Application Note AN-S-396

用离子色谱评估葡萄酒质量

Organic acid analysis using suppressed conductivity detection

Nature and concentration of organic acids are important parameters in enology. They affect organoleptic properties (color, flavor, and aroma), wine stability, and help to track alteration processes and the wine's authenticity [1]. Tartaric acid and malic acid represent the greatest fraction of organic acids in wine, coming from the grapes themselves. Free tartaric acid decreases during wine storage when it binds to other components and precipitates, and malic acid can metabolize to lactic acid. Other organic acids are formed as products during alcoholic fermentation [1] influencing flavor. Acetic acid for example causes an undesirable vinegar taste. Overall, monitoring of organic acids is crucial to improve flavor and quality, and to fulfill universal standardized criteria such as the International Code of Oenological Practices [2]. Analytically, organic acids can be properly determined with ion chromatography (IC) and suppressed conductivity detection. As a multicomponent method, inorganic acids can also be resolved which are also valuable tracers for wine quality and taste. This Application Note presents two IC methods for wine quality analysis: a fast isocratic screening method of major organic acids and anions including sulfite, and a complex monitoring method with a binary gradient to separate 15 organic acids. Inline Ultrafiltration was used for economical sample treatment.

SAMPLE AND SAMPLE PREPARATION

Samples of red and white wine were diluted (10–50-fold) in ultrapure water (UPW). To minimize oxidation, vials were capped with polyester lids. Using Inline Ultrafiltration, samples were automatically filtered through a 0.22 µm (regenerated cellulose) membrane prior to injection.

EXPERIMENTAL

Since all organic acids easily ionize, their conjugate bases can be analyzed by ion chromatography with suppressed conductivity detection.

For **fast screening analysis** the chromatographic separation was performed on A Metrosep A Supp 10 column with isocratic elution (**Figure 1**). Within less than 20 minutes the organic acids acetate, malate, tartrate, oxalate and the anions chloride, phosphate, sulfite, and sulfate are separated. Sulfite was calibrated separately to avoid a potential sulfate contamination. It was stabilized with 2-propanol (2% in working standard solutions). Although sulfite can be determined within in this multi-component run, for dedicated sulfite analysis please refer to the described methods [3].

