Established methods and comparison of 10 organic acids based on reversed phase chromatography and hydrophilic interaction chromatography

Tianyu Wang*, Mei Lin*, Xianju Feng, Peng Wang, Xuedan Cao and Weiqing Zhang

Zhejiang Citrus Research Institute, Zhejiang Academy of Agricultural Sciences, Taizhou, Zhejiang, China

ABSTRACT

High performance liquid chromatography (HPLC) methods have been established for simultaneously determining the 10 organic acids in fruit or soil. Two liquid chromatographic (LC) columns with reversed phase (RP) (Titan C18) and hydrophilic interaction (HILIC) (Poroshell 120 HILIC-Z) were selected to separate organic acids in molecular or ionic state, respectively. Meanwhile, the mobile phases had different solvents and pH values in the two states. Results indicated that these two columns can complement each other and avoid various matrix interferences. The limits of detection (LOD) of organic acids (S/N ≥ 3) were 0.002–0.521 μg∙mL⁻¹ in the molecular state and 0.003–0.459 μg∙mL⁻¹ in the ionic state, and the corresponding limits of quantification (LOQ) (S/N ≥ 10) were 0.007–1.737 μg∙mL⁻¹ and 0.009–1.530 μg∙mL⁻¹. The above methods can provide a technical support for the analysis of organic acid components in other matrices.

Métodos establecidos y comparación de 10 ácidos orgánicos con base en la cromatografía de fase inversa y la cromatografía de interacción hidrofílica

RESUMEN

Para determinar simultáneamente los 10 ácidos orgánicos presentes en la fruta o el suelo, en el presente estudio se establecieron métodos de cromatografía líquida de alto rendimiento (HPLC). Así, se seleccionaron dos columnas de cromatografía líquida (LC) de fase inversa (RP) (Titan C18) y de interacción hidrofílica (HILIC) (Poroshell 120 HILIC-Z), para separar los ácidos orgánicos en estado molecular o iónico, respectivamente. Mientras tanto, las fases móviles reportaron diferentes disolventes y valores de pH en los dos estados. Los resultados al respecto indican que estas dos columnas pueden complementarse entre sí y evitar diversas interferencias de la matriz. Los límites de detección (LOD) de los ácidos orgánicos (S/N ≥ 3) fueron de 0.002-0.521 μg∙mL⁻¹ en el estado molecular y de 0.003-0.459 μg∙mL⁻¹ en el estado iónico, y los correspondientes límites de cuantificación (LOQ) (S/N ≥ 10) fueron de 0.007-1.737 μg∙mL⁻¹ y de 0.009-1.530 μg∙mL⁻¹. En conclusión, los métodos anteriores pueden proporcionar un soporte técnico para el análisis de los componentes de ácidos orgánicos en otras matrices.

1. Introduction

As an important component of various samples such as fruits, soil and vegetables, the organic acid is one of the important indicators to measure the internal quality of fruit taste and flavor. It is also an important indicator to judge the processing, storage and maturation characteristics of fruits. For example, citrus mainly contains citric acid and malic acid (Yuan et al., 2018). At the same time, the sensory stimulation of various acids is also different, among which citric acid produces acid quickly and lasts for a short time; tartaric acid is slightly astringent, but the sour taste is refreshing (Dartiguenave et al., 2000). In addition, organic acids can reduce the pH value of the soil, improve the solubility of insoluble phosphorus compounds, form chelates with metal elements (iron, aluminum, calcium, manganese) in the soil, promote the release of phosphorus, and improve the utilization rate of phosphorus fertilizer, which plays an important role in the research of plant nutrition (Floch et al., 2009; Hussain et al., 2017; Rose et al., 2018). Therefore, the research on organic acids has been widely concerned by researchers.

At present, the analytical methods of organic acids reported in the literature mainly include spectrophotometry (Han et al., 2016; He et al., 2011), gas chromatography (GC) (Jham et al., 2002; Liu et al., 2022), ion chromatography (IC) (Fritz et al., 1984), HPLC (Scherer et al., 2012; Zong et al., 2015) and liquid chromatography tandem mass spectrometry (LC-MS/MS) (AliAbadi et al., 2022; Ehling & Cole, 2011). Among them, the specificity of spectrophotometry is poor to meet the requirements of high specificity and accurate characterization of organic acids. GC needs derivatization, which is complex, difficult to control and poor reproducibility. IC is easy to be disturbed by media and has high requirements for sample matrices, which is poor reproducibility and narrow application ranges. LC-MS/MS has narrow selection range of mobile phase, poor separation effect, and high equipment cost. As the main detection method of organic acids, HPLC has low cost and is easy to accept, but it has
many problems, such as many kinds of sample matrix, strong interference, and the detected few kinds of organic acids, Violeta et al. (2010) have studied the organic acid analysis in different citrus juices under RPLC, which contained only 6 kinds of organic acids.

