

FIAsTM in Germany:

Determination of sulphur dioxide

Many commercially available red and white wines have too low a content of free sulphur dioxide (SO_2), leading to insufficient protection against oxidation. On the other hand, too high a content of free SO_2 is not desirable either. What's needed is a fast and precise method for the determination of free SO_2 to give optimum protection of wine against oxidative effects.

Different methods for the determination of free and total SO_2 have therefore been evaluated here at the Institute for Wine Analysis and Beverage Research. Emphasis has been on comparison of different methods with the EU reference method. This is a distillation method that is too time-consuming for routine analysis, which means that in Germany fast alternative methods are used instead. This article takes a look at a new system for determination of SO_2 using Flow Injection Analysis (FIA).

The FIAsTM 5000 from FOSS is a well-proven system with components that are also used in the analysis of water, soil and foodstuffs. The system comprises two special modules for the determination of SO_2 [Möller, 2005], allowing simultaneous analysis of free and total sulphur dioxide in about 60 wine samples per hour. The method was developed at ETS Laboratories [G.Burns, I.Herve 2004] in cooperation with FOSS [S.Anderson 2004].

FIA method for determining free SO_2

Please see Figure 1. The samples are injected into an aquatic carrier (C) and merged with a diluted mineral acid (R1). The liberated SO_2 diffuses over a teflon membrane in a gas diffusion cell into a recipient stream (R2), which is reacted with the DNTB reagent (R3) to form a yellow dye that is measured in the detector (D).

Gas diffusion in FIA is a very elegant technique for separation of interferants, allowing sensitive and reliable determination of small amounts of free SO_2 .

FIA method for determining total SO_2

Please see Figure 2. The samples are in-



jected directly into a phosphate buffer (C, R1) and merged with the DTNB reagent (R2). After reaction at 50°C, the resulting yellow dye is diluted and separated from interferants by dialysis. An acceptor stream (R3) carries the product to the flow cell of the detector.

Calibration is carried out using ethanolic solutions of sodium disulphite in the range of 1-50 mg/l for free SO_2 and 5-250 mg/l for total SO_2 . In this concentration range a linear calibration function can be obtained. Samples with higher concentrations can be diluted. The ethanol in the FOSS Application Note can be replaced with methanol. The use of denatured ethanol is to be avoided.

Reference and other methods

For reference and comparison purposes, results for free SO_2 have been compared with the method described in EU Directive EEC

2676/90, and for total SO_2 with the distillation method according to IFU 7a. IFU 7a is a modified version of the EEC 2676/90 method, using stronger acid, which leads to slightly higher values for total SO_2 . In both methods phosphoric acid is used to expel the SO_2 into a receiver solution containing peroxide, for oxidation to sulphate. Subsequent titration with sodium hydroxide allows the calculation of SO_2 content.

Numerous alternative methods have also been compared with the reference methods. These have included direct colorization with DTNB [Berger, 2002], enzymatic and different titrimetric methods, direct iodometric titration using starch for free and total SO_2 , and titration using a double platinum electrode. Reducing substances that can interfere when using the titration methods have been determined separately and taken into account. The double platinum electrode is frequently used for titra-

using Flow Injection Analysis

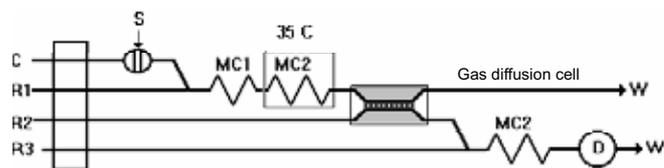
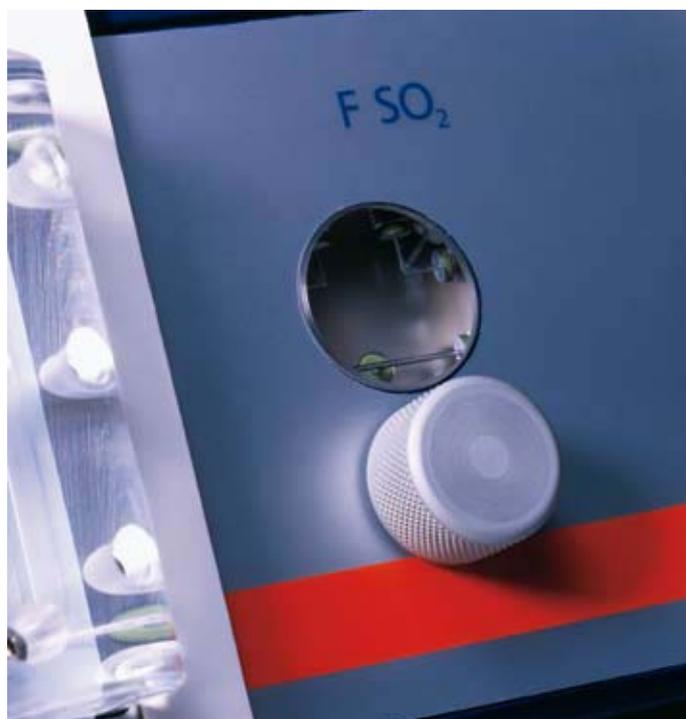


