Application Note AN-S-396

イオンクロマトクラフを使用したワイン品質の評価試験

Organic acid analysis using suppressed conductivity detection

ワインの品質評価において、イオンクロマトクラフィ(IC)は重要な分析手法の1つてす。ICを使用することて、ワイン中のさまさまな成分を定量し、その品質に関する重要な情報を得ることかてきます。ワインに含まれる有機酸の性質と濃度は、ワインの感覚特性(色、味、香り)、ワインの安定性、変質フロセスの追跡、ワインの正当性に影響を与えます[1]。ワイン中の有機酸の最大の割合は、フトウ自体から来る酒石酸とリンコ酸てす。遊離酒石酸は他の成分と結合し沈殿するため、ワインの貯蔵中に減少し、リンコ酸は乳酸に代謝する可能性かあります。他の有機酸は、アルコール発酵中に生成される生成物てす[1]。例えは、酢酸は望ましくない酢の味を引き起こします。全体的に、有機酸のモニタリンクは味と品質を向上させ、国際醸造慣行コートなとの普遍的な標準基準を満たすために重要てす[2]。分析的には、サフレッサと電気伝導度検出器を搭載したイオンクロマトクラフィ(IC)を使用して有機酸を適切に定量することかてきます。多成分法として、ワインの品質と味に貢献する無機酸も分解てきます。この技術資料ては、ワイン品質分析のための2種類のIC分析を紹介します。主要な有機酸および亜硫酸イオンを含む同一濃度法の高速スクリーニンク法と、15種類の有機酸を分離するための二元勾配法を用いた複雑なモニタリンク法てす。自動サンフル前処理のためにインラインウルトラフィルトレーションを使用しています。

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 m (regenerated cellulose) membrane prior to injection.

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]**.

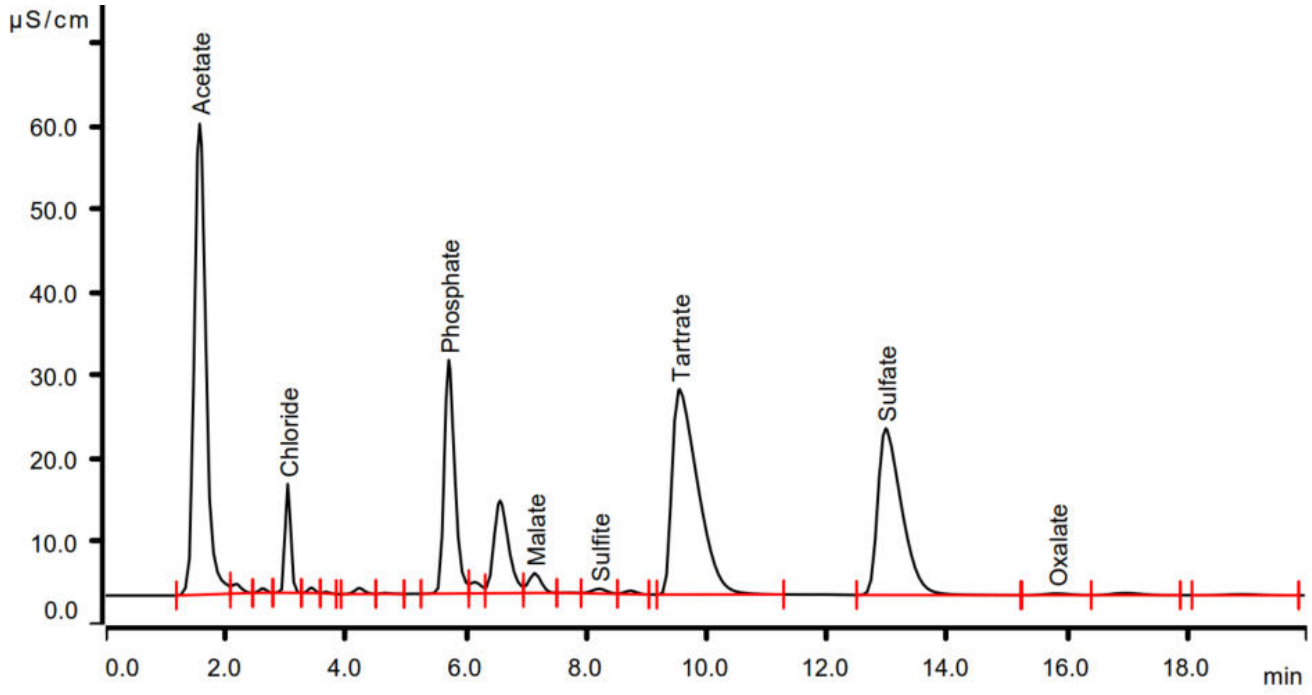

Figure 1. Fast screening analysis of major organic acids (acetate (not quantified), malate (105 mg/L), tartrate (1534 mg/L) and oxalate (<10 mg/L)) and major anions (chloride (22 mg/L), phosphate (818 mg/L), sulfite (29 mg/L), and sulfate (367 mg/L)) in a white wine sample (injection volume 20 µL) Isocratic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

