

# Determination of organic acids in fruit juices and white wines

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## Abstract

This application note presents a method using high performance liquid chromatography on NUCLEODUR C<sub>18</sub> Gravity-SB for the determination of organic acids in the most important fruit juices and white wines in food samples. Usage of HPLC phases that are stable in 100 % aqueous mobile phase systems helps to reduce the costs. Stationary phases with 5 µm particle size make the method suitable for simple HPLC-systems. The robustness and the stability of the method are tested by repeated sample injection. The influence of alcohol content in wine samples is also described.

## Introduction

The determination of organic acids is essential for quality control as well as for evaluating product authenticity [1, 2]. In addition the use of organic acids in fruit juices is regulated in many countries [3]. The analysis of organic acids in fruit juices and wines is a basic control parameter to ensure the quality and the authenticity of these products. In particular, the determination of organic acids using HPLC with UV detection is a low-cost and quick alternative method to the enzymatic quantification methods. The use of HPLC phases that are stable in 100 % aqueous mobile phase systems is important to reduce the costs. No amount of work and of time is required for the sample preparation necessary for the determination by HPLC. It was also our goal to develop a HPLC method with backpressure lower than 200 bar to make simple HPLC-systems usable for that method. That was the reason to choose a NUCLEODUR® C<sub>18</sub> Gravity-SB with 5 µm particle size. The combination of RP 18 phases with polystyrene-divinylbenzene phases is no longer necessary.

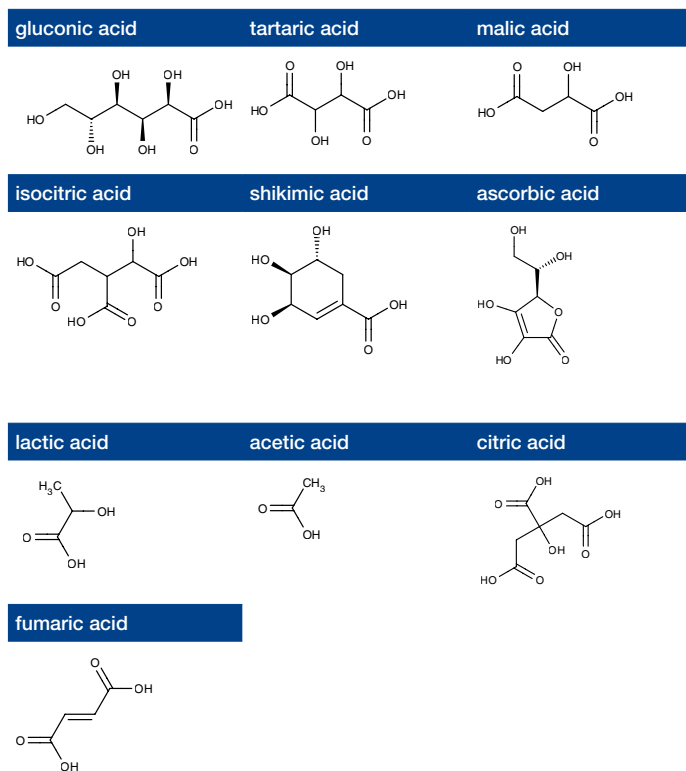


Figure 1: Organic acids in fruit juices and white wines.

## Sample preparation

- dilute 1 ml fruit juice or 1 mL wine with eluent to 5 mL
- filter sample solution through a syringe filter CHROMAFIL® Xtra PET-20/13 pore size 0.2 µm, (REF 729222)

## Chromatographic conditions

### Column:

EC 250/4.6 mm NUCLEODUR® C<sub>18</sub> Gravity-SB, 5 µm, (REF 760619.46)

### Eluent:

50 mmol NaH<sub>2</sub>PO<sub>4</sub>, pH 2.7 (addition of 6.0 g NaH<sub>2</sub>PO<sub>4</sub> and of 850 µL concentrated phosphoric acid to 1 L eluent)

Gradient: isocratic (25 min)