Based on the above problems, this study explored and analyzed the molecular structure characteristics of organic acids. The difficulty of organic acids detection is that the target compound is highly acidic and belongs to ionic compound, while ordinary liquid chromatography column is difficult to retain and separate. Therefore, selecting a suitable chromatographic column is the key to solving the analysis of organic acids. In recent years, the analysis of strongly polar compounds has developed rapidly. Among them, the emergence of new RP matrix or HILIC column can retain the molecular or ionic state of compounds, and provide a good technical support for the separation of polar or ionic compounds. In this study, several chromatographic columns suitable for the detection of anionic compounds were selected for detailed exploration and methodology evaluation based on the structural morphology of organic acid retention, binding group of chromatographic column, and buffer. Finally, two complementary chromatographic columns with different retention principles, designated as molecular state or ionic state, were screened out to establish HPLC methods for the analysis of organic acids in fruits or soils. The main organic acids include oxalic acid, tartaric acid, quinic acid, malic acid, shikimic acid, lactic acid, acetic acid, citric acid, succinic acid, and fumaric acid. This study can provide a theoretical reference for the development of organic acid analysis methods in the future.

2. Materials and methods
2.1. Instruments, chemicals and material
The instruments used in this study included HPLC 1260 (Agilent Technologies, California, USA), High-speed tissue crusher DS-1 (Shanghai Specimen Model Factory, Shanghai, China), Vortex MS3 (IKA, Staufen, Germany), High speed dispersed homogenization Machine FA25S (Shanghai Fluke Co., Shanghai, China), SK250KUDOS Ultrasonic Cleaner (Shanghai Science Guide Ultrasonic Instrument Co., Ltd., Shanghai, China), Sorvall ST 16 R Centrifuge (Thermo Fisher Scientific, Massachusetts, USA), Heraeus Fresco 21 centrifuges (Thermo Fisher Scientific, Massachusetts, USA), and Milli-Q Ultrapure Water Systems (Merck, Darmstadt, Germany).

Oxalic acid (≥98%), tartaric acid (≥99%), quinic acid (≥98%), malic acid (≥99%), shikimic acid (≥98%), lactic acid (≥98%), acetic acid (≥99%), citric acid (≥99%), succinic acid (≥99%), and fumaric acid (≥99%) were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Acetonitrile (G.R.) and methyl alcohol (G.R.) were purchased from Merck (Darmstadt, Germany). Potassium dihydrogen phosphate (A.R.) was purchased from Sinopharm Chemical Reagent Co., Ltd (Beijing, China). WCX adsorbents were purchased from Shanghai Anspectone Experimental Technology Co., Ltd (Shanghai, China). Ultrapure water was obtained by Milli-Q Ultrapure Water Systems. In addition, the citrus, blueberry, and soil samples were collected from farmers in Taizhou, Zhejiang Province (China).

2.2. Standard solution of organic acids
The oxalic acid, tartaric acid, quinic acid, malic acid, shikimic acid, lactic acid, acetic acid, citric acid, succinic acid, and fumaric acid were weighed into 100 mL brown volumetric flasks, respectively, and add the mixed solution of acetonitrile and water (1:1, v/v) to prepare standard solution of 1000 μg∙mL⁻¹. All organic acids standard solutions were stored at 4 °C for further use.

2.3. Mixed standard solution in the molecular state
The standard stock solutions were diluted by water to obtain the mixed standard solution in the molecular state with 5 μg∙mL⁻¹ oxalic acid, 50 μg∙mL⁻¹ tartaric acid, 50 μg∙mL⁻¹ malic acid, 1 μg∙mL⁻¹ shikimic acid, 20 μg∙mL⁻¹ lactic acid, 20 μg∙mL⁻¹ acetic acid, 100 μg∙mL⁻¹ citric acid, 200 μg∙mL⁻¹ succinic acid, 1 μg∙mL⁻¹ fumaric acid, and the single standard solution with 200 μg∙mL⁻¹ quinic acid. Then the solutions were diluted into a series of standard solutions for detection.

2.4. Mixed standard solution in the ionic state
The standard stock solutions were diluted by the mixture of acetonitrile and water (2:1, v/v) to obtain the mixed standard solutions I and II in the ionic state. The concentrations of solution I were set to be 100 μg∙mL⁻¹ oxalic acid, 100 μg∙mL⁻¹ tartaric acid, 100 μg∙mL⁻¹ malic acid, 10 μg∙mL⁻¹ shikimic acid, 100 μg∙mL⁻¹ lactic acid, 10 μg∙mL⁻¹ acetic acid, 200 μg∙mL⁻¹ citric acid, and 1 μg∙mL⁻¹ fumaric acid, respectively; and the concentrations of solution II were 100 μg∙mL⁻¹ succinic acid and 100 μg∙mL⁻¹ quinic acid. Then, the mixed solution was diluted into a series of standard solutions for detection.

2.5. Fruit sample preparation
The edible parts of fruits were crushed with a high-speed tissue homogenizer and filtered into a beaker through two layers of gauze. 2 g of juice were weighed into a plastic centrifuge tube and diluted to 50 mL with ultrapure water, mixed evenly, ultrasonic for 4 min, and centrifuged at 10,000 r.min⁻¹ for 5 min. The mixture was diluted 10, 100 and 500 times according to the target compound content, and passed a 0.22 μm inorganic filter membrane for further detection.

