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Comparison of different methods for O_2 and CO_2 measurement in wine

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The main aim of this study was to compare several instruments for carbon dioxide and oxygen measurement in wine. For carbon dioxide determination CarboQC, Orbisphere 3568 and Agitation Cylinder were used. Oxygen measurements were performed with PreSens, OxyQC and Orbisphere 3650. Sample bottles equally treated to have the same carbon dioxide or oxygen content were used for the measurements. Measurements were undertaken in the range from 0 to 2200 mg/l for carbon dioxide and in the range from 0 to 12 mg/l for oxygen. Developing correlation graphics and regression coefficients it was demonstrated that CarboQC and Orbisphere 3658 were effective for carbon dioxide measurement in the whole measurement range. Agitation Cylinder can also be used for this purpose at normal atmospheric pressure. Oxygen measurement with PreSens, OxyQC and Orbisphere 3650 showed good reliable results. Results obtained with all three instruments showed good correlation against each other and the initial pressure. So any of these devices can be used for oxygen determination in wine. However, more research should be made taking in consideration limitations of the proposed method for sample preparation. Counter pressure fillers should be used to obtain wine samples with gas concentration higher than saturation level.
Keywords: wine, gases, oxygen, carbon dioxide, analytical methods, luminescence, sensors

Methodenvergleich für die O_2- und CO_2-Bestimmung in Wein. *Das Hauptziel dieser Studie war der Vergleich unterschiedlicher Instrumente für die Messung von Kohlendioxid und Sauerstoff in Wein. Zur Bestimmung von Kohlendioxid wurden CarboQC, Orbisphere 3568 und der Schüttelzylinder verwendet. Die Sauerstoffmessungen wurden mittels PreSens, OxyQC and Orbisphere 3650 durchgeführt. Probeflaschen mit identer Behandlung und identen Kohlendioxid- und Sauerstoffgehalten wurden für die Untersuchung verwendet. Die Messungen wurden in einem Bereich von 0 bis 2200 mg/l für Kohlendioxid und von 0 bis 12 mg/l für Sauerstoff durchgeführt. Korrelationsgraphiken und Regressionskoeffizienten zeigten, dass CarboQC and Orbisphere 3658 für die Messung von Kohlendioxid über den gesamten Messbereich geeignet waren. Der Schüttelzylinder kann für diesen Zweck ebenfalls verwendet werden unter normalen atmosphärischen Druckverhältnissen. Die Sauerstoffmessung mittels PreSens, OxyQC und Orbisphere 3650 erbrachte reliable Ergebnisse. Diese zeigten bei allen drei Instrumenten gute Korrelation miteinander und mit dem Ausgangsdruck. Also kann jedes dieser Geräte für die Sauerstoffmessung in Wein verwendet werden. Allerdings sollten betreffend die Einschränkungen der vorgeschlagenen Methoden zur Probenaufbereitung weitere Untersuchungen durchgeführt werden. Gegendruckfüller sollten verwendet werden, um Weine mit Gaskonzentrationen über dem Sättigungsgrad zu erhalten.*
Schlagwörter: Wein, Gase, Sauerstoff, Kohlendioxid, Analysemethoden, Lumineszenz, Sensoren

Comparaison des méthodes de détermination des teneurs des vins en O_2 et en CO_2. *Le but principal de la présente étude consistait à comparer différents instruments de mesure de dioxyde de carbone et d'oxygène dans le vin. CarboQC, Orbisphere 3568 et le secoueur ont été utilisés pour déterminer le taux de dioxyde de carbone. Les mesures d'oxygène ont été réalisées à l'aide de PreSens, OxyQC et Orbisphere 3650. Des bouteilles d'échantillonnage traitées de manière identique et présentant des teneurs identiques en dioxyde de carbone et en oxygène ont été utilisées pour l'essai. Les mesures ont été réalisées dans une plage de 0 à 2200 mg/l pour le dioxyde de carbone et de 0 à 12 mg/l pour l'oxygène. Les diagrammes de corrélation et les coefficients de régression font apparaître que CarboQC et Orbisphere*

3658 convenaient pour la mesure du dioxyde de carbone dans toute la plage de mesure. Dans des conditions de pression atmosphérique normales, le secoueur peut également être utilisé à cette fin. La mesure de l'oxygène au moyen de PreSens, OxyQC et Orbisphere 3650 a donné des résultats fiables qui, pour les trois instruments, présentaient une bonne corrélation entre eux et avec la pression initiale. Chacun de ces appareils peut donc être utilisé pour mesurer la teneur d'oxygène dans le vin. D'autres essais relatifs aux limites d'utilisation des méthodes proposées pour la préparation des échantillons devraient toutefois être réalisés. Des tireuses isobarométriques devraient être utilisées pour obtenir des vins présentant des concentrations de gaz au-dessus du degré de saturation.

Mots clés : vin, gaz, oxygène, dioxyde de carbone, méthodes d'analyse, luminescence, capteurs

Among all gases that can be dissolved in wine, oxygen and carbon dioxide can be considered the most important. Although oxygen has a much lower solubility in wine than carbon dioxide, it can be of significant importance (Eder, 2009). Oxygen has to be considered as a very reactive chemical agent having potential to alter wine by oxidation. Different oxygen levels can have a great influence on color, aroma, flavor and the general perception of wine. The total capacity of oxygen absorption is 80 mg/l for white wines and 800 mg/l for reds (Moutounet et al., 1994).

The saturation value for oxygen at atmospheric pressure in water at 20 °C is 9.1 mg/l (Margalit, 2004). In wine these values are about 20 % lower. At 20 ºC and normal pressure oxygen can be dissolved in wine only up to 8.8 mg/l (Troost, 1988). However, at higher pressure and lower temperature more oxygen can be dissolved (Moutounet et al., 1994; Schmidt and Waidelich, 2008). Optimal concentrations can alter enormously for different wine styles.

In contrast to oxygen carbon dioxide has a much higher solubility but a lower reactivity with wine constituents (Table 1). It has a great impact on physical properties and the sensory perception of wine. The solubility of carbon dioxide in liquids depends on the temperature. Dissolved carbon dioxide is reversely converted with water into carbonic acid (H_2CO_3). Carbonic acid deprotonates into bicarbonate (HCO_3-) and carbonate (CO_3^{2}-). The relative concentration of either CO_2, H_2CO_3, HCO_3^- or CO_3^{2-} depends on the pH-value of the solution. At the pH-value of 3.0, which is common in wine, 99 % of the carbon dioxide is present in the anhydride gaseous form (Liger-Belair et al., 2006). The carbon dioxide solubility in wine will depend on alcohol and sugar content as well as on the total acidity and the extract level.