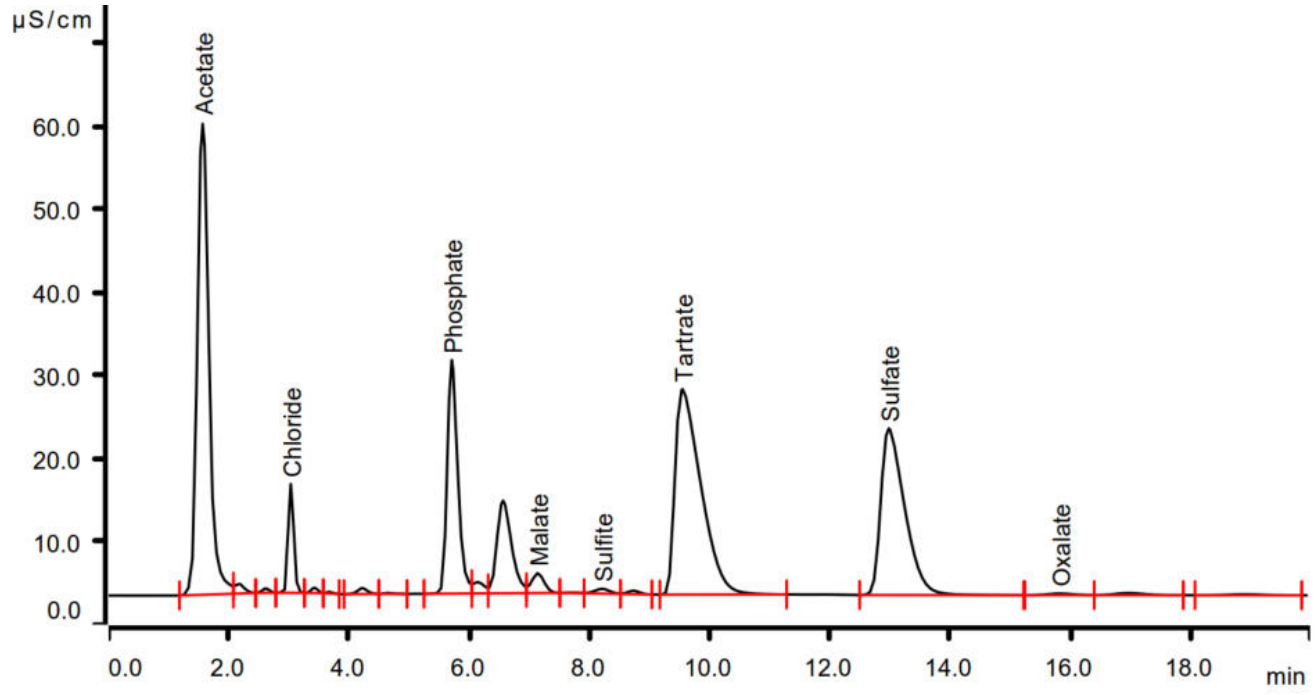

Figure 1. Fast screening analysis of major organic acids (acetate (not quantified), malate (105 mg/L), tartrate (1534 mg/L) and oxalate (<10 mg/L)) and major anions (chloride (22 mg/L), phosphate (818 mg/L), sulfite (29 mg/L), and sulfate (367 mg/L)) in a white wine sample (injection volume 20 µL) Isocratic elution was performed on a Metrosep A Supp 10 - 100/4.0 column using a carbonate eluent. (5.0 mmol/L Na2CO3 + 5.0 mmol/L NaHCO3 + 5 µmol/L HClO4, flow rate 1 mL/min, column temperature 35 ° C). Suppressed conductivity detection enables detection with a low background for detection in the lower mg/L range.