Flow rate: 0.4 mL/min

Temperature: 30 °C

Detection wavelength: UV: 205 nm

Injection volume: 10 µL

# Determination of organic acids in fruit juices and white wines

## Standard solution of target analytes of fruit juices

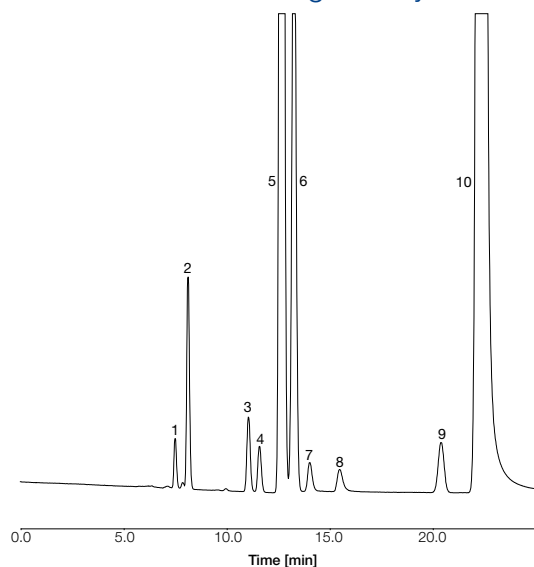


Figure 2: Chromatogram of aqueous standard solution of organic acids; UV at 205 nm.

peak number	analyte
1	gluconic acid
2	tartaric acid
3	malic acid
4	isocitric acid
5	shikimic acid
6	ascorbic acid
7	lactic acid
8	acetic acid
9	citric acid
10	fumaric acid

Table 1: Elution order of organic acids in aqueous standard solution.

## Apple juice

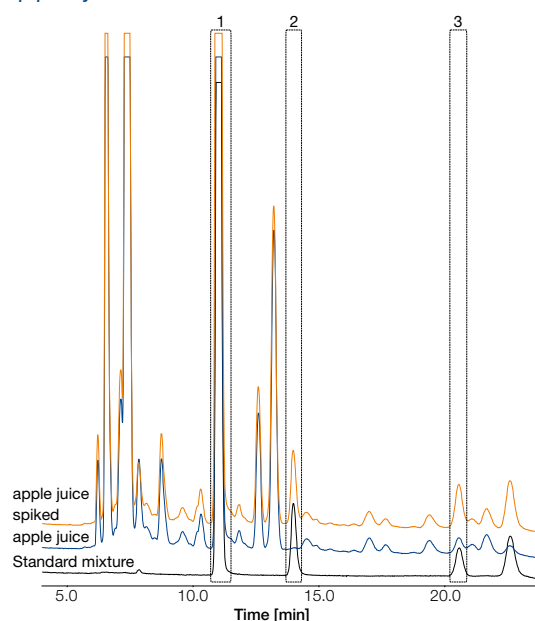


Figure 3: black: chromatogram of aqueous standard solution of organic acids, UV at 205 nm; blue: chromatogram of apple juice, UV at 205 nm; orange: chromatogram of spiked apple juice, UV at 205 nm.

### standard addition method

- add 0.6 mL of an aqueous solution of target analytes (250 µg/mL citric acid, 1.25 mg/mL lactic acid and 10 mg/mL malic acid) to 1 mL apple juice
- fill up with eluent to 5 mL

peak number	analyte	quantification with calibration curve (n=3)	quantification with standard addition method (n=3)
1	malic acid	6.41 ± 0.03 g/L	6.50 ± 0.03 g/L
2	lactic acid	0.1 ± 0.0 g/L	0.1 ± 0.0 g/L
3	citric acid	59.25 ± 0.03 mg/L	60.25 ± 0.03 mg/L

Table 2: Elution order and quantification of organic acids in apple juice.

