In Wine There is Truth - The Characterisation and Quantitative Analysis of Wine Using Spectroscopic Methods

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Nowadays, food and drinks are always a hot topic of discussion and in the focus of 'state of the art' analytical techniques. Food scandals all around the world from eggs to horsemeat, tainted wine, oil, and milk force the European Community to establish an integrated approach to food control. The target is a high level of food safety, animal health, animal welfare and plant health within the European Union through so-called 'farm-to-table' measures and monitoring, ensuring the effective functioning of the European market.

Due to globalisation, food is no longer a local product, but may be transported over thousands of kilometres from its source to where it is consumed. For this reason, the food needs cooling, sophisticated packaging and preservation to be robust during long transportation. Unfortunately, the additives for preservation as well as the packaging material are potential sources of contamination of food. Strict and steady control from the origin of the food to the final product is needed to protect consumers from undesired contaminations while guaranteeing a high level of quality.

From the very beginning, Shimadzu has been involved in the development of analytical methods related to European regulations and following guidelines focussing on consumer protection on a global scale. The common goal is to avoid contamination of air, water, soil and food in order to protect the health and safety of the population. This is achieved by controlling limits of maximum allowable concentrations of hazardous substances. Recent examples are the European drinking water regulation, the European food safety regulations, the recent food and packaging directive and the European wine regulation.

In all of these applications, it is essential to provide the right analytical tools in order to support accurate monitoring of harmful substances (such as antimony, cadmium and lead) in our environment. These tools consist of one or more analytical systems. A wealth of spectroscopic methods exists for residue analysis and quality control. The methods and type of instrumentation applied always depend on the composition and formulation of the samples to be measured.

Specific Tools for Specific Challenges

Spectroscopy is the method of choice when quick and simple information about the sample material is required, both qualitatively and quantitatively. The optimum spectroscopic method is determined by the sample characteristics. The widest application range is covered by UV-VIS spectrometers such as UV-1800 or UV-2600/2700 series, which are ideal for the quantitative and qualitative analysis of samples in absorption, transmission and reflection measurement modes.

FTIR spectrometers such as the IRTracer-100, from the near to the far IR region, allow specific identification of substances. These instruments are used in combination with a range of accessories including microscope, each accessory being selected according to the sample material and properties.

Fluorescence spectrometers capable of highly sensitive quantitative analyses complete the range of molecular spectroscopic methods suitable for the analysis of food and more. Elemental analysis is obviously the most important tool for quantitative analysis and requires precise systems such as X-ray fluorescence, ICP and atomic absorption spectrometers, e.g. the ICPE-9000 and AA-7000 series. These instruments are able to detect trace concentrations of hazardous components. Chromatography techniques help to analyse the food for pesticide residues, texture control can be done with material testing and monitoring of drinking water is done with TOC technique as a sum parameter.

Analytical Tools for Wine Analysis

Wine is one of the oldest cultural products in human history. Wines have been cultivated for over 8000 years. The oldest known archaeological evidence of winemaking is an 8000 year old wine and fruit press found near Damascus, in today's Syria. Awareness of the medicinal effects of wine also dates back to this time. The ancient Greek physician Hippocrates (460 – 377 B.C.) recommended wine diluted with water as a remedy against headaches and digestive disorders.

Winemaking is a rather simple process: freshly harvested grapes are crushed and the resulting juice (must) is collected. The must contains fermentable sugars and natural yeasts which, either by themselves or with the help of additional yeast cultures, start the fermentation process in which mainly ethyl alcohol and carbon dioxide are formed. The latter is a gas and escapes from the must. The fermentation process comes to a halt when all of the sugars are fermented or the alcohol concentration becomes too high and kills off the yeasts. At this point the must has turned into wine.

