The Analysis of Organic Acids in Foods and Beverages Using Reversed Phase HPLC

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Roles of Organic Acids in Foods & Beverages

- Flavor Compounds
- Indicators of Product Quality
- Purity Determinations using an Organic Acid Profile
- pH Control in Products
- Antimicrobial Agents
Common Food & Beverage Organic Acids

1. Acetic acid
2. Tartaric acid
3. Succinic acid
4. Malic acid
5. Citric acid
Challenges in Organic Acid Analysis

- Specific Assays
- Column Selection
  - Small carboxylic acids can be difficult to retain by reversed phase HPLC
  - Specialty columns for organic acid analysis
- Mobile Phase Selection
  - Reversed phase requires a highly aqueous mobile phase
  - Chain folding can occur with highly aqueous mobile phases and conventional C18 columns
- Reproducibility and Stability
  - The ideal analysis would provide stable and reproducible retention, even with completely aqueous mobile phases
Chain Folding

- Highly aqueous mobile phases can cause chain folding with conventional C18 columns
- Chain folding can result in a total loss of retention
- Exposure to completely aqueous mobile phases at ambient pressure (no flow) accelerates the chain folding process
Chain Folding on a Conventional C18 Column

Organic Mobile Phase

Highly Aqueous Mobile Phase
Stationary Phases

Columns with added polar functionalities:

- enhance the retention of polar compounds
- eliminate retention loss caused by chain folding (even in 100% aqueous mobile phase)
- can provide unique selectivity

Conventional C18

Ultra Aqueous C18

Ultra IBD

= polar functionality
The Ultra Aqueous C18 Column

- Provides reproducible retention
- Provides stability, even when stored in highly aqueous mobile phases
- Prepared using Type B, high purity silica
- True C18 phase (USP L1)
Chain Folding with a Conventional C18 Column

Sample: Organic Acids
Peak list: 1. glycolic acid, 5.4mg/ml
2. malonic acid, 4.2mg/ml
3. acetic acid, 7.8mg/ml
4. maleic acid, 0.06mg/ml

Inj. vol: 10µl
Column: Ultra C18
150 x 4.6mm, 5µm
Mobile Phase: 50mM potassium phosphate, pH 2.5
Flow: 1.0 mL/min.
Detector: UV @ 210nm
Stability of the Ultra Aqueous C18 Column

A. Initial

Same conditions, except column: Ultra Aqueous C18

B. After flow stopped 5min.
Stability of the Ultra Aqueous C18 Column

A. Initial

Same conditions, except column: Ultra Aqueous C18

B. After 3 Days

[Graphs showing chromatograms for initial and after 3 days samples]
Sample: Tobacco Extract
Peak list:
1. oxalic acid
2. pyruvic acid
3. malic acid
4. citric acid

Inj. vol: 20µl
Column: Ultra Aqueous C18
250 x 4.6mm, 5µm
Mobile Phase: 50mM potassium phosphate, pH 2.5
Flow: 1.0 mL/min.
Detector: UV @ 210nm
**Tobacco Extract: Ultra Aqueous C18 Column**

Sample: Tobacco Extract
Peak list:
1. oxalic acid
2. pyruvic acid
3. malic acid
4. citric acid

Inj. vol: 20µl
Column: Ultra Aqueous C18 250 x 4.6mm, 5µm
Mobile Phase: 50mM potassium phosphate, pH 2.5
Flow: 1.0 mL/min.
Detector: UV @ 210nm
Sample: Standard
Peak List:
1. Tartaric acid, 620 ppm
2. Malic acid, 460 ppm
3. Acetic acid, 1260 ppm
4. Citric acid, 580 ppm
5. Succinic acid, 540 ppm

Inj. vol: 10µl
Column: Ultra Aqueous C18
150 x 4.6mm, 5µm
Mobile Phase: 50mM potassium phosphate, pH 2.5
Flow: 1.0 mL/min.
Detector: UV @ 210nm
Foods & Beverage Organic Acids Standard

Sample: Standard

Peak List:
1. Tartaric acid, 620 ppm
2. Malic acid, 460 ppm
3. Acetic acid, 1260 ppm
4. Citric acid, 580 ppm
5. Succinic acid, 540 ppm

Inj. vol: 10µl
Column: Ultra Aqueous C18
150 x 4.6mm, 5µm

Mobile Phase: 98% 50mM potassium phosphate, pH 2.5:
2% acetonitrile

Flow: 1.0 mL/min.
Detector: UV @ 210nm
Organic Acids: Vinegar

Sample: 3% Vinegar in Mobile Phase
Peak List: 1. Acetic Acid
Inj. vol: 10µl
Column: Ultra Aqueous C18
Mobile Phase: 50mM potassium phosphate, pH 2.5
Flow: 1.0 mL/min.
Detector: UV @ 210nm
Organic Acids: Lemon-Lime Soft Drink

Sample: 10% Soda in Mobile Phase
Peak List: 1. Citric Acid
Inj. vol: 10µl
Column: Ultra Aqueous C18 150 x 4.6mm, 5µm
Mobile Phase: 50mM potassium phosphate, pH 2.5
Flow: 1.0 mL/min.
Detector: UV @ 210nm
Organic Acids: Orange-Mango Juice

Sample: 10% Juice in Mobile Phase

Peak List:
1. Malic Acid
2. Citric Acid

Inj. vol: 10µl

Column: Ultra Aqueous C18
150 x 4.6mm, 5µm

Mobile Phase: 50mM potassium phosphate, pH 2.5

Flow: 1.0 mL/min.

Detector: UV @ 210nm
Conclusions

- The Ultra Aqueous C18 column provides enhanced retention of organic acids.
- Stable and reproducible retention is possible, even with highly aqueous mobile phases.
- Columns with added polar functionalities can be advantageous for analyzing a wide range of polar compounds, including:
  - Carboxylic acids
  - Vitamins
  - Nutraceuticals
  - Pesticides