Analysis of organic acids from fruit juices by HPLC-UV

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Application benefits

- Analysis of organic acids from fruit juices by HPLC-UV
- Sensitive HPLC analysis on a NUCLEODUR® C18 OA column

MN products

REF 760688.46
NUCLEODUR® C18 OA, 5 µm, 150 x 4.6 mm

REF 729220
Syringe filters, labeled, CHROMAFIL® Xtra PET, 25 mm, 0.45 µm

REF 702107
Screw closure, N 9, PP, yellow, center hole, Silicone white/PTFE red, 1.0 mm septum thickness

REF 702293
Screw neck vial, N 9, 11.6 x 32.0 mm, 1.5 mL, flat bottom, amber, wide opening

MN application numbers

HPLC: 129180

Keywords

Organic acids, fruit juice, apple, orange, grape, syringe filter, HPLC

Introduction

Fruits and fruit juices contain organic acids like citric acid, malic acid, oxalic acid and so on [1].

Fruits and fruit juices are globally traded products. Therefore, monitoring of organic acids is an important parameter for quality control in the processing of juices and related products, as well as for the evaluation of the authenticity and purity of juices [2, 3].

In addition, the use of organic acids in foods and beverages is regulated in many countries, though regulations vary widely [4].

This work presents a simple and robust HPLC-UV method for the analysis of organic acids typically found in fruit juices. The chromatographic separation was successfully developed on a NUCLEODUR® C18 OA column and tested with three store-bought fruit juices (apple, orange, and grape juice).

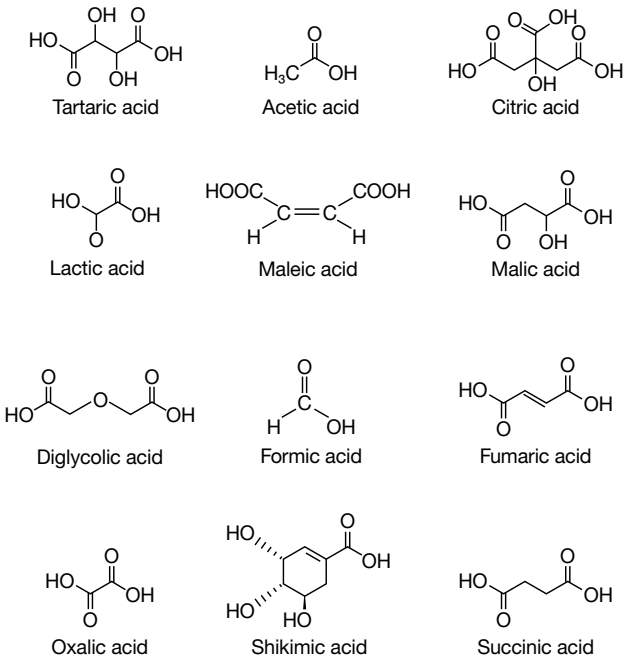


Figure 1: Typical organic acids in fruits und fruit juices

Sample pretreatment

1. Dilute sample 1 : 5 with Eluent A.
2. Centrifuge for 5 min at 4500 rpm.
3. Filtrate upper layer through a syringe filter CHROMAFIL® Xtra PET, 25 mm, 0.45 µm into a HPLC vial.
4. Sample is ready for injection.

Filtration test

1. 5 ml of standard mixture were pushed through a syringe filter CHROMAFIL® Xtra PET, 25 mm, 0.45 µm.

Peak Name	Concentration [mg/mL] in water	Recovery rate [%]
Oxalic acid	0.25	100.6 ± 0.55
Tartaric acid	1.00	99.7 ± 0.14
Formic acid	2.00	99.1 ± 0.42
Malic acid	2.00	99.9 ± 0.49
Shikimic acid	0.05	100.6 ± 0.16
Lactic acid	5.00	98.7 ± 0.57
Acetic acid	5.00	97.1 ± 0.73
Diglycolic acid	5.00	99.8 ± 0.97
Maleic acid	0.025	100.5 ± 0.92
Citric acid	5.00	99.9 ± 0.37
Succinic acid	5.00	100.9 ± 1.43
Fumaric acid	0.025	101.1 ± 0.74

Analysis by HPLC-UV

MN Appl. No. 129180

Chromatographic conditions

System	ThermoFisher Scientific UltiMate 3000 HPLC
Column	EC 150/4.6 NUCLEODUR® C18 OA, 5 µm (REF 760688.46)
Eluent A	25 mmol KH_2PO_4 in water (pH = 2.5, adjusted with phosphoric acid)
Eluent B	methanol
Flow rate	1.0 mL/min
Gradient	0% B for 8.0 min, from 0% to 65% B in 0.5 min, hold 65% B for 5.0 min, from 65% to 0% B in 0.5 min, hold 0% B for 6.5 min
Temperature	25 °C
Injection volume	3 µL
Detection	UV, 210 nm