Fig 1: Flow scheme for FIA determination of free SO_2 . C: Carrier (distilled water), R1: 1N HCl, R2: Phosphate buffer, R3: DNTB; MC: Mixing coil, D: Detector, W: Waste, S: Sample [Möller, 2005]

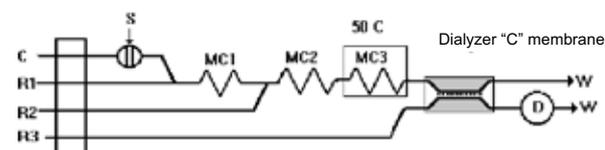


Fig 2: Flow scheme for FIA determination of total SO_2 . C: Carrier (phosphate buffer), MC: Mixing coil, D: Detector, W: Waste, R1: Phosphate buffer, R2: DNTB, R3: Distilled water, S: Sample [Möller, 2005]

tion of red wines, where a visual endpoint is difficult to detect.

Figure 3 gives an overview of the different analytical techniques used in determining SO_2 . Within each group, numerous variations of analytical conditions (temperature, acid, gas, oxidation agent) are possible. Most variegated is determination of total SO_2 where different direct methods as well as chromatographic methods may be applied.

For determining free SO_2 , iodometric titration is the routine method most frequently used, although the influences of interfering substances like ascorbic acid and phenols may be substantial. Even after correction for separately determined reducing substances, a significant difference compared to the reference method may remain.

To avoid artefacts from other volatile substances when using the reference method, the interference effect of different substances, including acetic acid (up to 20 g/l), acetaldehyde and sugar, was investigated.

No interference reaction in the receiver solution could be found for any of these substances. Due to the nitrogen carrier (gas), only easily released gases like CO_2 and SO_2 are transferred to the receiver solution, and not acetic acid and other substances that might be released by hot water steam distillation. More selective determination of the sulphate in the receiver solution, for example by gravimetry, nephelometry or ion chromatography, is also possible.

Generally, the reference method does not define 'true' values for sulphur dioxide but is a consensus method that is capable of producing comparable values if instructions are strictly followed. Legal limits for SO_2 are based on values obtained using the reference method and not on 'true' content. Investigations using a hyphenated HPLC – Biosensor system have shown that the actual content of total SO_2 can be as much as 33 per cent higher than that determined by the reference method [Patz and Galensa *et al.*, 1997]. In any case, in terms of legal limits, values obtained using the EEC

2676/90 method are binding.

FIA v EEC 2676/90

In the following, results obtained using the reference method are compared with those obtained using the FIA method. Different international red and white wines available in German retail outlets were analyzed.

Free sulphur dioxide

Figures 4 and 5 show the results for determination of free SO_2 in red and white wines using the FOSS FIAstar 5000 and the EEC 2676/90 method. The white wines covered a range of 0-37 mg/l free SO_2 and the red wines 5-41 mg/l. All samples were measured directly and without any dilution. The FIA method proved to be very suitable for these determinations.

The intercept of the regression lines for both methods is a little higher for red wines (+ 2,6 mg/l) than for white wines (+ 0,7 mg/l). This means that, on average, the FIA method produces slightly lower values for red wines than the EEC

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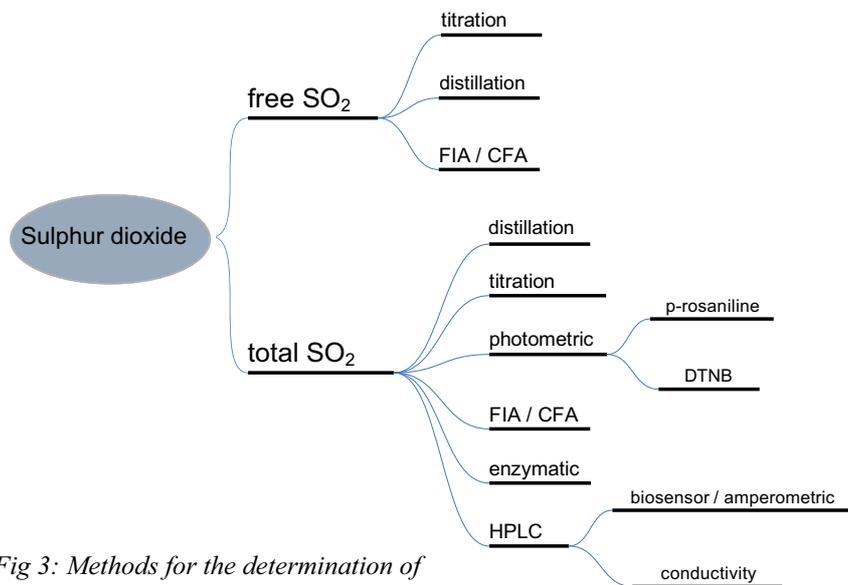


