



Application Note AN-S-396

Assessing wine quality with IC

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, 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].

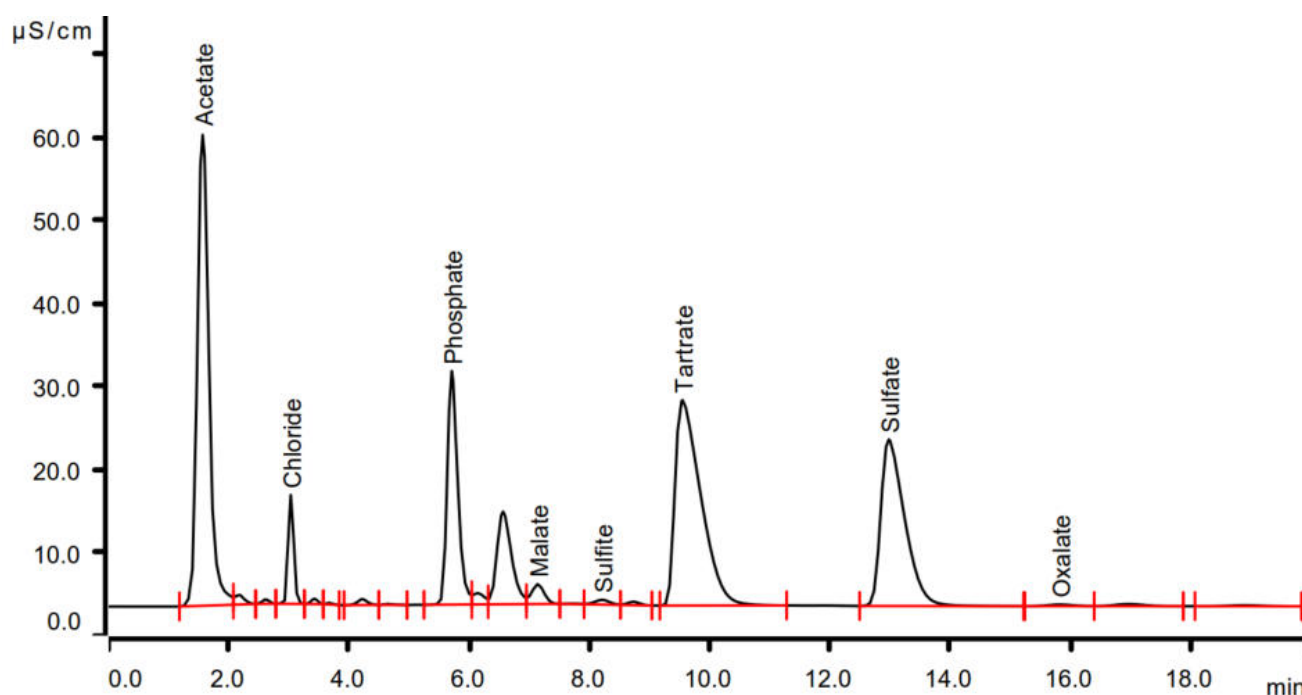


Figure 1. Fast screening analysis of major organic acids (acetate (not quantified), malate (105 mg/L), tartrate (1534 mg/L) and oxalate (<10 mg/L)) and major anions (chloride (22 mg/L), phosphate (818 mg/L), sulfite (29 mg/L), and sulfate (367 mg/L)) in a white wine sample (injection volume 20 µL). Isocratic 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.