Chromatograms of sample extracts

Figure 2: a

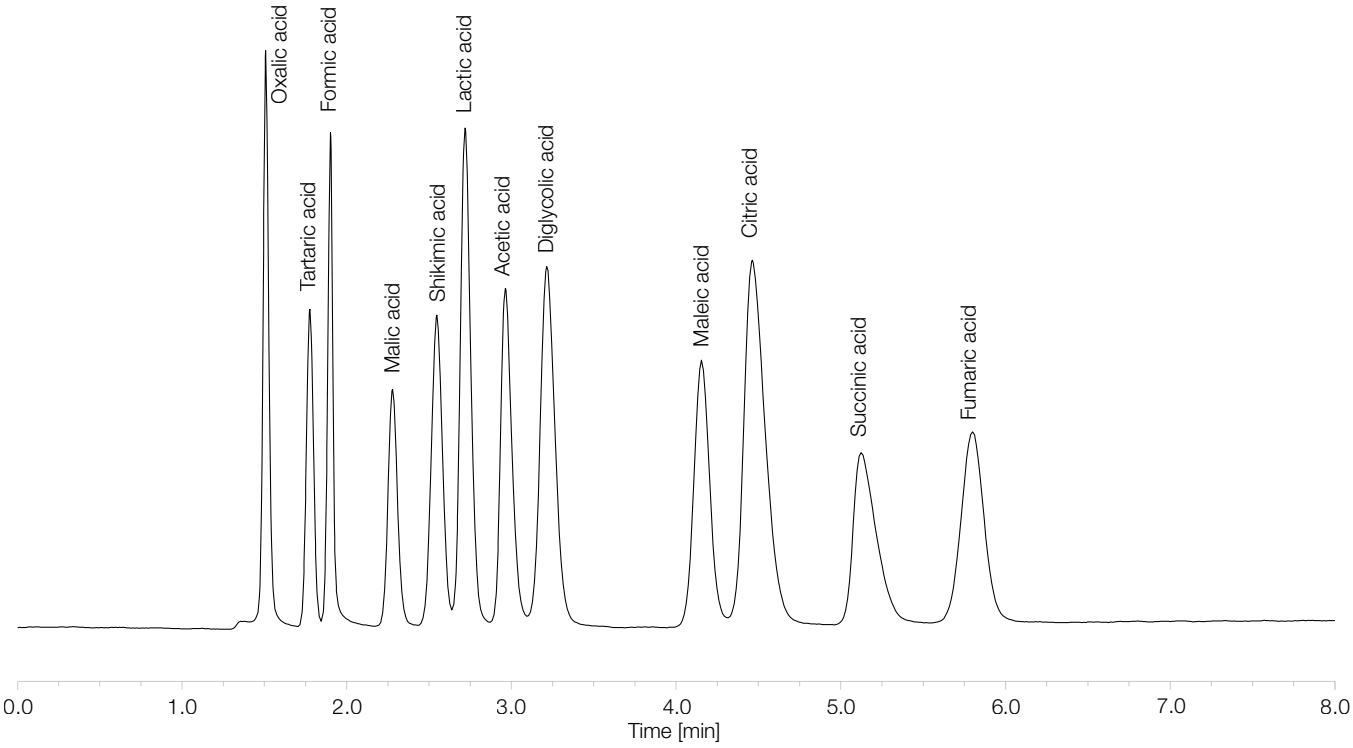


Figure 2: b

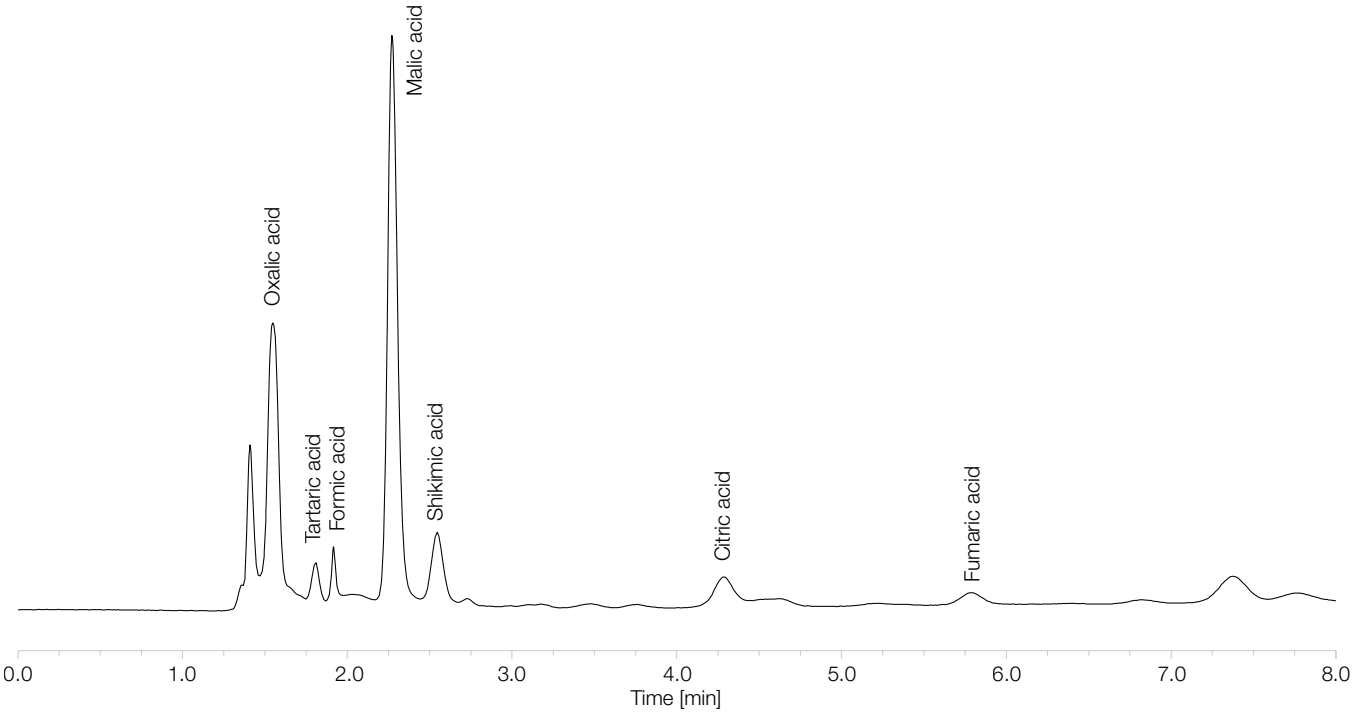