Carbon dioxide influences the taste of beverages. It is also important for the total pressure in a bottle. Carbon dioxide comes into wine mainly from natural processes (alcoholic and malolactic fermentation (El Haloui et al., 1988; Lonvaud-Funel, 1995), from post-fermentation processing or is enriched by sparging into wine (Knoll, 1990).

The solubility of carbon dioxide depends on the temperature and it is inversely related to the concentration of alcohol. Other contributing factors include reducing sugar levels and viscosity arising from polysaccharides and phenols. At atmospheric pressure and 20 ºC the solubility of carbon dioxide is 1500 mg/l (Zoecklein et al., 1995). Troost (1988) gives a value of 1460 mg/l.

Cellar operations also influence the concentration of carbon dioxide. Warm storage of wine accelerates degassing. Racking, fining and filtration may further reduce the concentration of carbon dioxide and at the same time lead to oxygen uptake (Vidal et al., 2001; Vidal et al., 2003; Vidal et al., 2004). The CO_2 content can be precisely installed right before the bottling of wine using direct sparging or a membrane contactor (Blank, 2010).

Carbon dioxide can provide tactile sensation, magnify the sense of acidity, and enhance odor intensity. Carbon dioxide is perceptible in water at 200 mg/l, and in wine about 500 mg/l (Peynaud, 1996). Carbon dioxide enhances the sense of acidity, thus reinforcing tannin and bitter elements and reducing the sense of sweetness (Zoecklein et al., 1995). Optimal carbon dioxide contents recommended in literature (Blankenhorn, 2002) are 400 to 800 mg/l for red wine, 0.7 to 1.5 g/l for white wine and 700 to 1200 mg/l for rosé wine. Hence the measurement of the concentration of dissolved oxygen and carbon dioxide is crucial to winemaking. Several analytical methods for gas measurement in liquids are available. Oxygen can be measured by the following methods:

- Titration (Azide-Winkler Method; Winkler, 1888). Accurate realization of the titration method is difficult to perform. Any oxygen uptake or loss during laboratory manipulations influences the result. The medium should be also free of any compounds that may oxidize the iodide.
- Potentiometric method based on voltage - difference due to chemical reaction on one

of the electrodes.
- Luminescence method based on changes in light absorption and reflection properties of oxygen sensitive material.

Measurement methods for dissolved carbon dioxide include:
- Titration method (OIV-MA-AS314-01). A wine sample is cooled to 0 °C. After setting the pH level at 10 to 11 with NaOH the titration with sulfuric acid solution is carried out. (OIV, 2008).
- Manometric method. The concentration of carbon dioxide is determined by measuring temperature and pressure in a bottle.
- Agitation method
- Conductivity method
- Optical method
- Method of multiple volume expansion

Materials and Methods

Sampling devices

Sampling is crucial, because the wine can lose CO_2 or take up oxygen at this step. Thus a special technique is helpful in order to transfer the samples from the container into the measurement device. Therefore we used two devices:
- Filling Device PFD (Anton Paar GmbH, Austria)
- Beverage Package Sampler Orbisphere 29971- 71 (Hach Lange GmbH, Germany)

Both tools are designed to transfer liquid samples from a closed container directly into the measuring chamber of a measuring instrument. They can be used with cans, glass and PET bottles. The PFD Filling Device was used for oxygen and carbon dioxide measurement as shown in Fig. 1.

Fig. 1: A sample is taken directly from a bottle using a pressure filling system (left), transferred to OxyQC (center) and after that to CarboQC (right)

Table 1: Solubility of selected gases in water at 293.15 K and partial pressure 1013.25 hPa (Haynes, 2010)

Gas	Solubility (mol)
Nitrogen (N_2)	1.274×10^{-5}
Oxygen (O_2)	2.501×10^{-5}
Carbon dioxide (CO_2)	7.07×10^{-4}

Table 2: Technical specifications of CarboQC

Measurement range	0 - 12 g/l (0 - 6 Vol.) at 30 °C 0 - 20 g/l (0 - 10 Vol.) < 15 °C
Temperature	-3 - 30 °C
Pressure	0 - 10 bar absolute
Repeatability	0.05 g/l (0.025 Vol.) CO_2

Measurement devices for carbon dioxide

CarboQC

CarboQC (Anton Paar GmbH, Austria) is a CO_2 measuring device based on the method of multiple volume expansion. Technical data given by Anton Paar is shown in Table 2.

Hach Lange Orbisphere 3658

Orbisphere 3658 (Hach Lange GmbH, Germany) is an instrument which determines the CO_2 concentration in liquids using the conductivity method (Fig. 2). Technical data provided by Hach Lange are shown in Table 3.

Table 3: Technical specifications of Orbisphere 3658

Measurement range	0 - 10 g/kg, 0 - 6 bar
Temperature	-5 - 35 °C
Pressure	0 - 10 bar absolute
Membrane	Hydrophobic membrane 29561A
Response time	1 min
Accuracy within ± 5 °C of calibration temperature	± 1% of reading; ± 0.025 g/kg or ± 16 mbar

Fig. 2: Orbisphere 3658

Agitation Cylinder

The agitation cylinder (Schliessmann, Germany) is a very simple and cheap instrument for the determination of carbon dioxide according to the method of Veitshöchheim (Jakob et al., 1997). The method is only applicable with non-carbonated beverages, such as still wine. The analysis is done manually. The whole equipment needed is a 100 ml volumetric flask with grounded neck, a plastic plug, a pipe airtight-mounted in the plug, a pinchcock and a teflon tip (Fig. 3). Due to the low costs and the simple operation the agitation cylinder is widely used in the wine industry. Technical data is given in Table 4. For measurement a thermometer and conversion tables are needed.

Table 4: Technical specifications of agitation cylinder

Measurement range	100 to 42 ml of initial sample volume*)
Temperature	0 - 20 °C
Recommended pressure	0 - 1 bar
Sample volume	100 ml

*) converting tables provided by producer

Measurement devices for oxygen measurement

PreSens

The PreSens (Precision Sensing GmbH, Germany) instrument is an oxygen measurement device based on the luminescence method. In Germany it is provided by Nomacorc Company and is known as NomaSense™.