2.6. Soil sample preparation
Soil samples (10 g) were weighed into a 50 mL plastic centrifuge tube and mixed with 20 mL of water. The mixture was vortexed for 1 min, ultrasonic for 20 min, and centrifuged at 10,000 r.min⁻¹ for 5 min. 2 mL of supernatant were taken, and 100 mg of WCX adsorbent were added. The supernatant was vortexed for 1 min and centrifuged at 10,000 r.min⁻¹ for 1 min, diluted 10, 100 and 500 times according to the target compound content, and then passed a 0.22 μm inorganic filter membrane for further detection.

2.7. HPLC for organic acids in molecular state
The instrument was equipped with chromatographic column (Titank C18, 250 mm × 4.6 mm I.D., 5 μm particle size) and
a diode-array detector (DAD). The mobile phase was comprised of a mixture of methanol and 40 mmol·L⁻¹ potassium dihydrogen phosphate solution (pH = 2.4) at a ratio of 2:98 (v/v) at a flow rate of 0.8 mL·min⁻¹. The injection volume was 20 μL, and the detection wavelength was set at 210 nm. The retention time was used for qualitative analysis, and the peak area was quantified by external standard method.

2.8. HPLC for organic acids in ionic state

The instrument was equipped with chromatographic column (Poroshell 120 HILIC-Z, 150 mm × 3.0 mm I.D., 2.7 μm particle size) and DAD. The mobile phase was comprised of a mixture of acetonitrile and 10 mmol·L⁻¹ Potassium dihydrogen phosphate solution (pH = 6.7) at a ratio of 81:19 (v/v) at a flow rate of 0.5 mL·min⁻¹. The injection volume was 20 μL, and the detection wavelength was set at 210 nm. The retention time was used for qualitative analysis, and the peak area was quantified by external standard method.

3. Results and discussion

3.1. Selection of chromatographic column bonded phase

The strongly polar compounds are not easy to retain and separate in conventional C18 columns. Some studies have found that the difficulty in the analysis of organic acids lied in the selection of liquid chromatographic columns. As a strongly polar compound, the requirements for the column are relatively high (Violeta et al., 2010). Based on the retention characteristics of molecular and ionic states of organic acids reported in the literature, several suitable chromatographic columns for strongly polar compounds were selected for comparative studies, such as Agilent ZORBAX SB-Aq (4.6 mm × 250 mm, 5 μm), Phenomenex Synergi Hydro-RP (4.6 mm × 250 mm, 5 μm), Phenomenex Titan C18 (4.6 mm × 250 mm, 5 μm), and Agilent HILIC-Z (3.0 mm × 150 mm, 2.7 μm). The column bonding structures are shown in Figure 1 (All pictures are from the corresponding suppliers).

The first three chromatographic columns were modified by special groups of silica-based C18 chemical bonded phase, and the last one adopted porous core-shell technology. These columns had different functions. For example, ZORBAX SB-Aq is fully porous, R1 is a proprietary bonded phase and it is used for the separation of polar compounds and can tolerate 100% aqueous phase. Synergi Hydro-RP is different from conventional C18 columns, and it is a polar endcapped C18 phase. The high surface area of 4 μm silica gel with dense bonding phase coverage allowed sufficient interaction between the analytes and the bonded phase, resulting in a highly retentive C18 stationary phase. Titan C18 is an organic silica gel hybrid matrix. In the siloxane network structure of silica gel, a more stable alkyl group in the siloxane network structure of silica gel is used to replace the unstable silicon-oxygen bridge connection under alkaline conditions, so that it not only has the good separation performance and mechanical strength of silica gel filler, but also has alkali resistance of polymer filler. Meanwhile, the peak symmetry and separation reproducibility can be ensured by strictly controlling the size distribution and surface morphology of particles. HILIC-Z is designed for the retention and high resolution separation of small molecule polar analytes. Its surface is porous core-shell filler particles and contains zwitterionic stationary phase, which can

![Figure 1. The bonded structure of chromatographic columns.](image)

**Figure 1.** The bonded structure of chromatographic columns.

**Figura 1.** Estructura de unión de las columnas cromatográficas.
significantly improve column efficiency and separate many strongly polar compounds or ionized compounds.

### 3.2. Performance of chromatographic column

The separation of 10 organic acids by four chromatographic columns was investigated. The results showed that the retention and separation effects of SB-Aq and Hydro-RP columns were not ideal. For the Hydro-RP column, the chromatographic peaks of oxalic acid and tartaric acid could not be separated, and oxalic acid was easy to overload the column and affect the peak of other organic acids. For SB-Aq column, it is unstable. After one month’s detection test, it was found that its retention and separation were significantly weakened, indicating that the high proportion of water can cause damage to the chromatographic column packing and is not suitable for the determination of organic acids. For Titank and HILIC columns, they have relatively stable column efficiency and separation effects, and are suitable for the development and application of organic acid detection methods after about 6 months of experiments, as shown in Figures 2 and 3.