Fig 3: Methods for the determination of sulphur dioxide in wine

		Bias [mg/l] SO ₂	SD of differences [mg/l] SO ₂	R ²
Free SO ₂	White wine	0,7	2,6	0,915
	Red wine	1,4	2,7	0,898
Total SO ₂	White wine	3,2	4,4	0,986
	Red wine	6,2	9,9	0,909

Table 1: Bias and standard deviation (SD) of difference between reference method and FIA method for determination of free and total SO₂ in red and white wines

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2676/90 method. When compared with the titrimetric methods used in routine analysis, the FIA method generates intrinsically more reliable and more precise results. The number of deviating results due to the composition of the wine (phenols and ascorbic acid) is highest for the titration methods.

The FIA method is excellent for fast and reliable determination of free SO₂. The reference method demands an analysis time of at least 15 min for a single determination, and the difference between duplicates is substantially higher than with the FIA method. With FIA, duplicate determinations can be performed within 2 min, generating simultaneous results for both free and total SO₂. Exact and reliable determination of free SO₂ is absolutely necessary, particularly for process control and during bottling.

During the Third Workshop on Wine Analysis, held in Germany in 2005, it was assessed that many white wines distributed in Germany had levels of free SO₂ that were too low to provide sufficient protection against oxidation. However, increased

use of L-ascorbic acid in UTA prevention makes a minimum of SO₂ necessary, as the degradation of the ascorbic acid results in peroxide, a strong oxidation reagent. Reliable and correct determination of free SO₂ is therefore a necessity.

NOTE: UTA is an unwelcome aging off-flavour that is predominantly found in young white wines. This off-flavour stems mainly from 4-amino-aceto-phenone, which is formed during storage. Wines smell 'like acacia flowers'. This off-flavour is a major problem because a lot of white wines are no longer drinkable after storage of no more than one or two years.

Total sulphur dioxide

Figures 6 and 7 show results for white wines (range 70-250 mg/l) and for reds (range 38-195 mg/l). Bias and standard deviations for red wines are clearly higher than for whites.

The mean bias between the FIA method and the reference method is 3 mg/l for white wines, and the standard deviation of difference (SD) is 4,4 mg/l. For red wines, the mean bias is 6 mg/l and the standard

deviation of difference (SD) is 10 mg/l. When compared with the direct titration method, FIA shows clearly better agreement with the reference method and significantly better repeatability.

The FIA method is therefore more reliable than the direct titration method, especially for higher sample throughputs. This should be emphasized here, as the titration method is approved for AP analysis.

NOTE: All German quality wines are awarded an official Approval Number (AP Number). For an AP Number to be awarded, the wine has to be examined by chemical and sensory analysis.

Table 1 gives a compilation of the results. When compared with the reference method, the FIA method shows systematically lower values for red wines, especially for total SO₂ (see Fig. 7).

When compared with the titration method, the FIA method is not sensitive to interferences e.g. from ascorbic acid, acetaldehyde, phenols or other reducing compounds. Interferences from residual sugars are very low, and can be disregarded up to a sugar concentration of about 100g/l.

For routine control of red wines, the negative bias of the FIA method for total SO₂ can be accounted for by applying the reference method before reaching legal limits for total SO₂ content.

Discussion

FIA is a fast and reliable method for determination of free and total sulphur dioxide in wine. The advantage of the method lies in the relatively high sample throughput of about 60 samples per hour, which makes it the method of choice if 30 or more determinations are made per day. Thanks to the speed of analysis, it makes sense to analyze several samples consecutively as against, say, one sample every 30 min. Results are comparable with those obtained using the reference method, especially in relation to results obtained using other approved methods. For total SO₂ in red wines, the FIA results are systematically about 10-15 mg/l lower than those obtained using the reference method. Compared with other fast methods, FIA produces results closest to the reference method. For single samples, the titration methods in use always produce outliers when compared with the reference method.