The measuring device (Fibox 3) is a stand-alone single channel fiber optic oxygen meter operating with an excitation wavelength of 505 nm. The light is transferred by a polymer optical fiber of 2 mm diameter and is connected to the instrument by an optical connector. Technical data of the device are described in Table 5.

Oxygen-sensitive PSt3 mini sensors were used for oxygen measurement. The sensors (spots) are made of oxygen sensitive material and can be glued with silicone directly into bottles made of clear glass. The measurement is performed without opening the bottle. Important technical data is given in Table 6.

Table 5: Technical specifications of Fibox 3 LCD instrument

Analog output (oxygen)	0 - 20 % air sat. ± 0,5 % air sat.
Analog output (temperature)	0 - 50 °C ± 1,5 °C
Acquisition time	Programmable (default 1 sec.)

Table 6: Technical specifications of oxygen sensor type PSt3

	Dissolved oxygen	Gaseous and dissolved oxygen
Measurement range	0 - 22 ppm	0 - 500 hPa
Limit of detection	15 ppb diss. oxygen	0.31 hPa
Accuracy (20 °C)	± 1 % at 100 % air saturation; ± 0,15 % at 1 % air saturation	

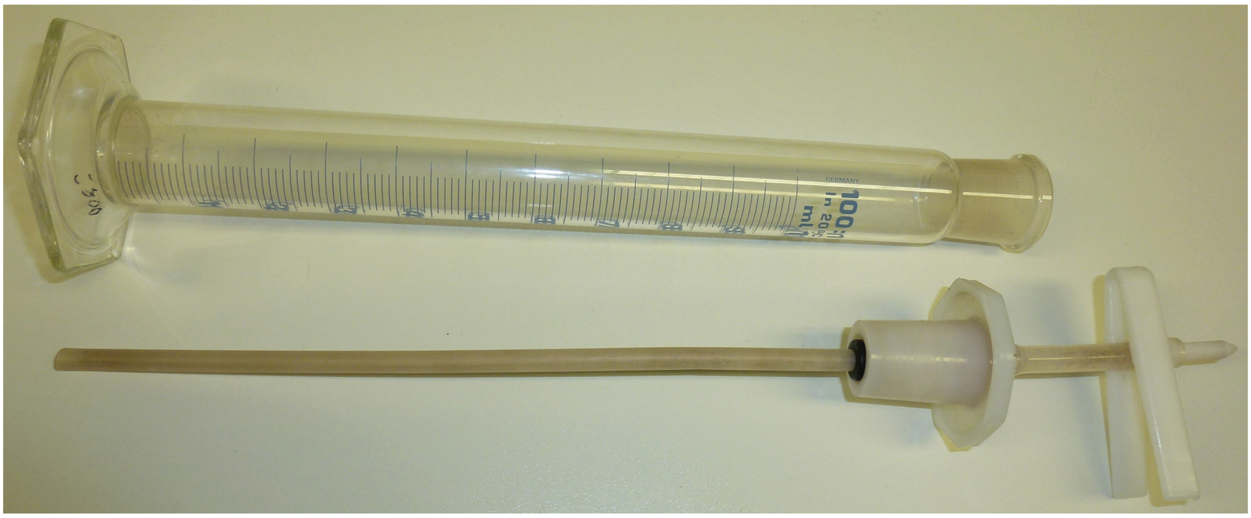


Fig. 3 : Agitation cylinder

OxyQC

OxyQC (Anton Paar GmbH, Austria) is an oxygen measurement instrument which uses the luminescence method for the determination of oxygen. The difference to the PreSens device is that oxygen measurement takes place in the chamber inside the instrument, where diodes, a sensor and the photodetector are located. Technical data is given in Table 7.

Table 7: Technical specifications of OxyQC

Measurement range	0 ppm to approx. 10 ppm O_2
Pressure	0.2 - 7 ± 0.5 bar
Temperature	0 - 30 °C
Repeatability	20 ppb
Response time	Less than 40 s

Hach Lange Orbisphere 3650

Orbisphere 3650 (Hach Lange GmbH, Germany) is a portable oxygen measurement device using the polarographic method (Fig. 4). It can be used for oxygen measurements in liquids. Orbisphere 3650 was equipped with a 25 µm 2956A membrane. The response time for 90 % of the signal at 25 °C is 7.2 seconds. Technical specifications of the device are given in Table 8 (manufacturer's data).

Table 8: Technical data of Orbisphere 3650

Power supply	Batteries: two C-type cells, NiCd or alkaline, each 26 x 50mm 2.4 - 3 volts total
Signal drift	<0.5 % of reading between service
Temperature	-5 - 60 °C
Instrument operating limits	-5 - 100°C
Display units	ppm/ppb (liquid)
Max. display resolution	1 ppb 0.001 ppm

Fig. 4: Orbisphere 3650

Gases

Compressed nitrogen (99.98 % purity) and compressed air gas (78.9 % nitrogen and 21.1 % oxygen) in bottles were provided by Lange & Co. GmbH, Germany.

Wine

A white wine of the variety 'Müller-Thurgau' from our institute was used for the study. Enological parameters are shown in Table 9.

Table 9: Standard enological analysis of Müller-Thurgau wine used for the experiment

Alcohol (g/l)	94.9
Glycerin (g/l)	4.8
Sugar (g/l)	1.4
Extract (g/l)	17.5
Reduced extract (g/l)	7.8
pH	3.28
Titratable acidity expressed in tartaric acid (g/l)	6.0
Tartaric acid (g/l)	1.9
Lactic acid (g/l)	n.d.
Volatile acids (g/l)	0.32
Potassium (g/l)	750
Calcium (g/l)	51
Magnesia (g/l)	96
Free SO_2 (g/l)	29
Total SO_2 (g/l)	82
CO_2 (g/l)	0.97
E 420 nm/1cm	0.052
Total phenols (Folin-Ciocalteu, g/l)	186

Carbon dioxide measurement

CarboQC

The wine samples in bottles were transferred to the measuring instruments by using the PFD Filling Device which is connected to nitrogen gas to push the wine sample through the system. In our experiment CarboQC and OxyQC were connected in series as shown in Figure 9. The sample was taken directly from the package. A bottle was placed into the chamber of the PFD Filling System. After closing the chamber, a lever on the Filling System was pushed down, which forced a needle inside the chamber to punch the screw cap on the bottle and get inside the liquid. Then 100 ml of beverage were forced into a glass measuring chamber of CarboQC by 6 bar nitrogen pressure. High pressure guaranteed no loss of gases due to sample transfer. Once the sample was in the measurement chamber, the line was closed automatically. The sample was agitated in the chamber until equilibrium between gas and liquid phases was reached. After that, the total pressure of gases (CO_2 + other gases present in the liquid) and temperature were measured. Then the volume of the measuring chamber was expanded a second time and further agitated. Each time the total system pressure and temperature were measured after agitation. Thus the measurements of pressure and temperature provided accurate information about the CO_2 content in the beverage as well as the concentration of other gases (predominantly oxygen and nitrogen). CarboQC was checked against destilled water and a commercial mineral water (Loewensteiner Classic), 10 bottles each. This was done in order to test the repeatability of the measurements. Standard deviation of these measurements did not exceed 2 mg/l CO_2.