Figure 2: c

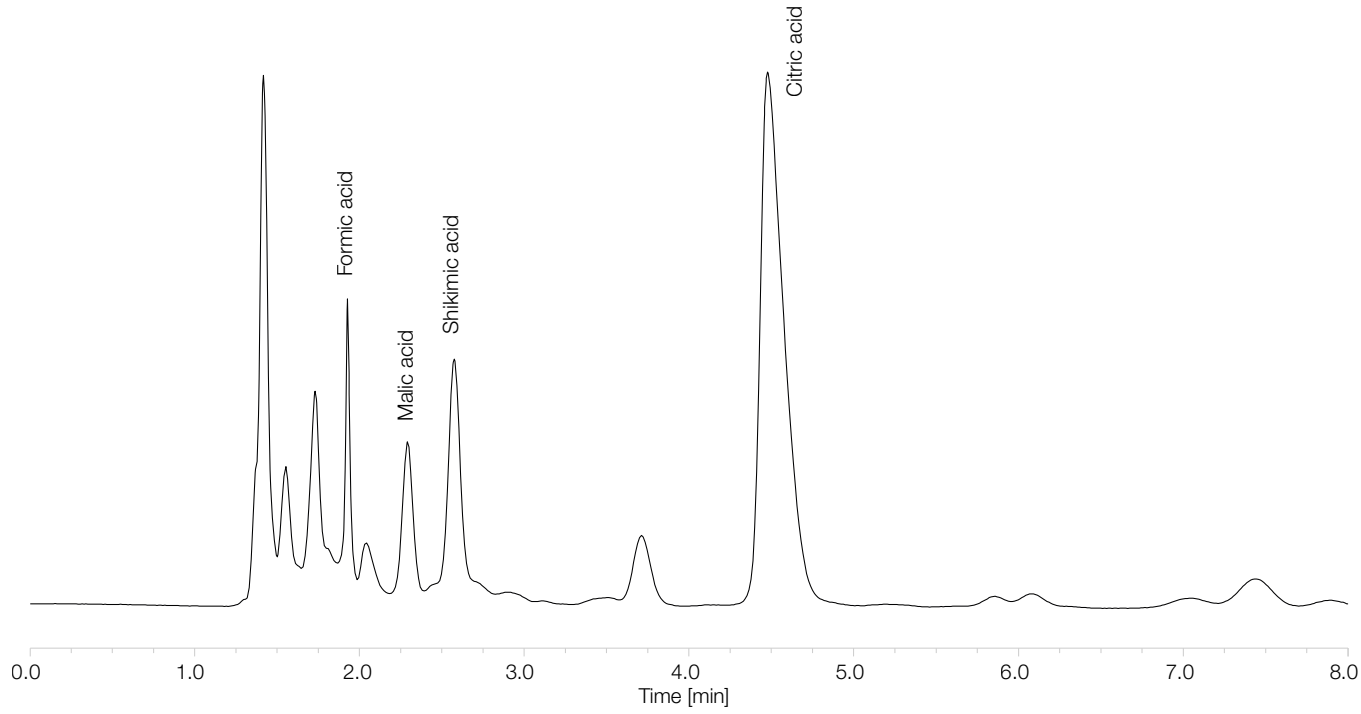


Figure 2: d

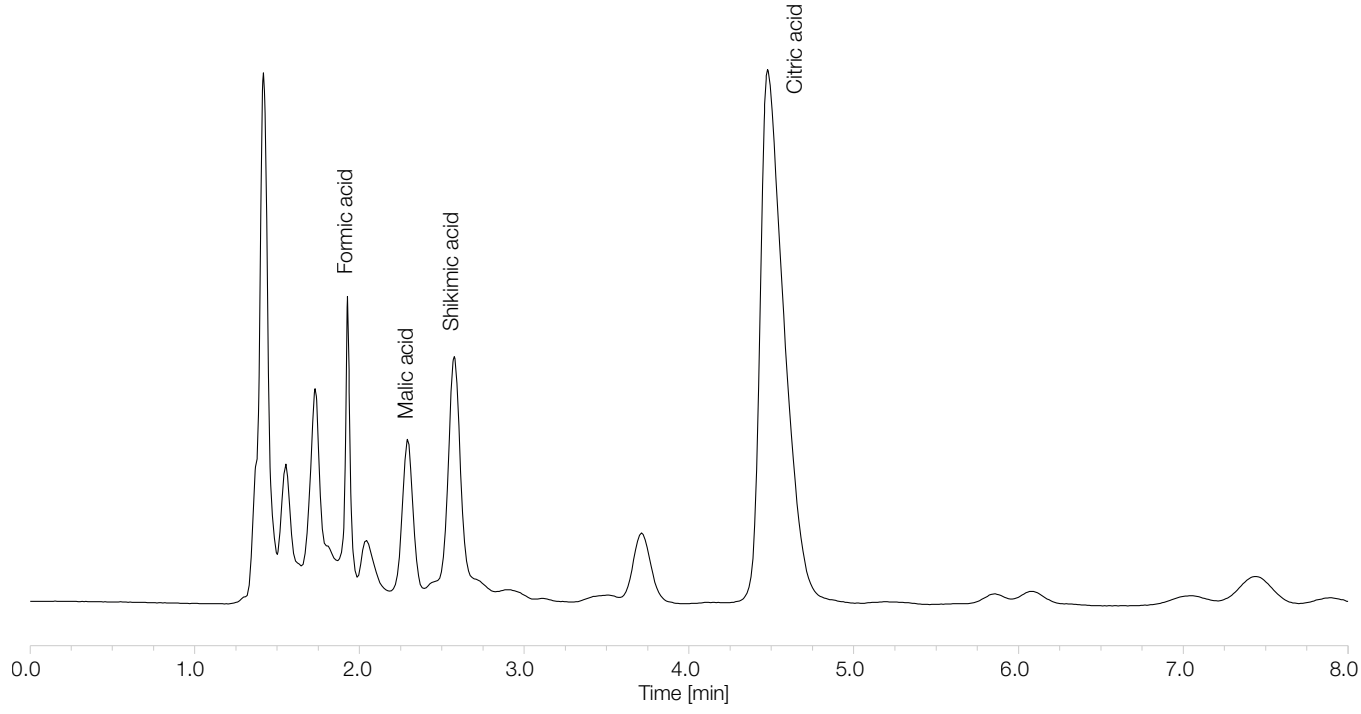


Figure 2: Chromatograms of samples: a = overlay of standard mixtures, b = apple juice, c = orange juice, d = grape juice.