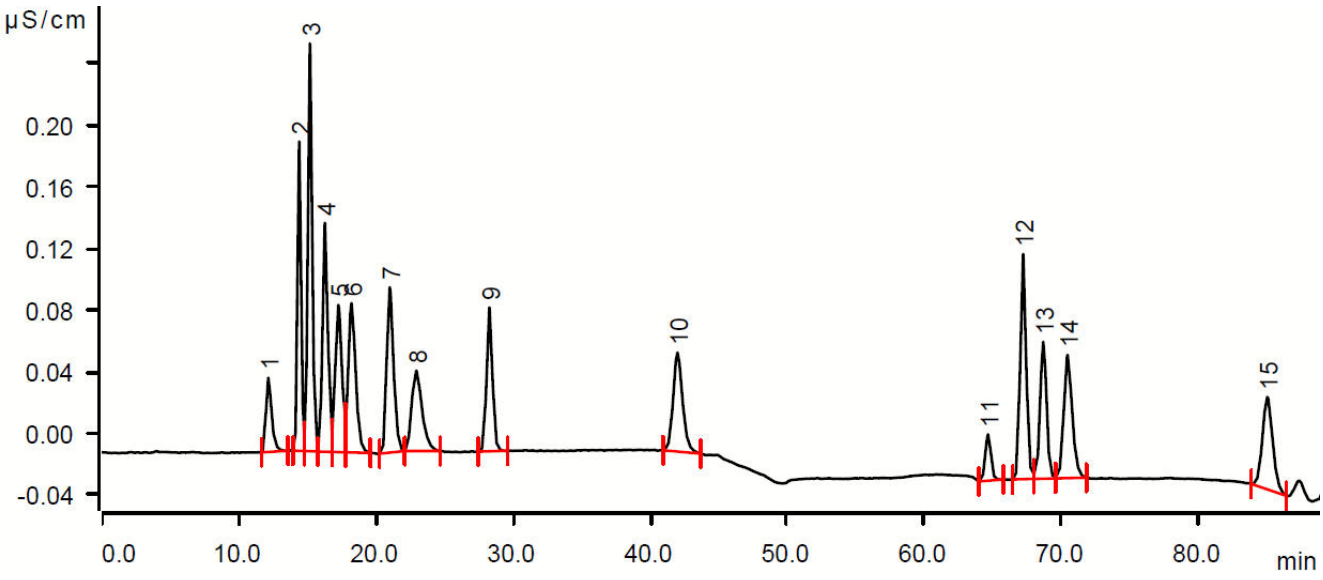

Figure 2. The figure shows the suppressed conductivity signal for the complex organic acid monitoring of gluconate (1), lactate (2), acetate (3), propionate (4), iso-butyrate (5), butyrate (6), methacrylate (7), valerate (8), methyl sulfate (9), dichloroacetate (10), malonate (11), malate (12), glutarate (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**.