Orbisphere 3658

The liquid that is going to be measured flows through the Beverage Package Sampler Orbisphere 29971-71 under pressure of nitrogen gas (1.5 to 2 bar) through the device at a certain speed (150 ml/min is recommended), passing a hydrophobic membrane, which separates the liquid from the gas before entering the measuring chamber with the sensor. CO_2 passes through the membrane into the measuring chamber. The measuring chamber is constantly flushed with air. The sensor determines the level of thermal conductivity corresponding to air, which is taken as a standard for "non-carbon dioxide media". Carbon dioxide diffusing through the membrane into the measuring chamber changes the thermal conductivity proportionally to the partial pressure of CO_2. Before starting the measurements the Orbisphere 3658 was calibrated with pure CO_2 at 1 bar, 1.5 bar and 2 bar pressure using an absolute pressure manometer.

Agitation Cylinder

The agitation cylinder is a simple tool to manually analyze the CO_2 concentration of non-carbonated beverages. A volume of 100 ml of wine is transferred into a measuring cylinder. The transfer should be carried out very carefully in order not to lose carbon dioxide before measurement. The cylinder is closed with a closure equipped with a tube in the center. The tube is closed with a clamp. After that the cylinder is shaken manually intensively and then stopped (CO_2 builds up pressure). Then the clamp should be opened to let the liquid out. Shaking and opening is carried out until no more liquid is leaving the cylinder. The closure and the tube have to be removed and volume and temperature of the remaining liquid are measured. The ejected volume is proportional to the carbon dioxide concentration in a beverage at a certain temperature. This residual volume in the cylinder is proportional to CO_2 concentration in g/l and temperature, and can be read directly from a table (Jakob et al., 1997).

Oxygen measurement

PreSens (NomaSense™)

The PreSens (NomaSense™) uses the principle of luminescence quenching for determination of the oxygen concentration (Fig. 5). In the absence of oxygen (Fig. 5; 1) a luminophore adsorbs light, and then emits luminescence. Oxygen deactivates the luminophore (Fig. 5; 2) due to energy transfer, and the measurable luminescence decreases. A relation between luminescence intensity and oxygen concentration is described in the *Stern-Volmer* equation (Fig. 6), where:
I: Luminescence intensity in presence of oxygen; I_0: Luminescence intensity in absence of oxygen; ṭ: Luminescence decay time in presence of oxygen; τ_0: Luminescence decay time in absence of oxygen; K_{SV}: Stern-Volmer constant (quantifies the quenching efficiency and therefore the sensitivity of the sensor); $[O_2]$: oxygen content.
The measuring system contains a light source (light emitting diodes - LED), a photodetector (photomultiplier - PMP), optical filters (OF), an optical fiber as signal transmitter and an optical sensor (Fig. 7). Minisensors (oxygen sensitive spots) are calibrated by the manufacturer at a certain pressure in an atmosphere with 0 % and 100 % of oxygen. After that, sensor spots are glued with silicon into white glass bottles (Fig. 8). The accuracy of the minisensors has been checked in empty bottles at atmospheric air pressure. Then the test bottles were filled with wine for measurement. To determinet he oxygen content the fiber is pointed directly at the minisensor through the glass. Oxygen partial pressure in hPa can be read from the instrument and calculated in mg/l. Temperature compensation can be done automatically by the software, or taken into consideration at further calculations (Huber, 2008).
Bottle preparation prior to test: Six clear glass bottles (1 liter) were equipped with two minisensors in each bottle, one for dissolved oxygen level close to the closure and the other one for dissolved oxygen concentration close to the bottom of the bottle. For the final calculations the average value was used. All bottles were flushed with nitrogen gas prior to bottling to eliminate oxygen and carbon dioxide. Every oxygen sensor was checked in the nitrogen atmosphere.

OxyQC

OxyQC also uses a luminescence-based method for oxygen measurement but it uses sensors in the measuring device as described above. The oxygen measurement was carried out simultaneously with the carbon dioxide measurement. The bottles were measured directly using the PFD Filling Device which was connected in series to the OxyQC and the CarboQC instruments (Fig. 9). The injected gas for the transfer of the wine sample to the measuring instruments was pure nitrogen gas. The OxyQC and the CarboQC were switched on for twenty minutes until the first measurement was taken in order to stabilize the signal inside the measuring chamber. Accuracy of the OxyQC can be tested with air, pure nitrogen gas and Na_2SO_3 solution. In the experiment the accuracy of the instrument was tested with Na_2SO_3 solution and checked against the corresponding table provided by the manufacturer. Zero-point of OxyQC was checked with an oxygen-free solution.