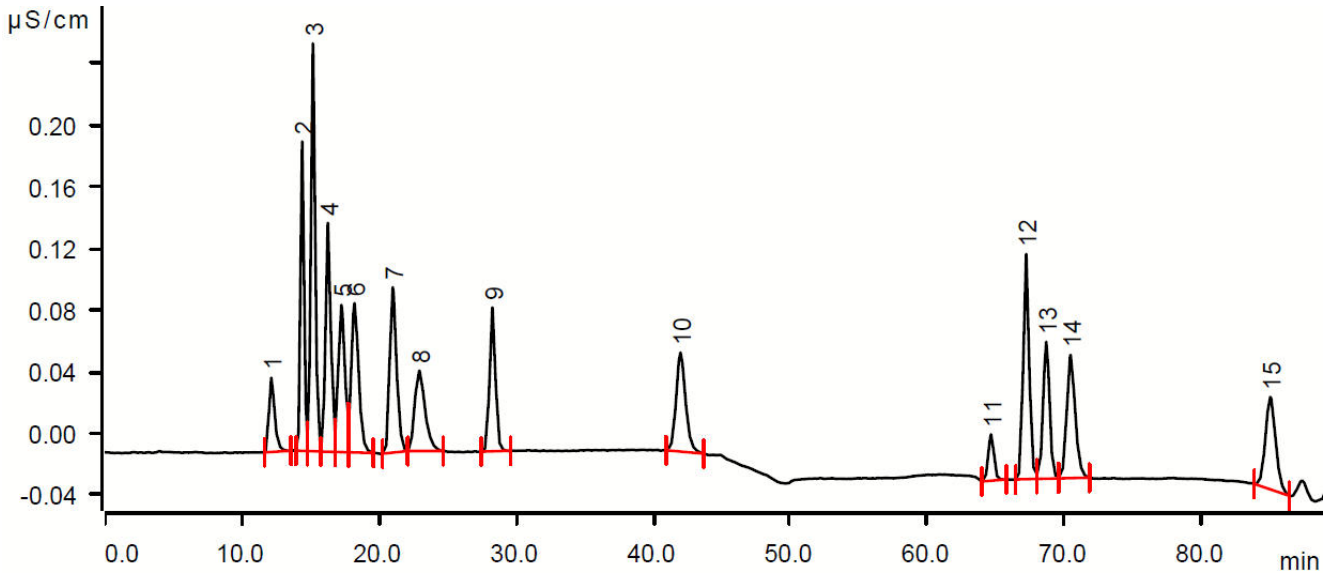

Figure 2. The figure shows the suppressed conductivity signal for the complex organic acid monitoring of gluconate (1), lactate (2), acetate (3), propionate (4), iso-butyrate (5), butyrate (6), methacrylate (7), valerate (8), methyl sulfate (9), dichloroacetate (10), malonate (11), malate (12), glutarate (13), adipate (14), and phthalate (15) in a 1 mg/L mixed standard (injection volume 20 µL). Separation was on a Metrosep A Supp 7 - 250/4.0 column with a binary gradient (eluent A: ultrapure water, eluent B: 6.4 mmol/L Na2CO3 + 2.0 mmol/L NaHCO3, flow rate 0.7 mL/min, column temperature 45 ° C).

A comprehensive view of the organic acid composition for **complex monitoring** can be obtained by separation with a Metrosep A Supp 7 column using a binary gradient (**Figure 2**). With the carbonate-UPW gradient the following 15 organic acids could be resolved: gluconate, lactate, acetate, propionate, isobutyrate, butyrate, methacrylate, valerate, methyl sulfate, dichloroacetate, malonate, malate, glutarate, adipate, and phthalate.

The experimental setup is shown in **Figure 3**.

