with ultrapure water in a 25 mL volumetric flask. A further calibration series is used for quantification, the concentrations of which correspond to the previous calibration series. However, due to the lack of reagent addition, this is performed without nitric acid and hydrogen peroxide (Table 2).

The ten calibration solutions and the one sample solution are then transferred to carefully labeled centrifuge tubes (background: the autosampler of the device requires a larger vessel opening) and analyzed together with the four centrifuge tubes of the digested sample using ICP-OES.

Table 1: Preparation of the calibration solutions for the digestion.

$\beta_{Mn,Cu,Fe,Zn}$ (ppb)	$V_{Stamm\text{-}Lösung}$ (µL)	V_{HNO3}	V_{H2O2}
0	0	5 mL	2 mL
40	20	5 mL	2 mL
200	100	5 mL	2 mL
500	250	5 mL	2 mL
1500	750	5 mL	2 mL

Table 2: Preparation of the calibration solutions for direct determination.

$\beta_{Mn,Cu,Fe,Zn}$ (ppb)	$V_{Stamm\text{-}Lösung}$ (µL)	V_{HNO3}	V_{H2O2}
0	0	-	-
40	20	-	-
200	100	-	-
500	250	-	-
1500	750	-	-

4 Evaluation & protocol

As part of the experiments, the element contents in various samples are to be determined using ICP-OES and TXRF. To enable a statistical statement to be made, an error must be specified for each determined variable (in this case the concentration).

4.1 TXRF

You will receive the raw data of the analyses 1-2 days later by e-mail. These consist of a .txt file for each measurement, which contains the x and y values of the respective X-ray fluorescence spectrum (units keV or counts) as well as a .csv file with the automatically calculated integrals of the signals. The self-explanatory columns "Net" and "Backgr." as well as "Sigma" as absolute standard deviation are relevant here.

A special feature of TXRF compared to relative methods such as ICP-OES is the type of calibration. Only an internal standard is required to determine the analyte concentrations due to low matrix influences - there is no need to prepare a dilution series as in the case of external calibration (see ICP-OES experiment) or standard addition, for example. The following equation can therefore be used to determine the concentration of the respective analyte in the respective sample:

$$\frac{c_A \cdot S_A}{N_{A,Net}} = \frac{c_{IS} \cdot S_{IS}}{N_{IS,Net}} \quad (2)$$

c: Concentration (the index indicates whether it is the internal standard (IS) or the analyte (A)); S: Device-specific relative sensitivities (see below); N: Net signal area of the respective X-ray fluorescence line. The respective analyte concentration can be determined by simply rearranging the equation. The uncertainty of the respective concentration should be calculated using Gaussian error propagation (show derivatives to be calculated, calculated derivatives and values used in an example) with two significant digits of the error. Assume the relative sensitivities to be error-free. The uncertainty of the concentration of the internal standard has already been calculated and is ± 23 µg/L. Enter the determined concentrations together with the respective uncertainties for all seven measurements in a clear table and additionally correct the concentrations of the digested samples by the concentrations from the blank sample. Do not forget the additional dilution factor for the digested sample. How do the determined concentrations of the direct and digested samples differ and what does this tell you about the matrix effects? **Also compare the values with the results from the ICP-OES experiment in a clear table.**

Also graph all the spectra obtained. Choose the scaling of the y-axis so that the signals of the analytes are recognizable (this will cut off some signals). Also consider and research what triggers the four intense signals present in all samples at approx. 1.75 keV, approx. 3 keV, approx. 17 keV and approx. 17.5 keV. In order to be able to recognize and classify the signals, you must create another graph with a complete y-axis.

Tabelle 3: Relative sensitivities of the analyte K-lines.

Analyte	Relative sensitivity K-line
Mn	0,361703
Fe	0,459260
Cu	0,749690
Zn	0,871383
Ga	1

Furthermore, the detection limit $c_{A,LOD}$ of the method should be determined for the respective analytes. In X-ray fluorescence methods, this is usually calculated directly from the spectrum of the sample (how is it determined in ICP-OES, for example?). The reason for this is the lack of an uncontaminated "blank red wine" and the dependence of the background on the nature of the sample carrier. Equation (3), which contains the net signal area of the background $N_{A,Bkg}$, is used for this purpose. Assume dimensionless signal areas for the calculation. **Calculate the detection limits of the method for a measurement and compare them with the results from the ICP-OES experiment.**

$$c_{A,LOD} = \frac{3 \cdot c_A \cdot \sqrt{N_{A,Bkg}}}{N_{A,Net}} \quad (3)$$

Finally, compare TXRF and ICP-OES with regard to the criteria listed in the introduction. Also make a statement regarding the possible suitability of TXRF as a direct method due to negligibly small matrix effects.

4.2 ICP-OES

First, the signals obtained must be corrected by the respective blank values. In both calibration series, the correction is made by subtracting the intensities at 0 ppb from the respective signals. In the case of open digestion, the blank digestion represents the blank value to be subtracted. In the case of direct determination, the signal intensity of the 0 ppb calibration point of the associated calibration series is used as the blank value (the direct determination sample contains 75% ultrapure water).

The calculation of the concentrations sought is carried out using the sufficiently known linear regression. The uncertainty of the respective concentration is to be calculated using Gaussian error propagation (show derivatives to be calculated, calculated derivatives and values used in an example).