Orbisphere 3650

Orbisphere 3650 uses an electrochemical method of oxygen measurement and was developed by Clark (Fig. 10) (Ribéreau-Gayon et al., 2006). The apparatus consists of two electrodes, a silver anode and a gold cathode, linked by potassium chloride gel. They are separated from the medium by a membrane selectively permeable to oxygen. The difference of potential established between two electrodes is modified by oxygen, circulating through the membrane. Before starting the measurement Orbisphere 3650 was calibrated with air and adjusted according to the oxygen content in air at the measured temperature and atmospheric pressure at the present moment. Then, Orbisphere 3650 was connected to the Beverage Package Sampler Orbisphere 29971-71 (Hach Lange GmbH, Germany) and to Orbisphere 3658 as shown in Figure 11. After a bottle

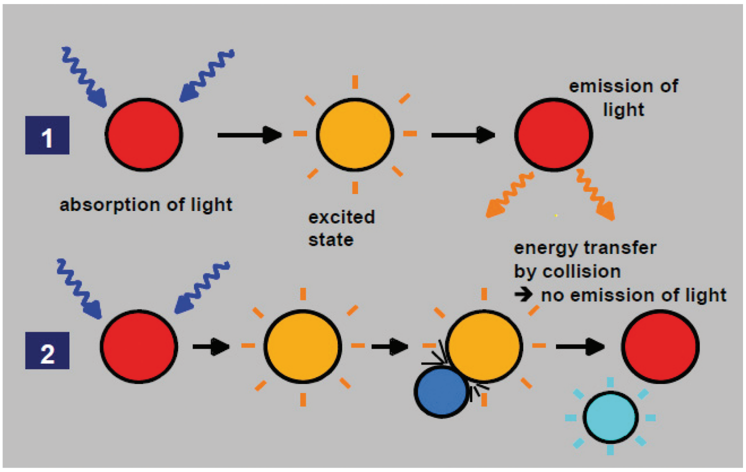


Fig. 5: Principle of dynamic quenching of luminescence by molecular oxygen (Huber, 2008)

$$\frac{I_0}{I} = \frac{\tau_0}{\tau} = 1 + K_{sv} * [O_2]$$

Fig. 6: *Stern-Volmer* equation

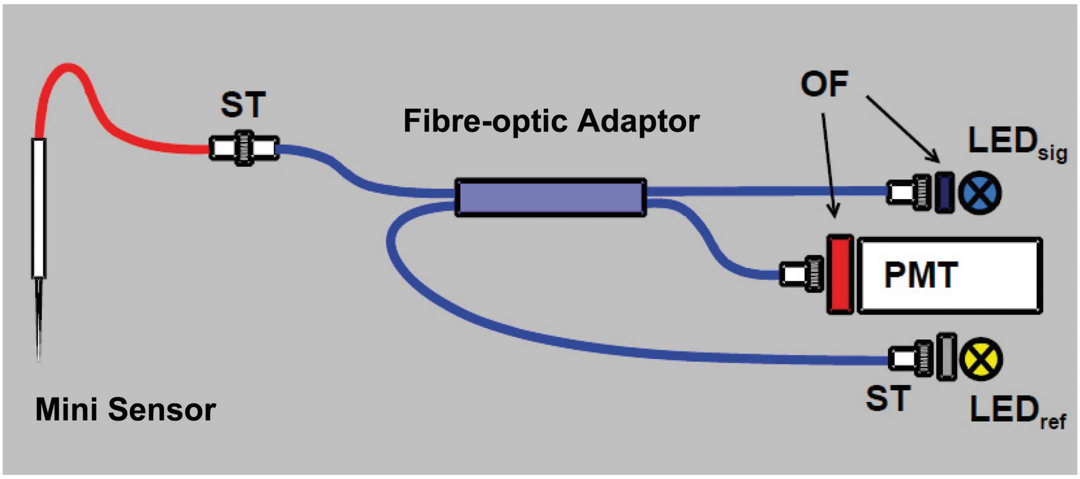


Fig. 7: Schematic of PreSens (Huber, 2008)

is placed in the Beverage Sampler, a gasket seals the bottle and then a hollow needle breaks the screw cap and gets into the wine. The wine is forced through both devices by nitrogen gas pressure (1.5 to 2.0 bar). Measurement is fixed as soon as the value stabilizes.

Sample preparation

In order to obtain wine samples with different concentrations of oxygen or carbon dioxide stainless steel beverage kegs of 20 l volume were used. 20 l kegs were filled with 10 l wine. The wine was degassed by sparging with nitrogen gas for one hour with a stainless frit. Wine temperature was 15 °C. After degassing the wine the headspace of the kegs was pressurized either with CO_2 or with compressed air. In both cases six different pressure levels were applied: 0; 0.5 bar; 1.0 bar; 1.5 bar; 2.0 bar; 2.5 bar. The kegs were shaken for ten minutes by constantly applying the adjusted pressure to allow the gases to dissolve and to reach gas equilibrium in the whole volume. Then six 1 l bottles were filled with wine from the keg one after another using a hose mounted to the keg and fed by the pressure in the keg. Then the bottles were sealed with screw caps

Fig. 9: Set-up for the simultaneous measurement of O2 and CO2 with the connection in series PFD Filling Device (left), OxyQC (center) and CarboQC (right) (image: Anton Paar GmbH

Fig. 8: Two minisensors (spots) mounted in a bottle sealed with screw-cap; the top sensor is in the headspace and the other measures the dissolved oxygen in wine

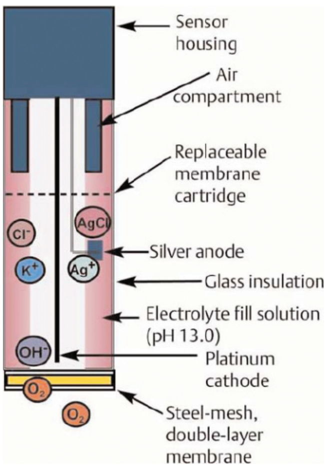


Fig. 10 (left): Polarographic method of oxygen measurement with Orbisphere 3560

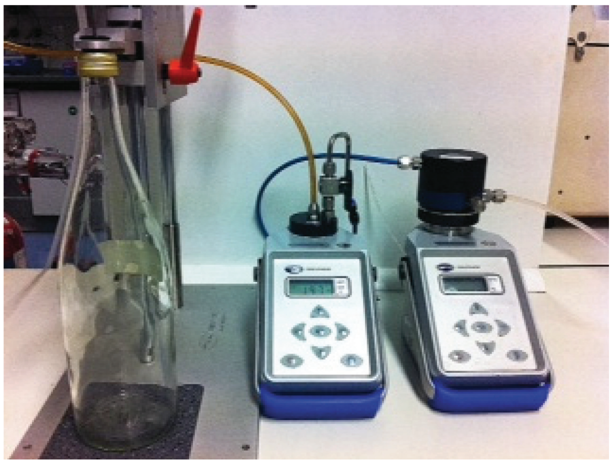


Fig. 11: Sample is taken by a Beverage Package Sampler (left), transferred to Orbisphere 3650 (center) and then to Orbisphere 3658 (right)

Fig. 10a: At the cathode: $O_2 + 2H_2O + 4e^-$ $4OH^-$

At the anode: $Ag + KCl$ $AgCl + e^- + K^+$

immediately. For each pressure level two bottling sessions took place. Thus a total of 144 bottles were prepared (six bottles for each pressure level × two gases × six pressure levels × two repetitions). All bottles were filled brimful without headspace.
All bottles were shaken for five minutes and were left standing for five more minutes before measurement to reach gas equilibrium in the whole volume. After that the bottles were measured with all instruments according to the experimental protocol (Table 10). After measurements with OxyQC and CarboQC, and after measurements with Orbispheres screw caps on bottles were destroyed in the process of sample taking and were changed for new ones. Nitrogen was used to force the wine through the measurement devices.