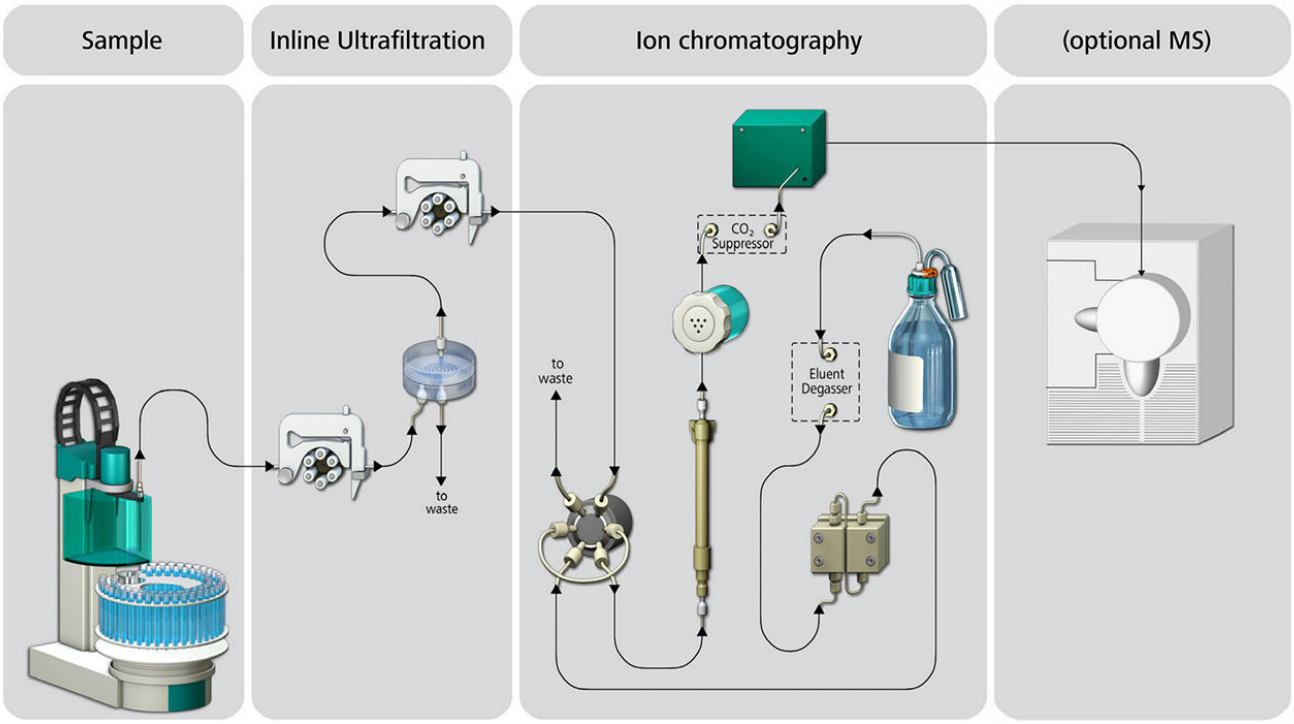

Figure 3. Schematic flow path for fast screening analysis of organic acids and anions with ion chromatography and suppressed conductivity detection. As sample preparation step, Inline Ultrafiltration is used to optimize the overall analysis in terms of laboratory time and laboratory expenses. After introduction of the sample (858 Professional Sample Processor) the sample passes the Ultrafiltration cell. The samples are filtered with a 0.2 µm regenerated cellulose membrane. Up to 100 samples depending on the matrix can be analyzed before changing the membrane and with less than 0.1% carryover speeding up this unavoidable process in routine analysis. After injection and separation with a high capacity anion column sequential suppression removes cations and carbonate resulting in a very low background signal in the conductivity detector. For the complex organic acid monitoring a second high pressure pump and a mixing capillary need to be added to the system. Connection of the outlet of the conductivity detector to a mass spectrometer can be a valuable addition for peak confirmation and even better detection limits.

RESULTS

The **fast screening analysis** of organic acids and anions took less than 20 minutes. Tartrate was the major organic acid in both samples and phosphate and sulfate the dominant anions, with slightly lower contents in white wine for tartrate and sulfate (**Table 1**). Triplicate injections showed a relative standard deviation of less than 2% for both the white wine and the red wine (**Table 1**).

Table 1. Organic acids and anions quantified in a red and white wine sample. Sample dilution was performed in UPW with a dilution factor of 10 (50 for tartrate). Samples were analyzed with the fast screening analysis resolving major organic acids and anions in wine samples.

Analyte	Red wine (mg/L) (RSD)	White wine (mg/L) (RSD)
Chloride	60 (0.03%)	22 (0.04%)
Phosphate	771 (0.2%)	818 (0.1%)
Malate	92 (0.1%)	105 (0.2%)
Sulfite	27 (2%)	29 (0.4%)
Tartrate	1756 (0.1%)	1534 (0.6%)
Sulfate	553 (0.01%)	367 (0.01%)
Oxalate	<10	<10

A gradient elution improved peak resolution for a complex monitoring analysis of 15 organic acids. Suppressed conductivity detection enabled a sensitive detection in a working range of 0.1 to 5 mg/L.

Both methods show excellent performance in the lower mg/L range. Detection of the suppressed conductivity signal omits interferences from UV-active components seen with UV-detection. Sample preparation with **Inline Ultrafiltration** makes this unavoidable (generally manual) step both time and cost effective while also guaranteeing column protection.

CONCLUSION

Ionic composition profiles in wines are readily quantified with IC and conductivity detection. Ion exchange chromatography allows the simultaneous determination of inorganic anions and organic acids in one run, in contrast to ion-exclusion which only separates organic acids. With the **fast multi-component screening analysis** sample throughput in laboratories can be maximized. Sample preparation can be facilitated with Inline Ultrafiltration, protecting the column and enhancing instrument performance. Further increase of the economic potential can be achieved by combination with Metrohm Inline Dilution including the possibility of automatic calibration. The manual error-prone dilution step of samples and standards is omitted while laboratory time is saved, and accuracy and precision improved.

The **complex organic acid monitoring** with suppressed conductivity detection benefits from higher sensitivity compared to UV-detection methods and reduced interferences from UV-active sugars and phenols in such wine samples.