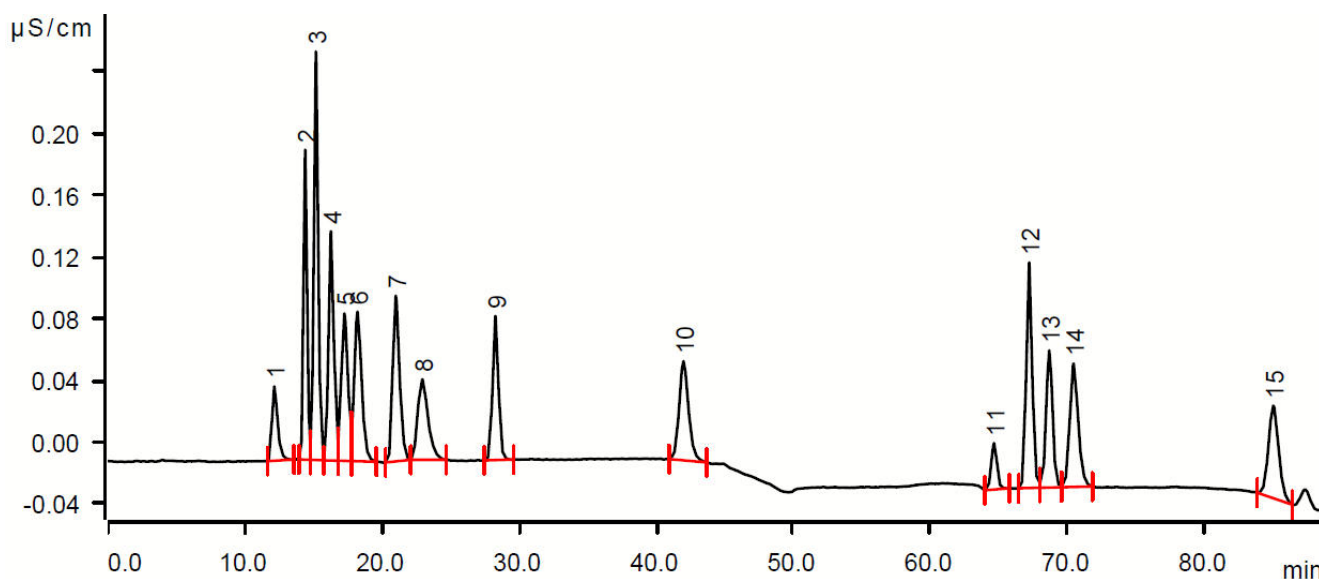


Figure 2. The figure shows the suppressed conductivity signal for the complex organic acid monitoring of gluconate (1), lactate (2), acetate (3), propionate (4), iso-butyrate (5), butyrate (6), methacrylate (7), valerate (8), methyl sulfate (9), dichloroacetate (10), malonate (11), malate (12), glutarate (13), adipate (14), and phthalate (15) in a 1 mg/L mixed standard (injection volume 20 μ L). 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 Na_2CO_3 + 2.0 mmol/L NaHCO_3 , flow rate 0.7 mL/min, column temperature 45 $^\circ\text{C}$).

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**.

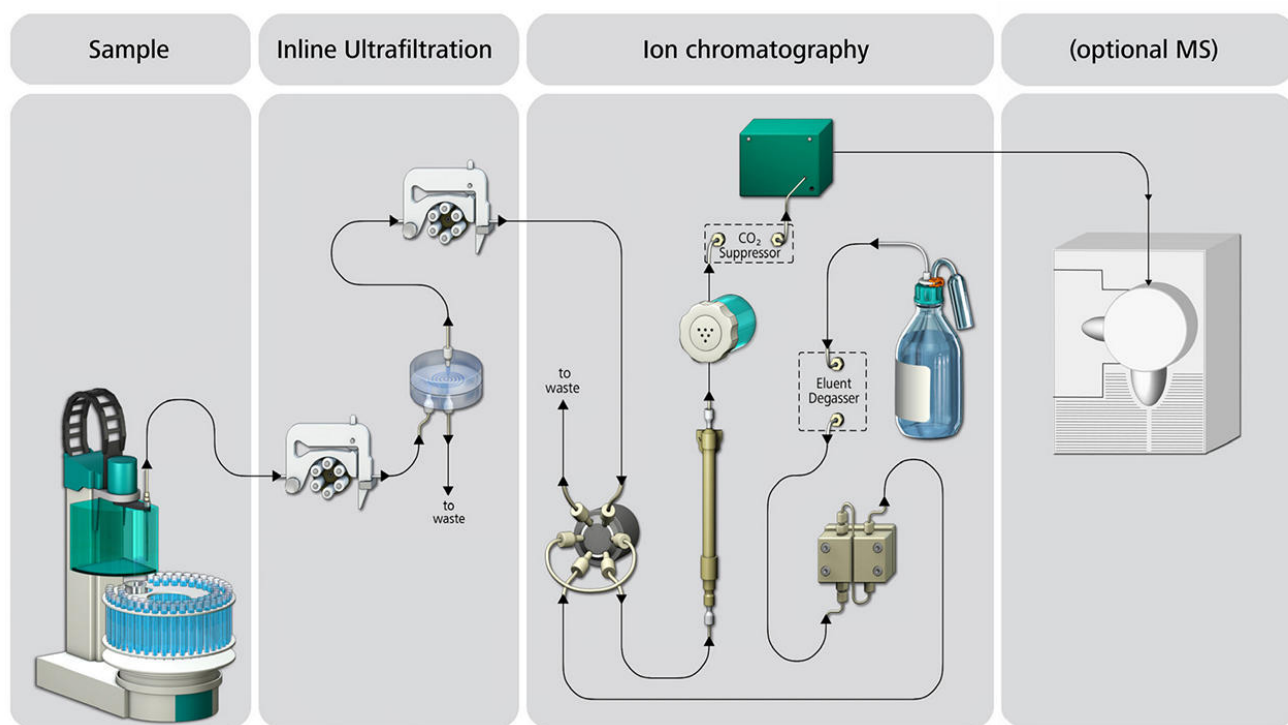


Figure 3. Schematic flow path for fast screening analysis of organic acids and anions with ion chromatography and suppressed conductivity detection. As sample preparation step, Inline Ultrafiltration is used to optimize the overall analysis in terms of laboratory time and laboratory expenses. After introduction 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