Reliable determination of free SO₂ content is only possible with FIA or the reference method. For fast and precise determination of free SO₂ before and after bottling, FIA is the most reliable method in terms of

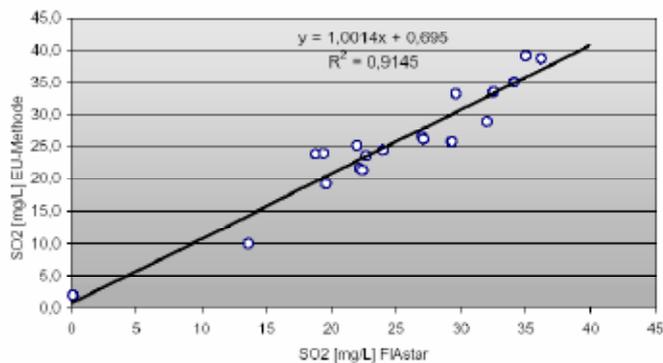


Fig 4: Free sulphur dioxide in white wines: FIAstar™ v EEC 2676/90

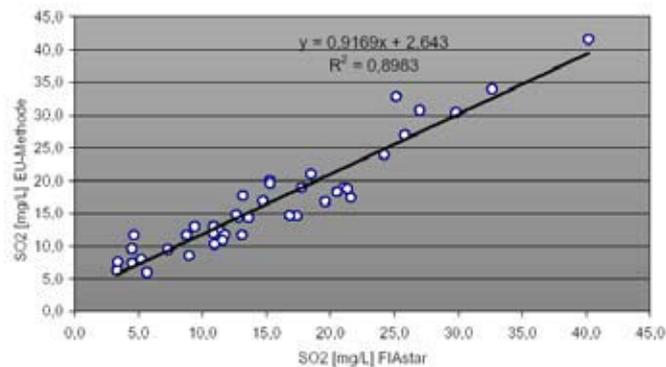


Fig 5: Free sulphur dioxide in red wines: FIAstar™ v EEC 2676/90

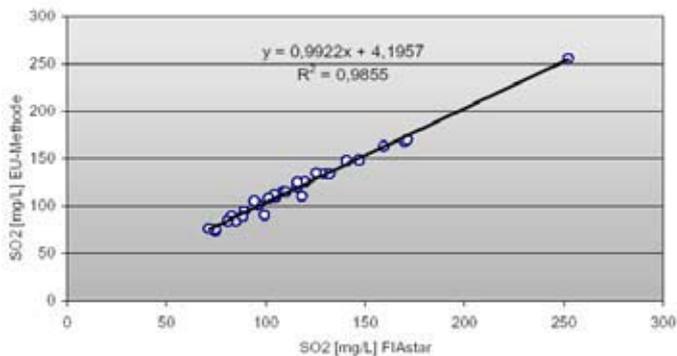


Fig 6: Total sulphur dioxide in white wines: FIAstar™ v EEC 2676/90

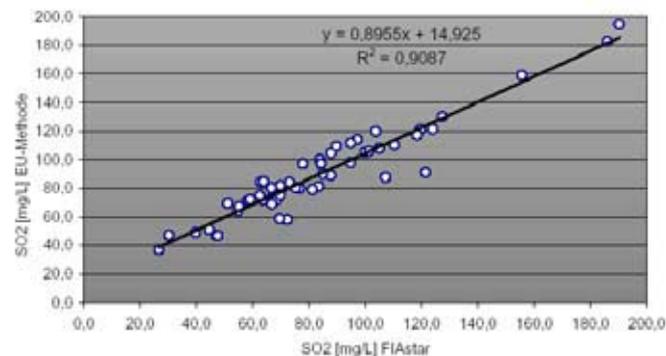


Fig 7: Total sulphur dioxide in red wines: FIAstar™ v EEC 2676/90

accuracy as well as repeatability.

For total sulfur dioxide, the same is valid as for any other fast method: they are all suitable for fast screening, and should be validated by the reference method when close to limit values. When compared with titration methods, FIA is not, or is much less, prone to interferences from ascorbic acid, phenols, sugars and acetaldehyde.

Conclusions

FIA is a fast and reliable method that permits high sample throughputs. Daily calibration using freshly prepared standards, and validation using a quality control sample, are part of GLP and should be applied to all analyses.

For production control purposes, the

method delivers very reliable results for free SO₂, which is of high practical value. For determination of total SO₂, especially in red wines, the method may show lower results than those obtained using the reference method. However, this bias can be corrected.

The FIA method is considerably less prone to interferences and errors than titration methods. Accurate control of actual content of free SO₂ is a necessity, and here FIA is the method of choice.

During 2006 the FIA method was compared with the official reference method in a major ring test in Germany. Satisfying results were obtained. Approval for the purposes of German AP analysis is therefore only a question of time.

References

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- IFU methods can be found under: <http://www.ifu-fruitjuice.com/>
- The authors can be contacted at patz@fa.gm.de



FIAstar™ method allows simultaneous analysis of free and total sulphur dioxide in wine

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