# Determination of organic acids in fruit juices and white wines

## Orange juice

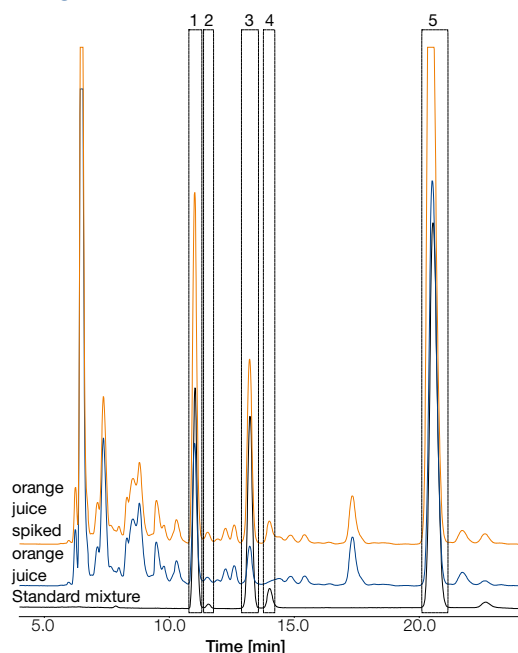


Figure 4: black: chromatogram of aqueous standard solution of organic acids, UV at 205 nm; blue: chromatogram of orange juice, UV at 205 nm; orange: chromatogram of spiked orange juice, UV at 205 nm.

### standard addition method

- add 0.6 mL of an aqueous solution of target analytes (15 mg/mL citric acid, 150 µg/mL isocitric acid, 1.25 mg/mL lactic acid, 5 mg/mL malic acid and 500 µg ascorbic acid) to 1 mL orange juice
- fill up with eluent to 5 mL

peak number	analyte	quantification with calibration curve (n=3)	quantification with standard addition method (n=3)
1	malic acid	2.04 ± 0.24 g/L	2.08 ± 0.24 g/L
2	isocitric acid	108 ± 0.0 mg/L	111 ± 0.0 mg/L
3	ascorbic acid	63 ± 2 mg/L	78 ± 2 mg/L
4	lactic acid	0.2 ± 0.0 g/L	0.2 ± 0.0 g/L
5	citric acid	9.52 ± 0.00 g/L	9.69 ± 0.00 g/L

Table 3: Elution order and quantification of organic acids in orange juice.

## Grape juice

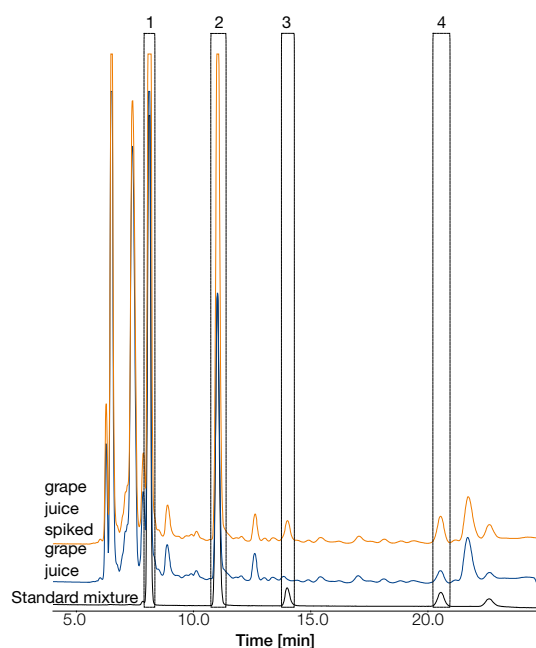


Figure 5: black: chromatogram of aqueous standard solution of organic acids, UV at 205 nm; blue: chromatogram of grape juice, UV at 205 nm; orange: chromatogram of spiked grape juice, UV at 205 nm.

### standard addition method

- add 0.6 mL of an aqueous solution of target analytes (500 µg/mL citric acid, 5 mg/mL tartaric acid, 1.25 mg/mL lactic acid and 7.5 mg/mL malic acid) to 1 mL grape juice
- fill up with eluent to 5 mL

peak number	analyte	quantification with calibration curve (n=3)	quantification with standard addition method (n=3)
1	tartaric acid	2.74 ± 0.00 g/L	2.70 ± 0.00 g/L
2	malic acid	4.27 ± 0.00 g/L	4.14 ± 0.24 g/L
3	lactic acid	0.2 ± 0.0 g/L	0.2 ± 0.0 g/L
4	citric acid	0.27 ± 0.00 g/L	0.27 ± 0.00 g/L

Table 4: Elution order and quantification of organic acids in grape juice.

