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HPLC Separation of Common Organic Acids in Foods and Beverages

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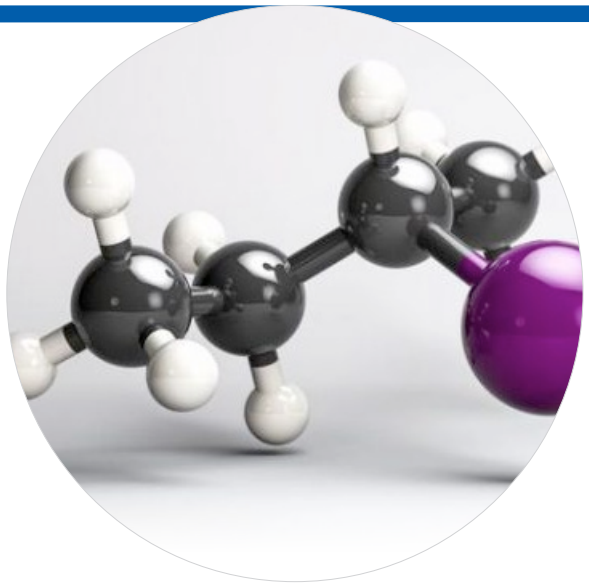


Introduction

Low molecular weight organic acids (usually aliphatic acids) occur in a wide variety of food products and arise from many different sources. Many are characteristic of the source material, such as the varying array of acids that appear in different fruit species. Others are created in the production or processing of foods, such as the microbial processes that create beer, wine and cheese, and yet others arise through food degradation or spoilage. Finally, 'foreign" organic acids (or, unexpected combinations of acids) may unintentionally appear in foods owing to processing errors or – worst case" - intentionally occur as a result of economically motivated adulteration, such as diluting an expensive fruit juice with an inexpensive juice or with water. Therefore, the ability to accurately identify and quantify organic acids in foods and beverages is an essential step in assuring the safety, quality and integrity of consumer products.

Experimental Approach

Although there are a relatively small number of commonly occurring organic acids that can be found in food products (**Table 1**), there are myriad combinations of these specific acids and their concentrations that characterize the broad universe of food types. Consequently, it is impractical to employ a broad spectrum analyte screen to characterize all organic acids in all types of foods. Therefore, it would be helpful to have a practical approach to quickly zero in on a specific LC system that could be effectively and rapidly optimized.

Table 2 displays various combinations of acids typically analyzed by certain classes of food producers, as ascertained through a written survey of Phenomenex food customers. As is seen, certain combinations of analytes (notably the first six) are quite common to multiple classes of food analyses. Other analytes appear infrequently and are associated only with specific food categories.

Based upon these commonalities, standardized liquid chromatography screening schemes were developed for common combinations of organic acids based upon consideration of analyte chemistry and LC stationary/mobile phase interactions. These schemes represent logical method development starting points, absent consideration of matrix interactions which must be dealt with in subsequent method optimization. An optimum scheme was selected and then evaluated in the Application Laboratory to confirm the efficacy of analytical approach.

Table 1.
Organic Acids Commonly Found in Foods and Beverages

Name	Formula
1. Malic	$C_4H_6O_5$
2. Citric	$C_6H_8O_7$
3. Acetic	$C_2H_4O_2$
4. Lactic	$C_3H_6O_3$
5. Tartaric	$C_4H_6O_6$
6. Succinic	$C_4H_6O_4$
7. Propionic	$C_3H_6O_2$
8. Formic	CH_2O_2
9. Quinic	$C_7H_{12}O_6$
10. Fumaric	$C_4H_4O_4$
11. Oxalic	$C_2H_2O_4$
12. Ascorbic	$C_6H_8O_6$
13. Orotic	$C_5H_4N_2O_4$
14. Saccharic	$C_6H_{10}O_6$
15. Pyruvic	$C_3H_4O_3$
16. Uric	$C_5H_4N_4O_3$