### 3.3. Solvent effect of standard sample

Methanol, acetonitrile, and water were selected as solvents to prepare organic acid standard samples, respectively. Due to many kinds of organic acids and low detection wavelength, there was interference of impurities in the solvents when using Titank column for analysis. we had tried several kinds of solvents from different company, such as Merk, Honerwell et al, which had the same interference of impurities. The LC conditions refered to section 2.7. As shown in Figure 4, it appeared serious interference by using acetonitrile and methanol at 5.2 min and 4.1 min, respectively, which affects the peak appearance of tartrate acid, quinic acid, malic acid, and shikimic acid, while water as the solvent had no interference. Therefore, water was selected as the dilution solvent in the molecular state of organic acids. In HILIC ionic mode, acetonitrile is usually selected as the solvent, because the solvent with low polarity could obtain a good peak shape by reducing the site competition between solvent and target compound. The LC conditions refered to section 2.8. As shown in Figure 5, considering the solubility of organic acids, a mixture of acetonitrile and water (2:1, v/v) was selected as the best dilution solvent.
Figure 3. Chromatogram of 10 organic acids on the HILIC column. Peak 1–10 represent lactic acid, acetic acid, quinic acid, shikimic acid, tartaric acid, fumaric acid, succinic acid, malic acid, oxalic acid, and citric acid.

Figure 3. Cromatograma de 10 ácidos orgánicos en la columna HILIC. Los picos 1–10 representan el ácido láctico, el ácido acético, el ácido quínico, el ácido shikímico, el ácido tartárico, el ácido fumárico, el ácido succínico, el ácido málico, el ácido oxálico y el ácido cítrico.

Figure 4. Chromatograms of different solvents on the Titank column.

Figura 4. Cromatogramas de diferentes disolventes en la columna Titank.
3.4. Selection of mobile phase

Referring to the structural formula and acidity coefficient of 10 organic acids (Table 1), different organic acids were kept in molecular or ionic state by adjusting the pH value of the buffer above or below pKa, and then achieved their separation on the two above-selected chromatographic columns.

Molecular state: methanol-water, acetonitrile-water, acetonitrile-potassium dihydrogen phosphate, and methanol-potassium dihydrogen phosphate were selected in the Titan column analysis, respectively. The results showed that the separation degree of various organic acids by using methanol as mobile phase was greater than that by using acetonitrile, because the elution capacity of acetonitrile in the C18 column was greater than that of methanol, making the peak speed of the target compounds faster and affected the previous separation of the compound. With the increase of salt concentration, the peak shape was greatly improved since the buffer salt could shield the unmodified hydroxy groups on the silica gel base and reduce the competition of target compound sites. When the salt concentration reached 0.04 mol L⁻¹, a good peak shape could be obtained. Meanwhile, the lower the pH value, the better the molecular state in the solution can be retained on the chromatographic column. Therefore, 0.04 mol L⁻¹ potassium dihydrogen phosphate-methanol (pH = 2.4) was finally selected as the mobile phase.
phase based on the pKa of various organic acids and the tolerance of the chromatographic column.

Ionic state: Acetonitrile is usually used as the organic phase in the HILIC column due to the strong polarity of methanol. The higher the pH value of the mobile phase and the larger the proportion of inorganic phase, the faster the peak time. The higher the buffer salt concentration, the slower the peak time and the wider the peak shape, the effect of buffer salts is important for HILIC separation (Berthelette et al., 2020; Zbornikova et al., 2019). 0.01 molL⁻¹ potassium dihydrogen phosphate-acetonitrile (pH = 6.7) was finally selected as the mobile phase.

### Table 1. Structural formula and acidity coefficient of 10 organic acids.

| Organic acid   | Structural formula | \( pK_1 \) | \( pK_2 \) | \( pK_3 \) |
|----------------|-------------------|----------|----------|----------|
| Oxalic acid (a) | ![Structure](image) | 1.22     | 4.19     | –        |
| Tartaric acid (b) | ![Structure](image) | 3.04     | 4.37     | –        |
| Quinic acid (c)  | ![Structure](image) | 4.27     | –        | –        |
| Malic acid (d)   | ![Structure](image) | 3.46     | 5.13     | –        |
| Shikimic acid (e) | ![Structure](image) | 5.19     | –        | –        |
| Lactic acid (f)  | ![Structure](image) | 3.86     | –        | –        |
| Acetic acid (g)  | ![Structure](image) | 4.75     | –        | –        |
| Citric acid (h)  | ![Structure](image) | 3.13     | 4.76     | 6.40     |
| Succinic Acid (i) | ![Structure](image) | 4.21     | 5.64     | –        |
| Fumaric acid (j) | ![Structure](image) | 3.02     | 4.83     | –        |
Table 2. The linear equation, LODs and LOQs of 10 organic acids.