# Determination of organic acids in fruit juices and white wines

## White wine standard

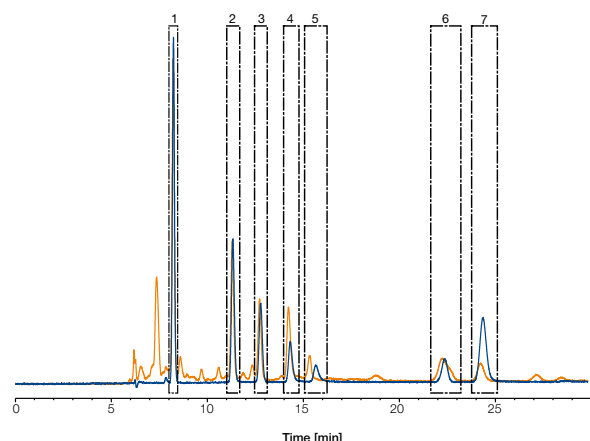


Figure 6: blue: chromatogram of aqueous standard solution of organic acids with 2.5 vol% ethanol, UV at 205 nm; orange: chromatogram of diluted white wine standard (with 2.5 vol% ethanol), UV at 205 nm.

peak number	analyte	quantification with calibration curve (n=3)
1	tartaric acid	$2.27 \pm 0.00$ g/L
2	malic acid	$2.53 \pm 0.01$ g/L
3	shikimic acid	$32.99 \pm 0.11$ mg/L
4	lactic acid	$3.70 \pm 0.04$ g/L
5	acetic acid	$0.96 \pm 0.01$ g/L
6	citric acid	$1.19 \pm 0.01$ g/L
7	fumaric acid	$6.76 \pm 0.06$ mg/L

Table 5: Elution order and quantification of organic acids in white wine standard.

## White wine, vitis: Weißer Burgunder

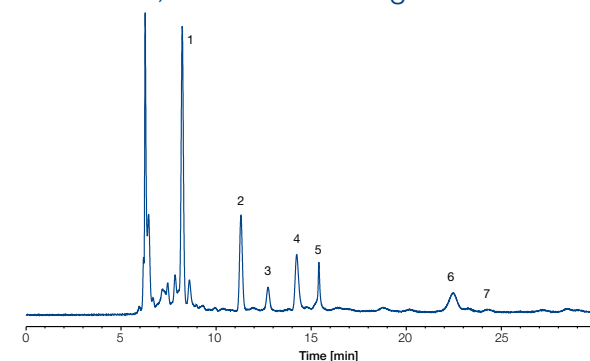


Figure 7: blue: chromatogram of wine, vitis: Weißer Burgunder, UV at 205 nm.

peak number	analyte	quantification with calibration curve (n=3)	quantification with standard addition method (n=3)
1	tartaric acid	$2.07 \pm 0.00$ g/L	$2.16 \pm 0.00$ g/L
2	malic acid	$1.77 \pm 0.00$ g/L	$1.87 \pm 0.00$ g/L
3	shikimic acid	$9.66 \pm 0.11$ mg/L	$15.13 \pm 0.18$ mg/L
4	lactic acid	$2.72 \pm 0.02$ g/L	$2.71 \pm 0.02$ g/L
5	acetic acid	$1.15 \pm 0.01$ g/L	$1.25 \pm 0.01$ g/L
6	citric acid	$0.73 \pm 0.01$ g/L	$0.80 \pm 0.01$ g/L
7	fumaric acid	n. q.	n.q.

Table 6: Elution order and quantification of organic acids in white wine, vitis: Weißer Burgunder.

## White wine, vitis: Chardonnay

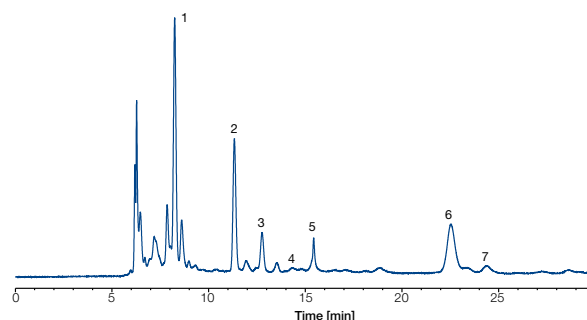


Figure 8: blue: chromatogram of wine, vitis: Chardonnay, UV at 205 nm.

peak number	analyte	quantification with calibration curve (n=3)
1	tartaric acid	$1.85 \pm 0.00$ g/L
2	malic acid	$2.45 \pm 0.00$ g/L
3	shikimic acid	$17.89 \pm 0.13$ mg/L
4	lactic acid	$0.11 \pm 0.00$ g/L
5	acetic acid	$1.07 \pm 0.02$ g/L
6	citric acid	$1.91 \pm 0.24$ g/L
7	fumaric acid	n. q.