In addition, the detection limit of the method used should be calculated. The detection limit x_{LOD} indicates the concentration at which the method can still qualitatively distinguish the analyte from background with sufficient statistical certainty. A measured value y_{LOD} is often assumed to be statistically reliable with at least k = 3 standard deviations above the blank value. y_b = mean value of the blank signal; s_b = standard deviation of the blank signal

$$y_{LOD} = y_b + k \cdot s_b \quad (1)$$

The actual detection limit x_{LOD} is then obtained by inserting y_{LOD} into the slope of the regression line. The regression must of course (!) be carried out again without blank value correction, i.e. with the signal intensity at 0 ppb as an additional point of the calibration function (otherwise the definition of the detection limit makes no sense, negative values are often obtained, please make this clear).

In addition to the plots of all calibration functions, the protocol should contain all specific concentrations, summarized in a single clear table. Like the detection limits, these should be compared with the values obtained from the TXRF experiment and discussed.

5 Notes on the colloquia

The colloquia on the ICP-OES and TXRF experiments are held daily before the experiments in the seminar room of the working group.

The following keywords should serve as an orientation for the preparation of the respective experiments.

General:

- Task for the respective experiment

- Microwave-assisted digestion

- Analytical quality numbers (e.g. sensitivity, detection capacity)

- Calibration strategies

ICP-OES:

- Structure of an ICP-OES system, in general and specifically of the GENESIS from SPECTRO used here (see e.g. brochures etc.)

- Detailed functionality of the individual components

- Inductively coupled plasma and excitation processes, energy transfer in plasmas

- Sample feeding techniques with advantages and disadvantages

- Design, advantages and disadvantages of various mono- and polychromators

- Detectors in emission spectrometry

- Matrix effects, spectral interferences and how to avoid them

- Comparison with other elemental analytical techniques, e.g. (CS-)AAS, ICP-MS, TXRF, LIBS. What are the respective advantages/disadvantages and which fields of application result from this?

TXRF:

- Physical principle of X-ray fluorescence

- Generation of X-rays

- Interaction of X-rays with matter

- Refractive index and total internal reflection

- Structure of a TXRF system and function of individual components

- Fields of application of TXRF

- Sample preparation and calibration strategies

- Matrix effects

- TXRF vs. XRF, advantages and disadvantages of the methods

- XRF systems with advantages and disadvantages

6 Sources and useful literature

- **G. Schlemmer, L. Balcaen, M.W. Hinds and J.L. Todolí, *Elemental Analysis*: *An Introduction to Modern Spectrometric Techniques,* De Gruyter, Berlin, Boston, De Gruyter Textbook, 2019.**
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A Appendix: Error calculation in Analytical Chemistry

The following is a brief explanation of how errors are usually calculated in analytical chemistry. Strictly speaking, this sentence is already incorrect, as a distinction is made between error and uncertainty. Errors are, for example, systematic errors such as overfilling the volumetric flask, which are usually a) not quantifiable and b) only cause a deviation in either a positive or negative direction. The uncertainty, on the other hand, is a measure of the width of the statistical distribution of the respective values (an interval, therefore indicated by ±). This is made up of the individual contributions of various "uncertainty sources" such as volumetric flasks, pipettes and balances. The term "error" for the "uncertainty" actually meant has nevertheless become established in everyday language and is often used synonymously.

As a rule, the "combined uncertainty" is used in experiments such as these, which squares the individual relative uncertainty contributions, adds them together and finally takes the square root of this value (with absolute uncertainty U_n of a component, e.g. ± 0.08 mL of a volumetric flask, the value S_n of the respective quantity, e.g. 25 mL of the volumetric flask). The combined uncertainty is therefore something like a relative total uncertainty and then only needs to be multiplied by the specific concentration.

$$u_c = \sqrt{\sum_{n=1}^{N} \left(\frac{U_n}{S_n}\right)^2} \cdot c \qquad (4)$$

In the case of the ICP-OES experiment, this would include the following contributions: Uncertainty of the volume of the volumetric flask of the sample, uncertainties of the 5 mL pipette for measuring the sample, uncertainty of the concentration resulting from the uncertainty in the calculation of the equalization line (contains the residual standard deviation, see basic lecture Analytical Chemistry or relevant literature). The prerequisite for this calculation is that the individual error contributions do not correlate, i.e. are independent of each other: for example, a high uncertainty in the volume of the volumetric flask does not affect the uncertainty of the volume of the pipette.

The situation is different with contributions from the creation of the calibration series, such as the uncertainties of pipettes, volumetric flasks, commercially available standard solutions, temperatures, etc. ("x-error" in the calibration line). These contributions correspond to the residual standard deviation and the concentration uncertainty from the calculation of the calibration line

and cannot be taken into account using the combined uncertainty. As these are small contributions, they are generally neglected. If they do need to be included, Monte Carlo simulations have become established as an extended method. To simplify matters, the final calculation of the result is repeated up to 10^6 times, each time with random values of the variables involved, taking into account the respective standard deviation. The total uncertainty can be calculated from the distribution of the results. Depending on the number of variables involved and the complexity of the calculation formulas, the calculation effort varies greatly, but is in principle manageable with Excel sheets. It has been shown that the uncertainties obtained using this method are only slightly larger, so that the combined uncertainty is preferred in most cases due to its simplicity.

For further information, please refer to the very detailed and mostly easy-to-understand EURACHEM/CITAC Guide: "Quantifying Uncertainty in Analytical Measurements" (https://www.eurachem.org/images/stories/Guides/pdf/QUAM2012_P1.pdf).