Tabla 2. Ecuación lineal, LOD y LOQ de 10 ácidos orgánicos.

| Organic acid   | State | Linear range (µg·mL⁻¹) | Linear equation | Correlation coefficient (r) | LOD (µg·mL⁻¹) | LOQ (µg·mL⁻¹) |
|----------------|-------|-------------------------|------------------|-----------------------------|---------------|---------------|
| Oxalic acid    | RPLC  | 0.011–500               | y = 16.7140x-1.9244 | 0.9998                      | 0.011         | 0.040         |
| Tartaric acid  | HILIC | 0.255–200               | y = 5.7531x + 0.3755 | 0.9996                      | 0.255         | 0.850         |
| Quinic acid    | RPLC  | 0.521–600               | y = 0.4019x-0.0936  | 0.9997                      | 0.521         | 1.737         |
| Malic acid     | HILIC | 0.137–200               | y = 2.8859x + 4.1860 | 0.9998                      | 0.137         | 0.459         |
| Shikimic acid  | HILIC | 0.004–100               | y = 39.6360x-1.2697 | 0.9999                      | 0.004         | 0.013         |
| Lactic acid    | RPLC  | 0.014–100               | y = 23.7140x-0.6122 | 0.9999                      | 0.014         | 0.049         |
| Acetic acid    | RPLC  | 0.032–40                | y = 0.5330x-1.5556  | 0.9999                      | 0.032         | 0.107         |
| Citric acid    | HILIC | 0.028–100               | y = 8.8956x + 6.1220 | 0.9998                      | 0.028         | 0.094         |
| Succinic acid  | RPLC  | 0.329–300               | y = 2.1772x + 1.4633 | 0.9995                      | 0.329         | 1.097         |
| Fumaric acid   | RPLC  | 0.050–100               | y = 167.7400x-18.3470 | 0.9996                      | 0.002         | 0.007         |

Figure 6. Chromatogram of organic acids in different fruit samples. Peak 1–10 represent oxalic acid, tartaric acid, quinic acid, malic acid, shikimic acid, lactic acid, acetic acid, citric acid, succinic acid, and fumaric acid.

Figura 6. Cromatograma de ácidos orgánicos en diferentes muestras de fruta. Los picos 1–10 representan el ácido oxálico, el ácido tartárico, el ácido quínico, el ácido málico, el ácido shikímico, el ácido láctico, el ácido acético, el ácido cítrico, el ácido succínico y el ácido fumárico.
3.5. Linear range, LOD and LOQ

According to the preparation and detection conditions of the mixed standard solution, the linear regression analysis was carried out with the peak area (mAU) as the ordinate (y) and the standard solution concentration (µg·mL⁻¹) as the abscissa (x). As shown in Table 2, each component had a good linear relationship within the corresponding concentration range, and the correlation coefficient r ≥ 0.9995, which meets the analysis requirements (Chinese Pharmacopoeia Commission, 2020). The LODs of 10 target compounds with signal-to-noise ratio (S/N) ≥ 3 were 0.002–0.521 µg·mL⁻¹ in molecular state and 0.003–0.459 µg·mL⁻¹ in ionic state, and the corresponding LOQs with S/N ≥ 10 were 0.007–1.737 µg·mL⁻¹ and 0.009–1.530 µg·mL⁻¹ (Table 2). Comparing the LOD and LOQ of each organic acid determined with HPLC (Scherer et al., 2012; Zong et al., 2015) and many other methods (Lin et al., 2011), our results were much lower. And the organic acids of interest could be quantified in citrus, blueberry and soil samples. However, the concentrations of organic acids in the current studies were at levels well above the LOQ.

3.6. Sample adaptability experiment

The two chromatographic methods of 10 organic acids basically showed the opposite situation of the retention order of substances. When the molecular state method is used for determination in soil samples, the interference of impurity peaks on oxalic acid is easy to occur due to the early peak time of oxalic acid, which cannot be accurately qualitative and quantitative. However, when the ionic state is used for the determination of organic acids, the peak time of oxalic acid is late and avoids the interference of impurities, so the content of oxalic acid could be calculated accurately. The content of organic acids in the soil can be determined by ion chromatography. Acetic acid, quinic acid, fumaric acid and succinic acid interfere with each other, so this method includes 7 kinds of organic acids for determination in soil samples such as acetic acid (g), shikimic acid (e), tartaric acid (b), fumaric acid (j), malic acid (d), oxalic acid (a), and citric acid (h). All acids are shown in numbers (Table 1). Similarly, when citrus and other fruit samples were measured in ionic state, the peak time of lactic acid was earlier and was easily interfered by impurities.