A meticulous quality control procedure is essential, and during each stage of the production process spectroscopic methods such as AAS-, ICP-, FTIR-, and UV-VIS spectroscopy are applied for quality assurance or for product characterisation. For the quantitative determination of essential elements such as potassium, sodium, calcium and magnesium as well as undesired components such as copper, lead, arsenic and cadmium, ICP spectrometry is the method of choice since a simultaneous instrument such as the ICPE-9000 allows a fast and precise analytical procedure.

The Colour of Wine

Wine is a natural product including substances such as sugars, wine acids and alcohol. Depending on the soil where it was grown, trace elements may have an important impact on the final properties. Other elements like potassium (K) in combination with the grapes influence the colour. This can be white, red or different depending on theoretical classification and practical subjective identification by the human eye. High potassium concentration is combined with red wine because of an equilibrium between potassium, tartaric acid and the anthocyane pigments complex, which is responsible for the red colour of the wine.

Anthocyane is a natural colour found in plants such as leaves or grapes. About 250 different structures of these anthocyane complexes are known. They all have the common structure of the Flaven molecule which will be substituted at 7 possible positions from simple OH-group to complex sugar molecules.

Definition of wine colour requires analysis of the absorption spectra of wine samples. Physically, the colour is a light characteristic, measurable in terms of intensity and wavelength. In the case of grapes and wine, we loosely regard them as RED since the anthocyane pigments absorb the green portion of the visible spectra, giving to the human eye the sensation of colour red.

For better understanding of wine colour analysis, the absorption spectrum generated by a UV spectrophotometer is used. According to the classical Sudraud method, an absorption spectrum of the wine sample in a 1 cm quartz cuvette is recorded in the range of 350 to 700 nm as shown in *Figure 1*. The intensity I is calculated as the sum of the absorbance values measured at 420 nm and 520 nm and the Tone T as the ratio of the absorbance at 420 nm and 520 nm. It was observed that the A520 became smaller as the wine aged. The tone also varies and is below 1 for young wines and greater than 1 for older wines in particular, for red wines the colour changes from ruby red up to orange red.

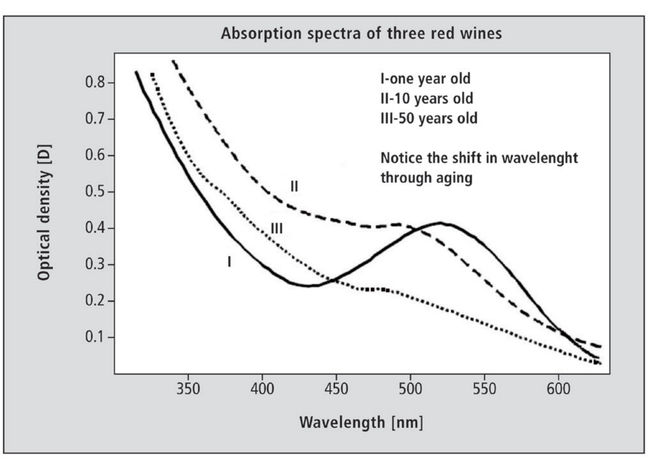

Figure 1. Absorption Spectra of red wine

Quantitative Analysis Using ICP-OES Spectrometry

Quality standards are defined in national wine regulations such as the German 'Weinverordnung'(Bundesgesetzblatt Teil1 Nr. 32) from 22 May 2002, with the latest revision in 2012. It includes the classification of wines from different locations, as well as the production process, alcohol concentrations and the maximum allowable concentrations of the elements listed in *Table 1*.

Table 1. Maximum allowable concentration of elements in wine

Element	Max. concentration [mg/L]
Al	8.00
As	0.10
B	80.00
Cd	0.01
Cu	2.00
Pb	0.25
Sn	1.00
Zn	5.00

For quantitative determination of the elements in the required concentration range, ICP is the most preferable tool for quality control due to high sensitivity, a wide dynamic range and a high sample throughput.

The vacuum system on an ICP instrument allows precise analysis of elements in the lower UV range under extremely stable conditions. The use of a vertically mounted mini torch allows a cooling gas flow rate of only 10 L/min. The system setup for determination of low concentration heavy metals in wine has been optimised using the mini torch in the dual view mode for axial and radial plasma observation. It enables determination of high concentration elements such as alkaline and alkaline earth and the heavy metals at the same time.