Table 10: Order of measurements and required volume of wine

1	PreSens	Non-invasive
2	OxyQC and CarboQC	~ 100 ml
3	Orbisphere 3650 and 3658	300 - 500 ml (until stable values were reached)
4	Cylinder	100 ml

Statistical evaluation

For the correlation regression analysis and statistical evaluation XLSTAT Software was used (Version 2011.2.08).

Results and Discussion

Carbon dioxide

Results of CO_2 measurements with CarboQC, Orbisphere 3658 and agitation cylinder are shown in Table 11. Ideally the pressure of CO_2 applied in the keg should be equal to the pressure of CO_2, measured in wine after bottling. But in the experiment carbon dioxide was partially lost during the bottling operation of wine oversaturated with CO_2 (1.5, 2.0 and 2.5 bar CO_2-pressure). These losses happened during the bottling operation and are unavoidable under normal pressure bottling. They only can be avoided by using counter pressure filler systems. However, all bottles were bottled equally, so the error due to carbon dioxide loss due to bottling can be considered equal for all samples. In addition to the loss due to bottling we found a second CO_2 loss with the measuring technique. There is an obvious loss of CO2 using the agitation cylinder in wines with a CO_2 concentration higher than about 1400 mg/l. This can be explained by the sample preparation in the open volumetric flask. During filling and adjusting the volume already a loss of CO_2 takes place. In contrast to this, both other analytical methods show higher values. This can be explained by the fact, that the wine was transferred within closed systems into the measuring devices without any loss during measuring. The correlation of carbon dioxide concentrations (mg/l) measured with CarboQC, Orbisphere 3658 and agitation cylinder is shown in Figure 12. The regression coefficient in the full pressure range up to 2.5 bar for the agitation cylinder is at 0.811. The sealed systems CarboQC and Orbisphere 3658 have considerably higher regression coefficients of 0.941 and 0.922. Thus the results clearly confirm the earlier statement, that the agitation cylinder cannot be used for carbonated wine.
In order to evaluate the CO_2 measuring methods for non-carbonated beverages, we graphed obtained data only for the CO_2 pressure up to 1.0 bar. Figure 13 shows measurement results only in the range from 0 to 1 bar CO_2 pressure applied to the kegs prior to bottling. Regression coefficients corresponding to this interval and to the whole data are given in Table 12. Results obtained with CarboQC and Orbisphere 3658 showed good correlation with the whole pressure range in keg. The agitation cylinder only can reach a satisfying regression coefficient in the interval from 0 to 1 bar CO_2 overpressure in the keg. At higher pressures the measurement with this instrument is not accurate. It can be explained with a fact that dissolved carbon dioxide is lost during cylinder filling. However, at normal atmospheric pressure measurements with agitation cylinder were very accurate and comparable to the other techniques.
To compare the measurement systems against each other the correlation between values obtained with the instruments were calculated for the whole CO_2 pressure range from 0 to 2.5 bar (Fig. 14; Table 13). Values obtained with CarboQC and Orbisphere 3658 strongly correlate (R^2 = 0,99). Regression coefficients of data obtained with these both devices against agitation cylinder are lower, although still at significant level.

Oxygen

The oxygen concentration in the keg was adjusted with different levels of compressed air applied to the partially filled kegs. Oxygen content in air is 20.9 %,

Table 11: Average carbon dioxide content values (mg/l; each value n = 2 × 6 bottles) and standard deviation, based on measurements of 12 sample bottles

CO_2 pressure (bar)	Instrument	Average CO_2	Standard deviation
0	CarboQC	208	6.2
	Hach Orbisphere	32	1.5
	Agitation Cylinder	8	17.5
0.5	CarboQC	718	12.2
	Hach Orbisphere	683	13.0
	Agitation Cylinder	612	75.5
1.0	CarboQC	1382	15.3
	Hach Orbisphere	1369	20.6
	Agitation Cylinder	1295	52.3
1.5	CarboQC	1655	20.2
	Hach Orbisphere	1465	43.8
	Agitation Cylinder	1368	32.9
2,0	CarboQC	1899	7.9
	Hach Orbisphere	1797	11.3
	Agitation Cylinder	1387	38.8
2.5	CarboQC	2063	6.5
	Hach Orbisphere	1988	14.0
	Agitation Cylinder	1571	55.6

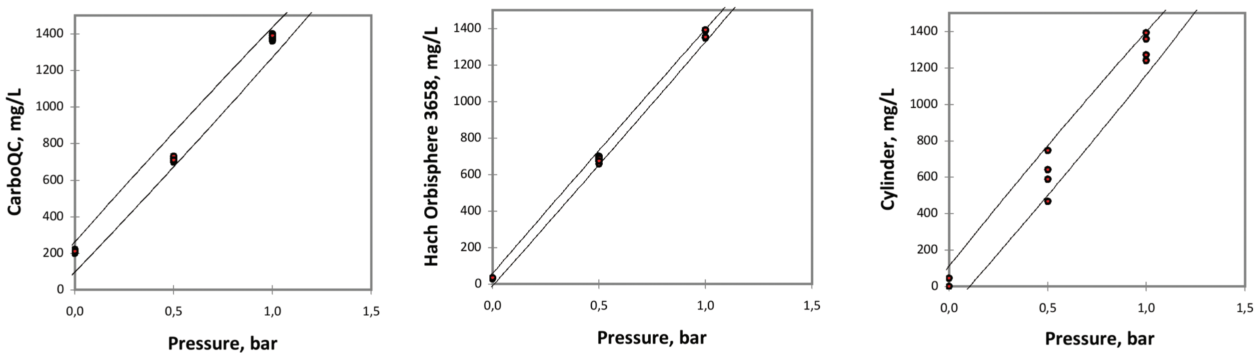
Table 12: Regression coefficients (R^2) for CO_2 measurements