---

**Table 3.** Recoveries and relative standard deviations (RSD) of organic acids in the citrus, blueberry and soil.

| Organic acid | Sample type | Added 1 | Added 2 | Added 3 |
|--------------|-------------|---------|---------|---------|
|              | Background content (mg·kg⁻¹) | Recovery (%) | RSD (%) | Recovery (%) | RSD (%) | Recovery (%) | RSD (%) |
| Oxalic acid | Citrus | 0.0 | – | – | – | – | – |
|             | Blueberry | 0.0 | – | – | – | – | – |
|             | Soil | 1.0×10² | 102.7 | 1.9 | 108.9 | 2.0 | 102.7 | 0.8 |
| Tartric acid | Citrus | 6.7×10² | 95.9 | 7.3 | 102.8 | 4.9 | 108.1 | 3.0 |
|             | Blueberry | 0.0 | – | – | – | – | – |
|             | Soil | 0.0 | 97.4 | 2.1 | 91.9 | 1.5 | 94.7 | 3.0 |
| Quinic acid | Citrus | 0.0 | – | – | – | – | – |
|             | Blueberry | 1.7×10² | 93.7 | 2.7 | 102.4 | 2.1 | 95.8 | 3.5 |
|             | Soil | 0.0 | – | – | – | – | – |
| Malic acid | Citrus | 2.9×10² | 94.8 | 2.9 | 93.9 | 0.6 | 92.9 | 6.4 |
|             | Blueberry | 6.4×10² | 103.8 | 3.1 | 98.0 | 2.9 | 99.8 | 1.1 |
|             | Soil | 0.0 | 85.7 | 4.4 | 90.7 | 2.9 | 90.4 | 3.0 |
| Shikimic acid | Citrus | 5.0×10² | 108.3 | 2.1 | 102.1 | 6.3 | 110.5 | 3.9 |
|             | Blueberry | 6.3 | 95.8 | 3.2 | 94.0 | 4.2 | 100.1 | 4.2 |
|             | Soil | 0.0 | 101.5 | 6.3 | 103.5 | 3.0 | 97.3 | 1.5 |
| Lactic acid | Citrus | 0.0 | 87.8 | 3.5 | 91.0 | 4.2 | 89.1 | 6.3 |
|             | Blueberry | 0.0 | 95.5 | 2.1 | 90.9 | 5.2 | 89.0 | 1.4 |
| Acetic acid | Citrus | 0.0 | 95.7 | 2.1 | 89.4 | 0.4 | 95.6 | 3.2 |
|             | Blueberry | 9.3×10² | 98.4 | 2.2 | 104.2 | 3.9 | 104.1 | 2.8 |
|             | Soil | 0.0 | 98.6 | 1.0 | 93.3 | 4.2 | 102.8 | 4.2 |
| Citric acid | Citrus | 6.3×10² | 110.3 | 3.8 | 107.2 | 0.4 | 98.4 | 3.2 |
|             | Blueberry | 9.2×10² | 94.2 | 4.2 | 98.5 | 1.9 | 95.0 | 2.4 |
|             | Soil | 2.0×10² | 94.2 | 2.2 | 96.3 | 3.5 | 90.4 | 7.4 |
| Succinic acid | Citrus | 0.0 | 96.4 | 7.4 | 94.5 | 4.0 | 97.9 | 1.4 |
|             | Blueberry | 0.0 | 102.3 | 2.1 | 108.3 | 1.5 | 98.5 | 3.1 |
|             | Soil | 0.0 | – | – | – | – | – |
| Fumaric acid | Citrus | 2.0 | 99.0 | 1.1 | 95.7 | 3.1 | 91.9 | 5.3 |
|             | Blueberry | 0.0 | 95.3 | 3.2 | 98.1 | 4.1 | 96.1 | 2.5 |
|             | Soil | 2.0×10⁻¹ | 92.6 | 6.3 | 94.9 | 2.8 | 102.7 | 4.0 |

---

**Table 4.** Contents of 10 organic acids in citrus, blueberry and soil samples.

| Organic acid | Organic acid content (mg·kg⁻¹) |
|--------------|--------------------------------|
|               | Citrus1 | Citrus2 | Citrus3 | Blueberry4 | Blueberry5 | Blueberry6 | Soil7 | Soil8 | Soil9 |
| Oxalic acid   | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 124.2 | 303.9 | 125 |
| Tartric acid  | 665.7 | 624.2 | 748.4 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Quinic acid   | 0.0 | 0.0 | 0.0 | 2510.5 | 1315.0 | 1680.5 | 0.0 | 0.0 | 0.0 |
| Malic acid    | 297.0 | 150.5 | 450.4 | 82.5 | 30.0 | 63.8 | 0.0 | 0.0 | 0.0 |
| Shikimic acid | 52.2 | 16.0 | 142.0 | 8.5 | 6.5 | 6.3 | 0.0 | 0.0 | 0.0 |
| Lactic acid   | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Acetic acid   | 0.0 | 0.0 | 1.733 | 894.3 | 932.5 | 22 | 22 | 22 | 22 |
| Citric acid   | 6397.0 | 4302.5 | 5759.4 | 16216.3 | 9122.0 | 9150.5 | 280 | 18.9 | 9.0 |
| Succinic acid | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Fumaric acid  | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.3 | 0.2 | 0.2 |
determination of organic acids in fruits by molecular state method can meet the determination of more components. Due to the interference between tartaric acid and quinic acid, and their standard solutions can be prepared separately. Therefore, this method included 9 organic acids such as tartaric acid (b), quinic acid (c), malic acid (d), shikimic acid (e), lactic acid (f), acetic acid (g), citric acid (h), succinic acid (i), and fumaric acid (j). Chromatograms of organic acids in the fruit and soil samples are shown in Figures 6 and 7.