The wine samples were diluted twice with deionised water and then aspirated in the same way as aqueous solutions in the cyclone chamber.

The standard solutions have been prepared including an ethanol concentration of 6% in order to match the matrix.

Table 2. Shows a summary of the system parameters

Instrument	ICPE-9000
Generatorpower [kW]	1.2
Plasmagas [L/min]	10
Carriergas [L/min]	0.7
Observation	Axial/radial
Plasma	Minitorch
Sample Introduction	Coaxial nebulizer/xyclone chamber

The concentration of copper in wine is limited to a maximum concentration level of 2 mg/L. In case of higher copper concentrations the wine may have a metallic, bitter taste and the fermentation process will also be influenced by higher copper concentrations.

Copper in wine originates from the so-called Bordeaux mixture fungicide, which is a mixture of copper (II) sulphate ($CuSO_4$) and calcium hydroxide ($Ca(OH)_2$) solution used in vineyards to protect against downy mildew, powdery mildew and other fungi. Since the Bordeaux mixture is applied in large quantities, the copper accumulates in the soil and becomes a pollutant. This is why the Bordeaux mixture will most probably be banned in the European community as of 2016.

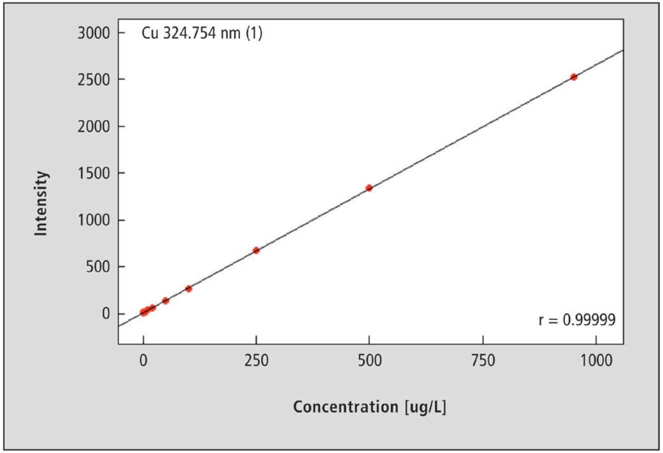

Figure 2. Copper Calibration

The calibration curve in *Figure 2* shows the standards with concentrations starting at 5 µg/L up to the maximum concentration of 1000 µg/L. The limit of detection is calculated with 0.5 µg/L (3 s). Furthermore the determination of arsenic and lead is important, as these elements still can be found in the environment generated from lead arsenate ($PbHAsO_4$) which has been used as an inorganic insecticide until 1988, after which it was officially banned.

Antimony in PET Bottles

Another element which is nowadays in the focus is antimony. Annual consumption of antimony trioxide in the United States and Europe is approximately 10,000 and 25,000 tonnes respectively. The main application is as flame retardant synergist in combination with halogenated materials. The combination of the halides and the antimony are key to the flame-retardant action for polymers helping to form less flammable chars.

Antimony trioxide is used as a catalyst in the production process for PET bottles. Elevated concentrations of Sb have been found in soft drinks such as cola and orange juices which are stored in PET bottles, as the antimony migrates from the plastic to the liquid and so accumulates in the drink. The migration process is accelerated in alcoholic beverages. Vodka samples from glass and PET bottles have been compared according to their antimony levels. It was found that the antimony concentration in vodka from a PET bottle can be as high as 20 µg/L in comparison to less than 1 µg/L in a glass bottle. The maximum allowable concentration of antimony in drinking water is 5 µg/L.

Since the latest development in the wine industry is the introduction of 75 cl PET bottles in supermarket shelves with wines from New Zealand, Australia, and France, further analytical investigations are in process to evaluate the antimony concentration in wines.