Chromatographic results from standard mixture

Peak number	Peak Name	Retention time [min]	Concentration [mg/mL]	Calibration range [g/L]	Correlation R^2
1	Oxalic acid	1.51	0.36	0.01–1.00	0.9995
2	Tartaric acid	1.78	1.51	0.10–10.00	1.0000
3	Formic acid	1.90	2.90	0.10–10.00	1.0000
4	Malic acid	2.28	3.07	0.10–10.00	1.0000
5	Shikimic acid	2.55	0.068	0.01–1.00	1.0000
6	Lactic acid	2.72	12.61	0.50 – 25.00	0.9999
7	Acetic acid	2.96	7.69	0.50 – 25.00	0.9998
8	Diglycolic acid	3.22	7.67	0.50 – 25.00	1.0000
9	Maleic acid	4.16	0.040	0.01–1.00	0.9999
10	Citric acid	4.46	8.28	0.50 – 25.00	1.0000
11	Succinic acid	5.13	7.64	0.50 – 25.00	0.9999
12	Fumaric acid	5.80	0.037	0.01–1.00	0.9998

Table 1: Chromatographic results of 12 organic acids from standard mixture. Retention times of additional organic acids can be found on our application database (MN application number 129180).

Chromatographic results from fruit juices

Peak Name	Amount in apple juice [mg/mL]	Amount in orange juice [mg/mL]	Amount in grape juice [mg/mL]
Oxalic acid	0.36	n. d.	0.64
Tartaric acid	0.19	n. d.	5.01
Formic acid	0.33	2.60	1.15
Malic acid	8.95	2.88	6.06
Shikimic acid	0.016	0.078	0.014
Lactic acid	n. d.*	n. d.	n. d.
Acetic acid	n. d.	n. d.	n. d.
Diglycolic acid	n. d.	n. d.	n. d.
Maleic acid	n. d.	n. d.	n. d.
Citric acid	0.59	17.24	0.98
Succinic acid	n. d.	n. d.	n. d.
Fumaric acid	0.0002	n. d.	0.032

Table 2: Chromatographic results of 12 organic acids from fruit juices, * n. d. = not detected.

Conclusion

This application note presents the reliable and successful determination of 12 organic acids using a special HPLC phase NUCLEODUR® C18 OA (organic acids). The chromatographic results for the separation of common investigated organic acids from fruits are given in table 1. The calibration range for the chromatographic separation and the R-Square for each acid is also listed.

The application was successfully tested for the determination of organic fruit juices as shown in figure 2 b-d. The amounts of organic acid found in three store-bought fruit juices are given in table 2.

References

[1] Validation of a HPLC method for simultaneous determination of main organic acids in fruits and juices, R. Scherer, Food Chemistry, Volume 135, Issue 1, 1 November 2012, Pages 150–154.

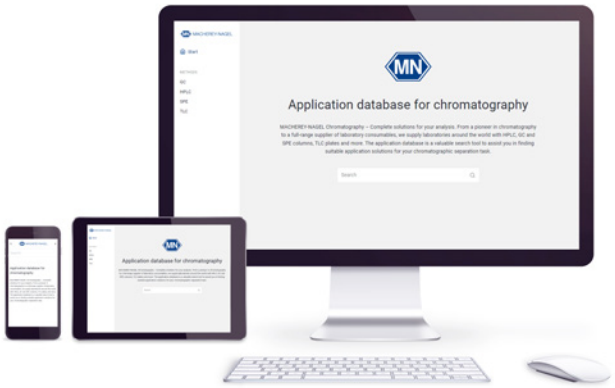
[2] DIRECTIVE 2012/12/EU OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 19 April 2012 amending Council Directive 2001/112/EC relating to fruit juices and certain similar products intended for human consumption.

[3] Leitsätze für Fruchtsäfte, Neufassung vom 27. November 2002 (BAnz. Nr. 46b vom 7. März 2003, GMBl 2003 S. 151), geändert durch die Bekanntmachung vom 07. Januar 2015 (BAnz. AT vom 27.01.2015 B1, GMBl 2015 S. 113).

[4] Code of Practice, AIJN - European Fruit Juice Association, Reference Guidelines (acerola, apple, apricot, aronia, banana, blackcurrant, carrot, coconut, cranberry, grape, grapefruit, guava, kiwi, lemon, lime, mandarin, mango, orange, passion fruit, peach, pear, pineapple, pomegranate, raspberry, sour cherry, strawberry and tomato).

The chromatography application database

- Free access to more than 3,000 application examples from HPLC, GC, TLC and SPE
- Free access to the MN application database: *https://chromaappdb.mn-net.com*



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