3.7. Recoveries and relative standard deviations

The recovery was examined by calculating the mean recoveries of the analysts using the standard addition method (Padilha et al., 2017). According to the sample detection methods in “1.3” and “1.4”, citrus, blueberry or soil samples with known organic acid content were weighed. Three fortified concentrations (approximately equivalent to 0.8, 1.0 and 1.2 times of the known matrix concentration for the existed acids, while 1.0 and 1.2 times of the corresponding minimum concentrations for others) were set for each substrate, and designated as Added 1, 2 and 3 (Table 3). Each sample was repeated five times. Considering there were different interferences in different substrates, the spiked step works as follows: the minimum concentrations of organic acids (b, d, e, f, g, h, i and j) added to citrus were 500.0, 2300.0, 40.0, 20.0, 20.0, 5000.0, 10.0, and 1.0 mg·kg⁻¹, respectively. For the blueberry, except the quinic acid was at 1300.0 mg·kg⁻¹, the variety of other acids were as the same as citrus but at different concentrations (50.0, 6.0, 20.0, 700.0, 7000.0, 10.0 and 1.0 mg·kg⁻¹, respectively). The minimum concentrations of organic acids (g, b, j, e, d, b, and h) added to soil were 2.5, 5.0, 0.2, 5.0, 5.0, 80.0 and 15.0 mg·kg⁻¹, respectively. The results showed that the recoveries of various organic acids in soil and fruit were 85.7%–110.5%, and the relative standard
deviations were 0.4%–7.4%, indicating that the methods have high accuracy and precision. We found that the recoveries of some organic acids were relatively low, such as malic acid in the soil was only 85.7%, which may be due to the slightly lower response value of the organic acid. While it had a greater impact when added to the soil matrix at low concentration. But when adding concentration increase, the recovery rate of organic acid could achieve 90% above. It showed that the higher concentration of the organic acid in matrix could enhance recovery, the result was also more accurate.

3.8. Determination of organic acids in the fruit and soil samples

The content of organic acids in the citrus, blueberry and soil samples was detected according to the established methods. The results showed that the organic acids in different samples were the same in type but different in content (Table 4). Tartaric acid, malic acid, shikimic acid, citric acid and fumaric acid were the main organic acids in citrus, where citric acid content was the highest with 4302.5–6397.0 mg·kg⁻¹. Blueberries were mainly composed of quinic acid, malic acid, shikimic acid, acetic acid and citric acid. The content of citric acid was the highest with 9122.0–16216.3 mg·kg⁻¹. The soil contained oxalic acid, acetic acid, citric acid and fumaric acid, in which oxalic acid content ranged from 124.2 to 303.9 mg·kg⁻¹.

4. Conclusions

In this study, HPLC methods for detecting 10 organic acids in fruit or soil were established based on the retention and separation of molecular and ionic states of organic acids on the chromatographic column. The LODs and LOQs of 10 organic acids in molecular state were 0.002–0.521 µg·mL⁻¹ and 0.007–1.737 µg·mL⁻¹, respectively. The corresponding values in ionic state were 0.003–0.459 µg·mL⁻¹ and 0.009–1.530 µg·mL⁻¹. Meanwhile, two different types of chromatographic columns were compared based on their molecular structure, and the two analytical methods could complement each other. The sample preparation of the two methods is simple and the separation efficiency is good, and the linear range, precision and accuracy can meet the requirements for the qualitative and quantitative analysis of trace organic acids in soil and fruits. This study provided a technical support for the determination of organic acids in different matrices.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

The work was supported by the National Agricultural Product Quality and Safety Major Project of China [GFP2019012]; the Zhejiang Provincial Natural Science Foundation of China [LY19C150001]; Zhejiang Provincial Agricultural Major Technology Collaboration Promotion Program of China [2018XTGSG011], and the Zhejiang Provincial Natural Science Foundation of China [No. LY19C150001].

References

AliAbadi, M. H. S., Karami-Osboo, R, Kobafard, F., Jahani, R., Nabi, M., Yazdanpanah, H., Mahboubi, A., Nasiri, A., & Faizi, M. (2022). Detection of lime juice adulteration by simultaneous determination of main organic acids using liquid chromatography-tandem mass spectrometry. Journal of Food Composition and Analysis, 105, 104223. https://doi.org/10.1016/j.jfca.2021.104223

Berthelette, K. D., Walter, T. H., Gilar, M., Gritti, F., MacDonald, T. S., & Soares, M. (2020). Evaluating MISER chromatography as a tool for characterizing HILIC column equilibration. Journal of Chromatography A, 1619, 460931. https://doi.org/10.1016/j.chroma.2020.460931

Chinese Pharmacopoeia Commission. (2020). Pharmacopoeia of the People’s Republic of China, Part III. China Medical Science Press.