CONTACT

Metrohm France
13, avenue du Québec - CS
90038
91978 VILLEBON
COURTABOEUF CEDEX

info@metrohm.fr

CONFIGURATION

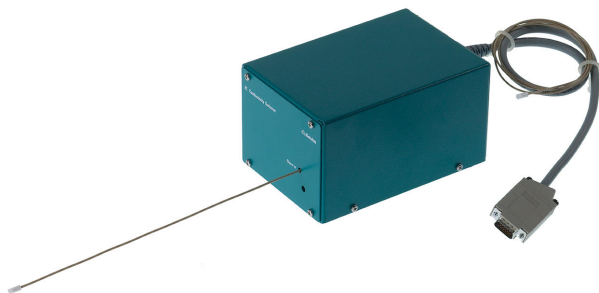


940 Professional IC Vario ONE/SeS/HPG

Le 940 Professional IC Vario ONE/SeS/HPG est l'appareil CI intelligent avec **suppression séquentielle** et **gradient haute pression binaire**. Il peut être développé avec le 942 Extension Module jusqu'au niveau d'un système gradient quaternaire. On peut utiliser un 800 Dosino pour la régénération du supprimeur. L'appareil peut être utilisé avec n'importe quelles méthodes de séparation et de détection.

Domaines d'application typiques :

- Applications en gradient pour les déterminations d'anions ou de cations avec suppression séquentielle



IC Conductivity Detector

Détecteur de conductivité haute performance compact et intelligent destiné aux appareils CI intelligents. Excellente constance de la température, tout le traitement du signal au sein du bloc de détecteur protégé et DSP – Digital Signal Processing – de la dernière génération garantissent une précision de mesure optimale. Grâce à la plage de travail dynamique, aucun changement de plage n'est nécessaire (même automatique).



Metrosep A Supp 7 - 250/4.0

Les produits secondaires du traitement de l'eau (disinfection by-products) sont soupçonnés d'être non seulement nocifs pour la santé, mais aussi cancérigènes. C'est la raison pour laquelle les oxohalogénures sont devenus l'objet de nombreuses recherches et de normes (p. ex. EPA 300.1 partie B, EPA 317.0, EPA 326.0). Il s'agit en premier lieu du bromate, produit du bromure lors de l'ozonisation de l'eau potable. La colonne Metrosep A Supp 7 - 250/4.0 est une colonne de séparation haute performance pour la détermination en parallèle des anions standard, des oxyhalogénures et de l'acide dichloracétique. Avec cette colonne, ces ions peuvent être déterminés jusque dans la gamme inférieure des $\mu\text{g/L}$ de manière sûre et précise. La sensibilité de détection élevée est atteinte par l'utilisation d'un polymère à base d'alcool polyvinylique de 5 μm , permettant d'obtenir un nombre de plateaux très élevé et, par conséquent, d'excellentes propriétés de séparation et de détection. En plus, par modification de la température, il est possible d'adapter la séparation aux exigences spécifiques de l'application.



858 Professional Sample Processor – Pump

Le 858 Professional Sample Processor – Pump traite des échantillons de 500 μL à 500 mL. Le transfert des échantillons s'opère soit au moyen de la pompe péristaltique bidirectionnelle à deux voies intégrée soit par un 800 Dosino.



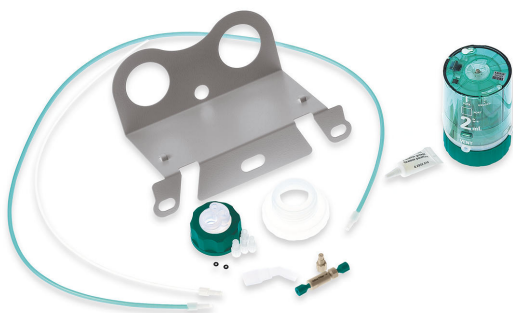
800 Dosino

Le Dosino 800 est un moteur de burette avec système de lecture/écriture pour les Unités de distribution intelligentes. Avec câble attachant (150 cm).



Rotor MSM-HC A

Rotor de supprimeur pour tous les instruments CI avec MSM-HC (Metrohm Suppressor Module à haute capacité)



Équipement CI : Dosino, régénération

Jeu d'accessoires pour le montage d'un Dosino pour la régénération automatique du Metrohm Suppressor Module (MSM).