If peak identity needs confirmation, or very low detection limits are required, the IC setup can be combined with a sensitive mass specific detector (**Figure 3**).

REFERENCES

1. Waterhouse et al. (2016), John Wiley & Sons, UK, ISBN 1118627806
2. International Organization of Vine and Wine (OIV) (2021), OIV, France, ISBN 978-2-85038-030-3
3. Metrohm, WP-065 Simplified sulfite determination in foods and beverages using ion chromatography

Internal references: AW IC US6-0249-062017; AW IC CH6-1266-012016

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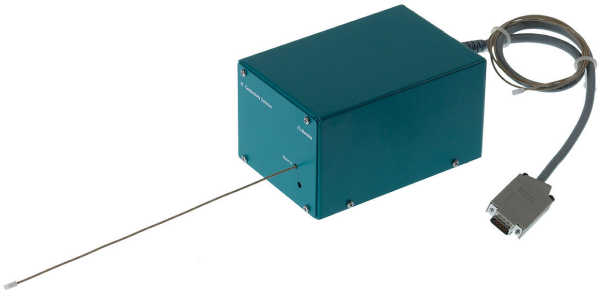
CONFIGURATION

940 Professional IC Vario ONE/SeS/HPG

940 Professional IC Vario ONE/SeS/HPG 是智能型离子色谱仪器,带有**序列抑制**和**二元高压梯度**。可使用 942 Extension Modul 将其扩展至四元梯度系统。可使用一台 800 Dosino 用于抑制器再生。该仪器可使用各种分离和检测方法。

典型的应用范围:

- 用于阴离子或阳离子测定的梯度应用,序列抑制法



IC Conductivity Detector

用于智能型离子色谱仪的紧凑智能型高性能电导检测器。不凡的温度稳定性,受保护的检测器端子板内的整个信号处理过程以及新一代的 DSP(数字式信号处理)均能确保测量的准确性。归功于动态工作范围,无需进行范围变更(也不是自动进行)。

Metrosep A Supp 7 - 250/4.0

水处理中的副产物(消毒副产物)不仅可能危害健康,甚至可能致癌。因此羰基卤化物成为许多检测方法及标准的目标(例如 EPA 300.1 方法 B 部分、EPA 方法 317.0、EPA 方法 326.0)。首先针对那些饮用水臭氧化过程中由溴化物中产生的溴酸盐。Metrosep A Supp 7 - 250/4.0 是可以同时测定标准阴离子、羰基卤化物和二氯乙酸的高效分离柱。借助此柱甚至在低 µg/L 范围内也可准确可靠地测定这些离子。使用 5 µm 的聚乙烯醇聚合物使其达到高度指示灵敏度,其分离度高,由此实现优秀的分离性能和指示灵敏度。此外,还可通过更改温度来针对特殊应用要求调整分离工作。

858 Professional Sample Processor – Pump

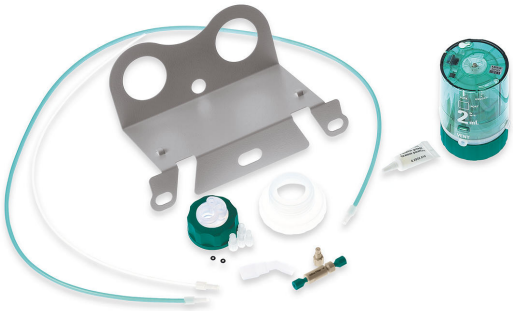
858 Professional Sample Processor – Pump 可处理体积在 500 µL 至 500 mL 之间的样品。进行样品转移时,既可以使用内置的双向双通道蠕动泵、也可通过 800 Dosino 来进行。

800 Dosino

800 Dosino 驱动头,带有可用于智能型加液单元的读/写硬件设备。带固定电缆。

MSM-HC A

适用于所有带 MSM-HC(高容量万通抑制器模块)的离子色谱仪的抑制器马达



IC Dosino

用于安装 Dosino 以实现万通抑制模块(MSM)自动再生的附件组。