Dartiguenave, C., Jeandet, P., & Maujean, A. (2000). Study of the contribution of the major organic acids of wine to the buffering capacity of wine in model solutions. American Journal of Enology and Viticulture, 51(4), 352–356. https://doi.org/10.5344/ajev.2000.900069-2

Ehling, S., & Cole, S. (2011). Analysis of organic acids in fruit juices by liquid chromatography-mass spectrometry: An enhanced tool for authenticity testing. Journal of Agricultural and Food Chemistry, 59(6), 2229–2234. https://doi.org/10.1021/jf104527e

Floh, C., Capowiez, Y., & Criquet, S. (2009). Enzyme activities in apple orchard agroecosystems: How are they affected by management strategy and soil properties. Soil Biology Biochemistry, 41(1), 61–68. https://doi.org/10.1016/j.soilbio.2008.09.018

Fritz, J. S., Duval, D. L., & Barron, R. E. (1984). Organic acid eluents for single-column ion chromatography. Analytical Chemistry, 56(7), 1177–1182. https://doi.org/10.1021/ac00271a027

Han, Z. J., Xu, J., Wei, X. Z., Liu, B. Y., Xue, G., He, B., & Yang, T. Z. (2016). Determination of organic acid in tobacco root by ferric hydroxamate spectrophotometry. Chinese Agricultural Science Bulletin, 32(9), 194–199. https://doi.org/10.11924/jissn.1000-6850.casb15090136

He, Y. C., Ma, C. L., Xu, J. H., & Zhou, L. (2011). A high-throughput screening strategy for nitrile-hydrolyzing enzymes based on ferric hydroxamate spectrophotometry. Applied Microbiology and Biotechnology, 89(3), 817–823. https://doi.org/10.1007/s00253-010-2977-5

Hussain, S. B., Shi, C. Y., Guo, L. X., Kamran, H. M., Sadka, A., & Liu, Y. Z. (2017). Recent advances in the regulation of citric acid metabolism in citrus fruit. Critical Reviews in Plant Sciences, 36(4), 241–256. https://doi.org/10.1080/07352689.2017.1402850

Jham, G. N., Fernandez, S. A., Garcia, C. F., & Silva, A. A. D. (2002). Comparison of GC and HPLC for the quantification of organic acids in coffee. Phytochemical Analysis, 13(2), 99–104. https://doi.org/10.1002/ypca.629

Lin, J. T., Liu, S. C., Shen, Y. C., & Yang, D. J. (2011). Comparison of various preparation methods for determination of organic acids in fruit vinegars with a simple ion-exclusion liquid chromatography. Food Analytical Methods, 4(4), 531–539. https://doi.org/10.1007/s12161-011-9204-6

Liu, C., Yang, P., Wang, H. L., & Song, H. L. (2022). Identification of odor compounds and odor-active compounds of yogurt using DHS, SPME, SAFE, and SBSE/GC-O-MS. LWT-Food Science and Technology, 154, 112689. https://doi.org/10.1016/j.lwt.2021.112689

Padilha, C. V. D. S., Miskinis, G. A., Souza, M. E. A. O. D., Pereira, G. E., Oliveira, D. D., Bordignon-Luiz, M. T., & Lima, M. D. S. (2017). Rapid determination of flavonoids and phenolic acids in grape juices and wines by RP-HPLC/DAD: Method validation and characterization of commercial products of the new Brazilian varieties of grape. Food Chemistry, 228, 106–115. https://doi.org/10.1016/j.foodchem.2017.01.137

Rose, T. J., van Zwieten, L., Claasens, A., Scanlan, C., & Rose, M. T. (2018). Phytotoxicity of soilborne glyphosate residues is influenced by the method of phosphorus fertiliser application. Plant and Soil, 422(1-2), 455–465. https://doi.org/10.1007/s11104-017-3482-8

Scherer, R., Rybka, A. C. P., Ballus, C. A., Meinhart, A. D., Filho, J. T., & Godoy, H. T. (2012). Validation of a HPLC method for simultaneous determination of main organic acids in fruits and juices. Food Chemistry, 135(1), 150–154. https://doi.org/10.1016/j.foodchem.2012.03.111
Violeta, N., Ion, T., & Ionica, M. E. (2010). HPLC organic acid analysis in different citrus juices under reversed phase conditions. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 38(1), 44–48. https://doi.org/10.15835/nbha3814569

Yuan, Z., Wei, Z. H., Wu, L. Z., Qi, L. T., Zong, Z. X., Cang, S. Z., & Cheng, X. H. (2018). Fruit sugar and organic acid were significantly related to fruit Mg of six citrus cultivars. Food Chemistry, 259, 278–285. https://doi.org/10.1016/j.foodchem.2018.03.102

Zbornikova, E., Knejzlik, Z., Hauryliuk, V., Krasny, L., & Rejman, D. (2019). Analysis of nucleotide pools in bacteria using HPLC-MS in HILIC mode. Talanta, 205, 120161. https://doi.org/10.1016/j.talanta.2019.120161

Zong, Y. Y., Lin, J., Xu, H., Jia, Z. H., & Yang, X. P. (2015). Optimization and validation of an HPLC-photodiode array detector method for determination of organic acids in vinegar. Journal of AOAC International, 98(2), 422–430. https://doi.org/10.5740/jaoacint.14-164