Table 7: Elution order and quantification of organic acids in white wine, vitis: Chardonnay.

## White wine, vitis: Müller-Thurgau

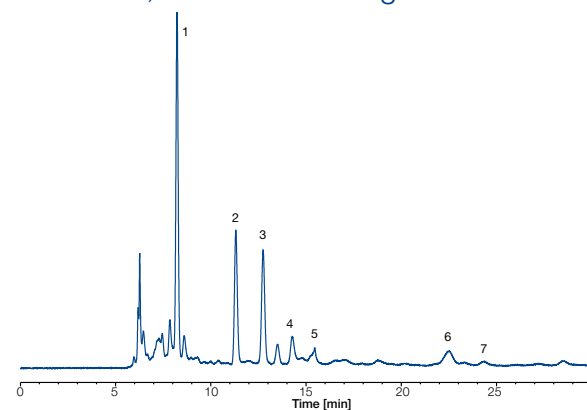


Figure 9: blue: chromatogram of wine, vitis: Müller-Thurgau, UV at 205 nm.

peak number	analyte	quantification with calibration curve (n=3)
1	tartaric acid	$2.57 \pm 0.01$ g/L
2	malic acid	$2.54 \pm 0.01$ g/L
3	shikimic acid	$52.41 \pm 0.08$ mg/L
4	lactic acid	$1.26 \pm 0.02$ g/L
5	acetic acid	$0.71 \pm 0.02$ g/L
6	citric acid	$0.52 \pm 0.03$ g/L
7	fumaric acid	n. q.

Table 8: Elution order and quantification of organic acids in white wine, vitis: Müller-Thurgau.

# Determination of organic acids in fruit juices and white wines

## White wine, vitis: Grüner Veltiner

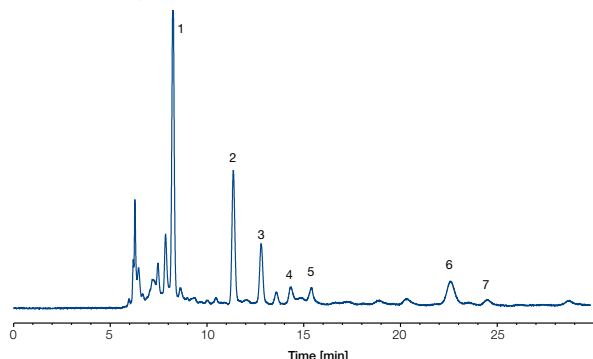


Figure 10: blue: chromatogram of wine, vitis: Grüner Veltiner, UV at 205 nm.

peak number	analyte	quantification with calibration curve (n=3)
1	tartaric acid	$2.27 \pm 0.00$ g/L
2	malic acid	$2.52 \pm 0.01$ g/L
3	shikimic acid	$26.59 \pm 0.05$ mg/L
4	lactic acid	$1.26 \pm 0.02$ g/L
5	acetic acid	$0.66 \pm 0.01$ g/L
6	citric acid	$0.83 \pm 0.01$ g/L
7	fumaric acid	n. q.

Table 9: Elution order and quantification of organic acids in white wine, vitis: Grüner Veltiner.

## Comparison of retention times after 10 times repeated sample injection (orange juice)

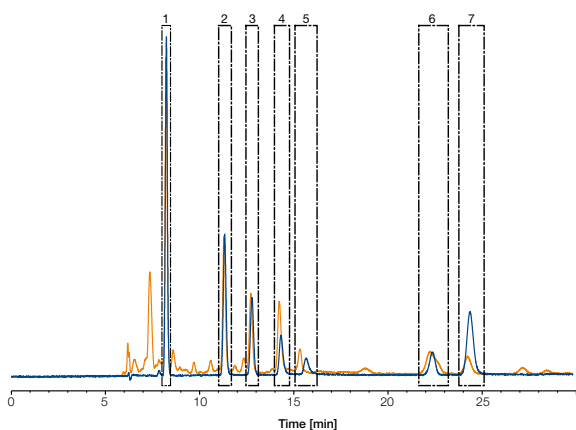


Figure 11: black: chromatogram of orange juice, 1st injection, UV at 205 nm; blue: chromatogram of orange juice, 5th injection, UV at 205 nm; orange: chromatogram of orange juice, 15th injection, UV at 205 nm.