Interval	CarboQC	Hach Orbisphere 3658	Cylinder
0 - 2,5 bar	0.941	0.922	0.811
0 - 1,0 bar	0.994	0.999	0.989

Table 13: Regression coefficients (R^2) for measurements taken with three different instruments against one another

Variables	CarboQC	Orbisphere 3658	Agitation Cylinder
CarboQC	-	0.990	0.946
Orbisphere 3658	0.990	-	0.957
Agitation Cylinder	0.946	0.957	-

Fig. 12: Correlation of carbon dioxide concentrations (mg/l) measured with CarboQC, Orbisphere 3658 and Agitation Cylinder in range of CO2 pressure from 0 to 2.5 bar



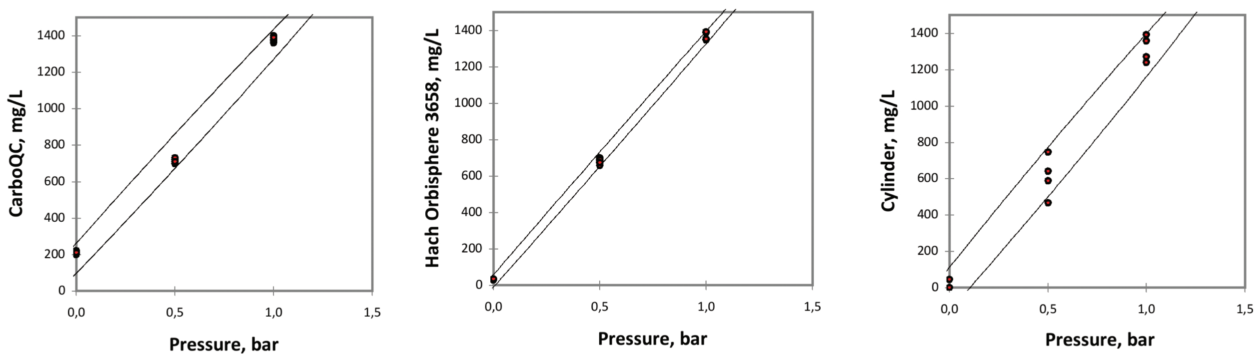


Fig. 13: Correlation of carbon dioxide concentrations (mg/l) measured with CarboQC, Orbisphere 3658 and Agitation Cylinder in range of CO2 pressure from 0 to 1 bar

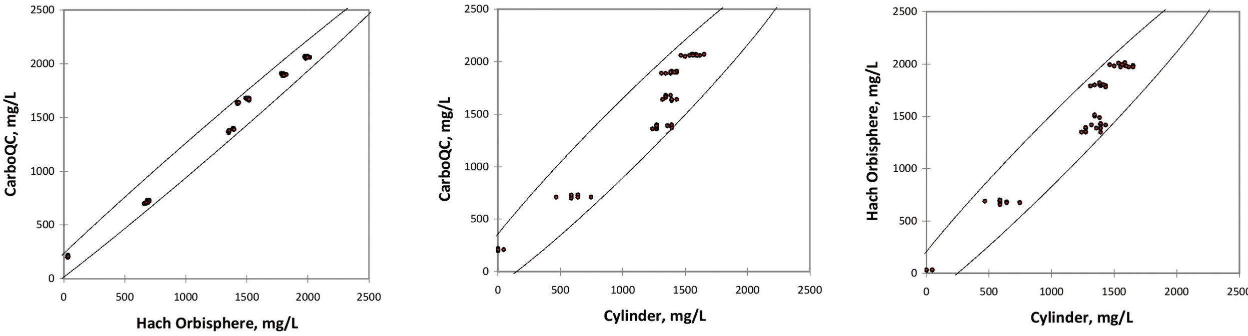


Fig. 14: Correlation of carbon dioxide measurements with CarboQC, Orbisphere 3658 and Agitation Cylinder

so the partial pressure of oxygen is also 20.9 % from the whole pressure in the keg (e.g. 1 bar will give the oxygen pressure of 209 hPa). Measurements obtained with PreSens and Orbisphere 3650 were read in hPa (partial pressure of oxygen) and then calculated in mg/l oxygen. It is not possible to get values in hPa with OxyQC. But as the instrument uses the same measurement principle and formulas as PreSens, measurements can be taken directly in mg/l and be comparable with data obtained from the other two devices.
The results of the oxygen measurement and standard deviation are shown in Table 14. Results obtained with all three instruments are very close. Obviously the results of the oxygen measurement show less deviation compared to the carbon dioxide measurement.
The correlation of the applied pressure in the kegs and the measured oxygen concentration is shown in Fig. 15 (pressure range in the keg from 0 to 2.5 bar) and Fig. 16 (pressure range 0 to 1.0 bar). Similar to the carbon dioxide the simple bottling technique without counter-pressure filler at atmospheric pressure leads to gas loss. OxyQC and PreSens, though having the same measurement principle, have showed slightly different results. The fact, that OxyQC has an isolated measurement chamber could be a possible explanation.
Orbisphere 3650 showed slightly lower values compared to PreSens and OxyQC; this can be explained as calibration inaccuracy or particularity of the method. It is possible, that some amounts of oxygen do not have enough time to diffuse through the membrane and react on the cathode. Due to the limited volume of a bottle, the volume taken for analysis with Orbisphere did not exceed 500 ml, which could be not enough to get a stable value. Or, another explanation could be, that some amount of oxygen is lost during sampling. Nevertheless, all values are very close and Orbisphere 3650 can be considered as a reliable instrument for oxygen measurement.
The saturation concentration of oxygen in wine is close to 8 mg/l. Supersaturated media may lose dissolved gases, when the overpressure is released. This is the reason, why after reaching the saturation value of 8 mg/l the oxygen concentration in sample bottles is no more strongly correlating with compressed air pressure. This can be proved by Table 15 that shows regression coefficients for three instruments in the range from 0 to 2.5 bar pressure and in the range from 0 to 1 bar pressure. As can be seen, regression coefficients in the range of pressure, not exceeding the saturation pressure, are higher. However, the filling method used during the experiment made it possible to reach oxygen concentrations much higher than the saturation level (up to 12 mg/l).
Fig. 17 and Table 16 show the correlation of oxygen measurement results obtained with three instruments. The figure shows, that results from all devices showed good correlation with one another. This is also proved by regression coefficients.