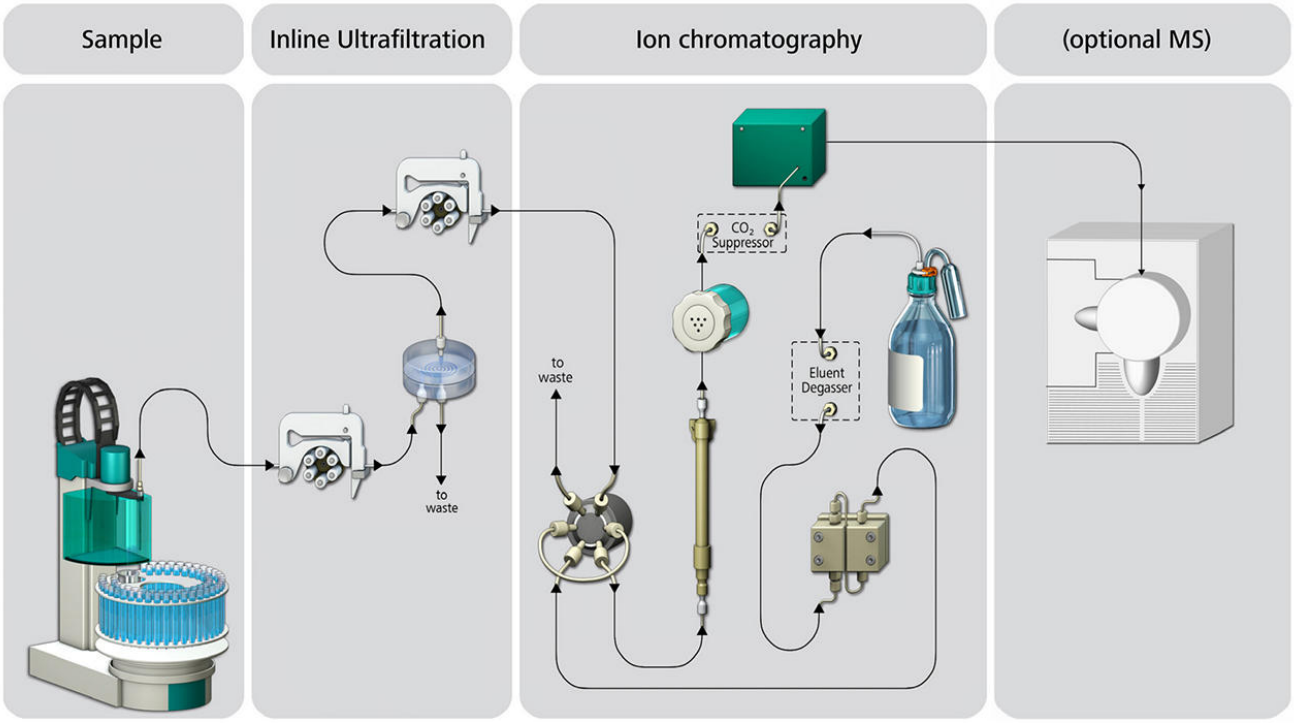

Figure 3. Schematic flow path for fast screening analysis of organic acids and anions with ion chromatography and suppressed conductivity detection. As sample preparation step, Inline Ultrafiltration is used to optimize the overall analysis in terms of laboratory time and laboratory expenses. After introduction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

RESULTS

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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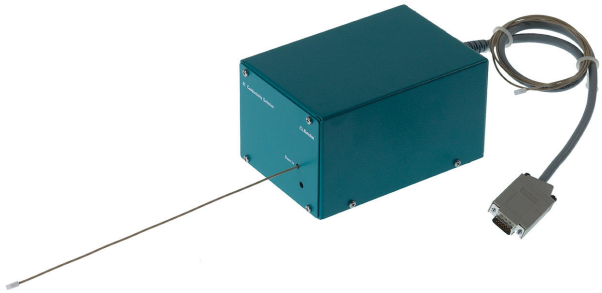
CONFIGURATION

940 Professional IC Vario ONE/SeS/HPG

940 フロフェッショナル IC Vario ONE/SeS/HPG は**連続サフレッション**と**ハイナリー高圧クラシエント**を備えたインテリシェントコンハクトIC装置てす。942 拡張モシュール を使用することてクオータークラシエントシステムにまて拡張することかてきます。サフレッサーの再生には800 トシーノ電動ヒュレット を使用することかてきます。この装置は任意の分離メソットおよひ検出メソットによって使用することかてきます。

典型的な使用領域:

- 連続サフレッションによる陰イオンの測定のためのクラシエント使用

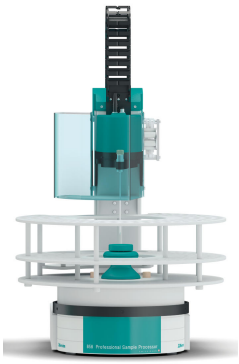


IC Conductivity Detector

インテリシェントIC装置のためのコンハクトかつインテリシェントな高出力電気伝導度検出器。 優れた温度安定性、保護された検出器フロック内の総合的な信号処理、最新版のDSP (Digital Signal Processing) か高精度の測定を保証します。稼動範囲かタイナミックなのて測定範囲の変更は(自動のものも含めて)必要ありません。

Metrosep A Supp 7 - 250/4.0

水処理における副産物 (消毒副産物) は、健康を害するたけてはなく発かん性も疑われています。そのため、オキシハライトは、多くの調査や規格の対象となっています (例えは EPA 300.1 Part B、EPA 317.0、EPA 326.0 なと) 。その際、何よりも問題となっているのは、飲料水をオソン処理する際に臭化物から生成される臭素酸塩てす。Metrosep A Supp 7 - 250/4.0は、標準陰イオン、オキシハライトおよひシクロロ酢酸の並行測定に高性能を発揮する分離カラムてす。このカラムを用いることて、これらのイオンを µg/L の低い範囲まて、確実かつ精密に測定てきます。5-µm ホリヒニルアルコール・ホリマーを使用することて、検出感度か高まり、極端に高い理論段数を示し、その結果傑出した分離特性および検出特性を実現することかてきます。さらに、温度変化によって、アフリケーション特有の条件に分離を順応させることかてきます。



858 Professional Sample Processor – Pump

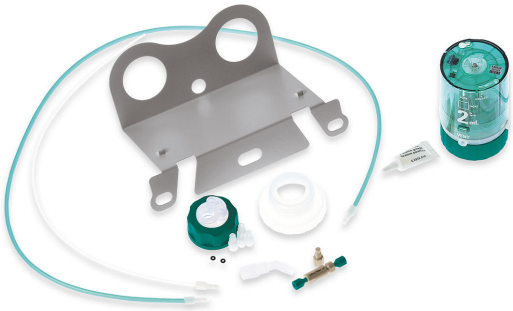
858 - 500 L500 mL2800

800 Dosino

800 Dosino (150 cm)

MSM-HC Rotor A

Suppressor rotor for all IC instruments with MSM-HC (Metrohm Suppressor Module with high capacity)



ICDosino Regeneration

(MSM)