peak number	analyte
1	malic acid
2	isocitric acid
3	ascorbic acid
4	lactic acid
5	citric acid

Table 10: Elution order of organic acids in orange juice after 10 times repeated sample injection.

## Recommended column reequilibration

- column should be rinsed with organic eluent after a sample sequence
- Eluent: 50 mmol  $\text{NaH}_2\text{PO}_4$ , pH 2.7 (addition of 6.0 g  $\text{NaH}_2\text{PO}_4$  and of 850  $\mu\text{L}$  concentrated phosphoric acid to 1 L eluent)
- Eluent B: methanol
- Gradient: 0-50 % B in 5 min, 50 % B for 10 min, 50-0 % B in 5 min, 0 % B for 15 min

## Results and discussion

This work illustrates a cheap and quick method for the determination of important organic acids in fruit juices and in white wine by HPLC. The chromatographic separation leads to sample-specific acid profiles. In respect of the quantification method, it should be noted that there was no significant difference between the standard addition method and the quantification with external calibration curve. For the analysis of fruit juices, ascorbic acid was successfully taken as analyte into the determination method. Because of less stability of ascorbic acid the determination should be done by the standard addition method. For all other organic acids the quantification with an external calibration curve was successful. The comparison of retention times show that after injection of 10 samples the chromatographic results were unchanged. The alcohol content of wine samples did not influence the separation of the organic acids due to a 1:5 sample dilution with an aqueous eluent. With a series of tests we confirmed that there was no significant influence of alcohol content on HPLC between 0 to 12.5 % by volume in wine samples. The results demonstrate that the determination of organic acids by HPLC is a robust method that can be used for large sample numbers. For column maintenance a flushing step with organic eluents (e.g., methanol) is recommended to remove non-polar compounds of matrix from the HPLC column. The presented method is a suitable alternative to enzymatic determination procedures of organic acids due to the low costs and the time-saving by simultaneous determination of all organic acids in one run.

## Literature

- [1] Verordnung über Fruchtsaft, einige ähnliche Erzeugnisse, Fruchtnektar und koffeinhaltige Erfrischungsgetränke (Fruchtsaft- und Erfrischungsgetränkerverordnung - FrSaftErfrischGetrV).
- [2] Regulation (EG) Nr. 1234/2007, Regulation (EG) Nr. 607/2009, Regulation (EG) Nr. 606/2009, Regulation (EG) Nr. 555/2008, Weingesetz, Weinverordnung, Wein-Überwachungsverordnung.
- [3] AIJN Code of Practice (CoP) for the Evaluation of Fruit and Vegetable Juices.

# Determination of organic acids in fruit juices and white wines

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## Additional information

The following applications regarding "Determination of organic acids in fruit juices and white wines" and further applications can be found on our online application database at [www.mn-net.com/apps](http://www.mn-net.com/apps):

MN Appl. No. 127440 (standard fruit juices)

MN Appl. No. 127450 (apple juice)

MN Appl. No. 127460 (orange juice)

MN Appl. No. 127470 (grape juice)

MN Appl. No. 127540 (standard white wine)

MN Appl. No. 127550 (weißer Burgunder)

MN Appl. No. 127560 (Chardonnay)

MN Appl. No. 127570 (Müller-Thurgau)

MN Appl. No. 127580 (Grüner Veltiner)

MN Appl. No. 127590 (repeated sample injection)

## Product information

The following MACHÉREY-NAGEL products have been used in this application note:

REF 729222, CHROMAFIL® Xtra PET-20/13

REF 760619.46, EC 250/4.6 NUCLEODUR® C<sub>18</sub> Gravity-SB, 5µm

REF 702293, Screw neck vials N 9, 1.5 mL

REF 702107, N 9 PP Screw cap, yellow, center hole,  
septum silicone white / PTFE red