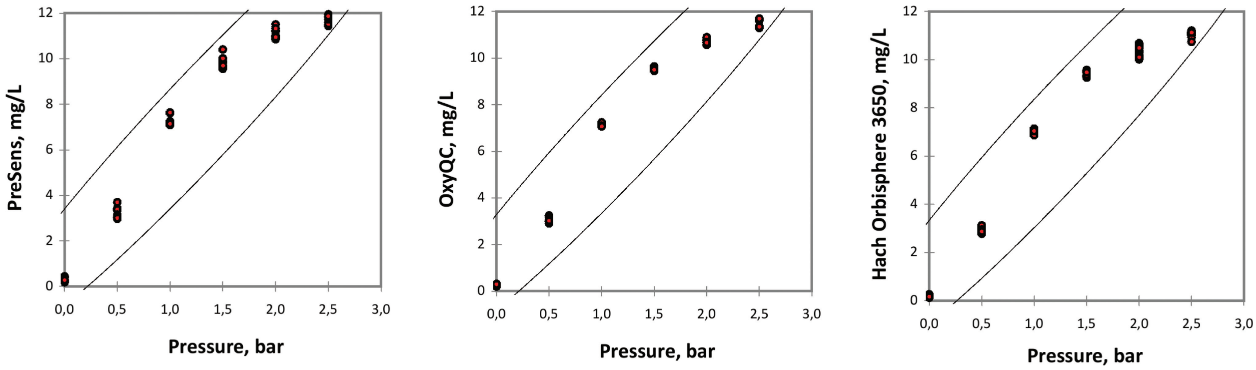


Fig. 15: Correlation of O2 measurements with OxyQC, Orbisphere 3650 and PreSens in range of air pressure from 0 to 2.5 bar

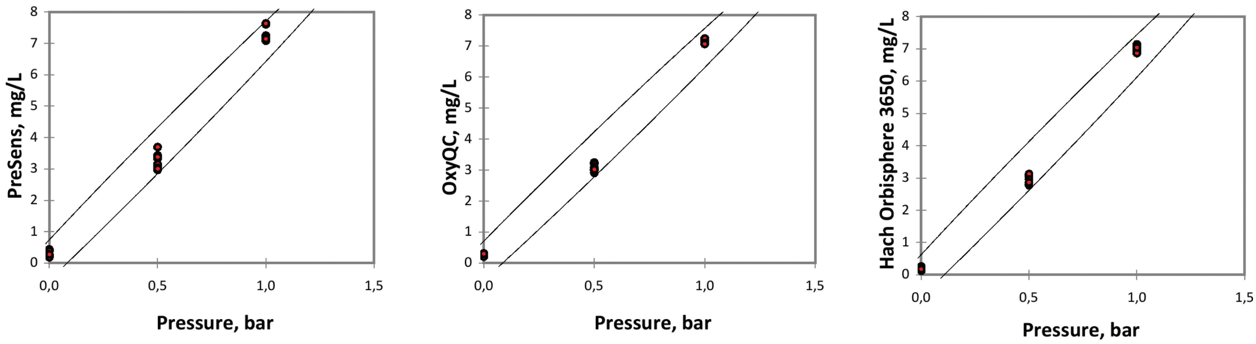


Fig. 16: Correlation of O2 measurements with OxyQC, Orbisphere 3650 and PreSens in range of air pressure from 0 to 1 bar

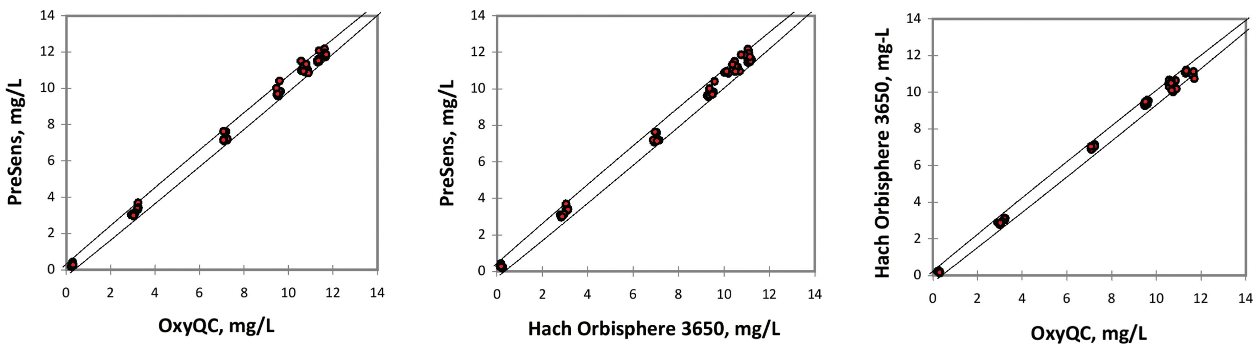


Fig. 17: Correlation of results obtained with PreSens, OxyQC and Orbisphere 3650

Table 14: Average oxygen content (g/l; each value n = 2 × 6 bottles) and standard deviation based on 12 measurements

Air pressure (bar)	Instrument	Average O_2	Standard deviation
0	PreSens	0.28	0.06
	OxyQC	0.28	0.85
	Hach Orbisphere	0.18	1.13
0,5	PreSens	3.24	0.23
	OxyQC	3.12	0.12
	Hach Orbisphere	2.97	0.13
1,0	PreSens	7.25	0.18
	OxyQC	7.15	0.07
	Hach Orbisphere	6.99	0.08
1,5	PreSens	9.81	0.41
	OxyQC	9.54	0.04
	Hach Orbisphere	9.39	0.03
2,0	PreSens	11.07	0.61
	OxyQC	10.71	0.47
	Hach Orbisphere	10.39	0.47
2,5	PreSens	11.74	0.36
	OxyQC	11.49	0.27
	Hach Orbisphere	11.05	0.36

Table 15: Regression coefficients (R^2) for three instruments against compressed air pressure in keg

Interval	PreSens	OxyQC	Orbisphere 3650
0 - 2,5 bar	0.935	0.936	0.926
0 - 1,0 bar	0.989	0.989	0.988

Table 16: Regression coefficients obtained with three instruments against one another

Variables	PreSens	OxyQC	Hach Orbisphere
PreSens	-	0.998	0.997
OxyQC	0.998	-	0.998
Hach Orbisphere	0.997	0.998	-

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