Table 2.
Industry Specific Organic Acid Analyte Targets

Organic Acid	Speciality Food Ingredients	Soft Drink & Juice Formulation	Wine Making	Beer Brewing	Dietry Supplements	Milk Processings
Malic	●	●	●	●	●	
Citric	●	●	●	●	●	●
Acetic	●	●		●	●	●
Lactic	●	●	●	●	●	●
Tartaric	●	●	●		●	
Succinic	●		●	●	●	●
Propionic	●					●
Formic			●			
Quinic		●			●	
Fumaric	●	●	●		●	
Oxalic						
Ascorbic			●			
Orotic						
Saccharic					●	
Pyruvic				●		
Uric						●

Experimental Conditions

The analytical column selected as optimum for the organic acid panel separations was Luna™ Omega 3 µm Polar C18 100A 250 x 4.6 mm. Part Number: 00G-4760-E0

Method Conditions

Column: Luna Omega 3 µm Polar C18 100 Å
Dimensions: 250 x 4.6 mm
Part Number: 00G-4760-E0
Mobile Phase: 100 mM Potassium Phosphate (monobasic), pH 2.5 for 30 minutes
Flow Rate: 1mL/min
Injection: 0.5 µL of 2 mg/mL standards
Temperature: 30 °C
Detection: UV @ 210 nm
Detector: Agilent® 1100 Quaternary HPLC system

The potassium phosphate mobile phase showed the best overall resolution for the full 15 analyte panel (**Table 3**) and was selected for further evaluation of the six industry-specific panels **Figures 1-6**). This mobile phase selection did not show good resolution of the formic and quinic acid pair. However, formic and quinic acid do not appear together in any of the industry-specific panels, so this lack of resolution was not considered problematical. The six most commonly occurring acids (malic, citric, acetic, lactic, tartaric and succinic) were all well separated.

Table 2.
Industry Specific Organic Acid Analyte Targets

Analyte	Retention Time (min)
Oxalic Acid	2.699
Tartaric Acid	3.207
Quinic Acid	3.412
Formic Acid	3.425
Malic Acid	4.184
Ascorbic Acid	4.678
Lactic Acid	5.027
Acetic Acid	5.378
Orotic Acid	5.480
Maleic Acid	6.636
Citric Acid	7.707
Succinic Acid	8.941
Fumaric Acid	9.400
Glucuronic Acid	12.608
Propionic Acid	12.938

Results and Discussion

Figures 1-6 show good chromatographic resolution for all selected analyte panels with a few exceptions. **Figure 1** exhibits noticeable trailing of the propionic acid peak in the Specialty Food Ingredients panel. However, propionic acid displays a symmetrical peak in the Milk Processing panel, suggesting an artifact in the first instance. The Wine Making panel shows good chromatographic resolution, but experience with wine analysis has shown that sample preparation to remove matrix interferences is a prerequisite for the effective analysis of real wine sample. All told, however, the chromatographic system presented here appears to be good starting point for the development of organic acid analysis methods for a wide variety of food products and ingredients. Obviously, to develop a complete method, additional sample preparation and optimization work will be required to deal with the unique matrix interferences and analyte ranges of specific food products. However, by beginning that process with a sound chromatographic approach, one can significantly accelerate the development process.

Finally, **Figure 7** shows the application of the above chromatographic approach to the analysis of actual milk sample to demonstrate further development work that could be done. A milk sample was fortified with the Milk Panel analytes at a concentration of 5mg/mL and 20 uL was injected. The Red trace is that of the actual milk sample and the Blue trace is that of the standard mix from **Figure 6**. **Figure 8** shows an expansion of the same chromatograms to show greater detail for smaller peaks. These data show that there are no matrix interferences in immediate proximity of the target analytes and suggests that with the adjustment of analyte ranges to better reflect real-world sample composition, one could directly proceed with the development of a quantitative method.

Figure 3.
Wine Panel

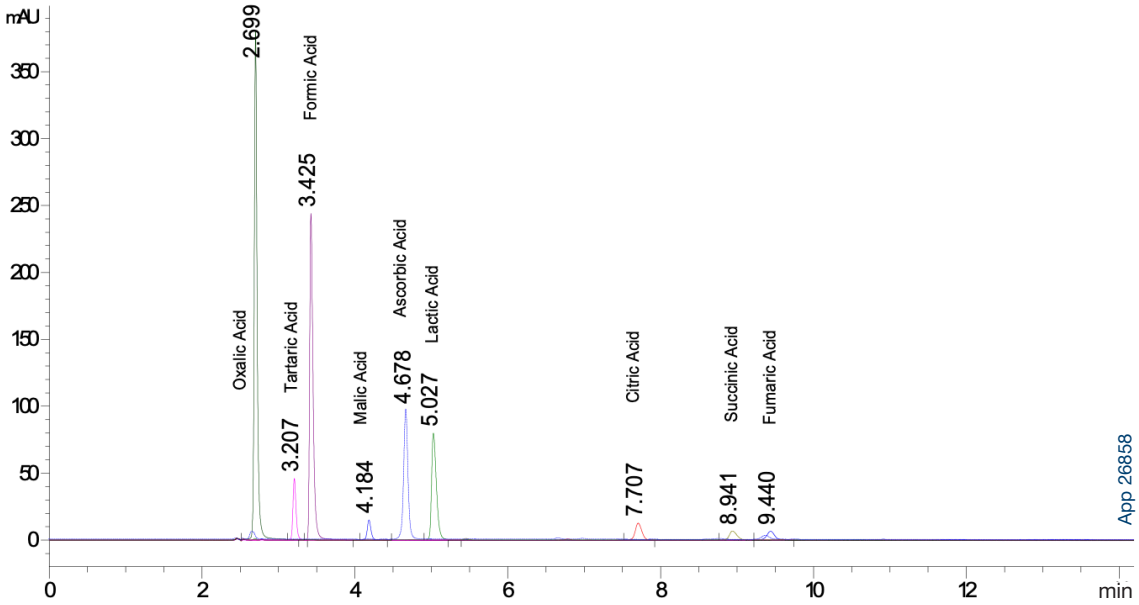


Figure 4.
Beer Panel

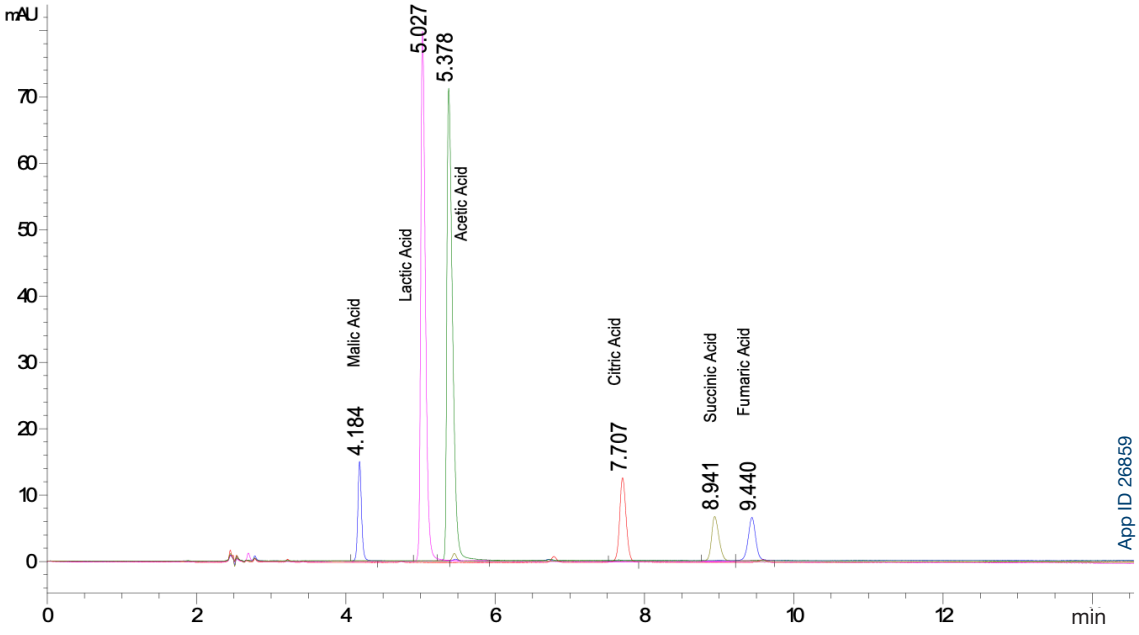


Figure 5.
Dietary Supplements Panel

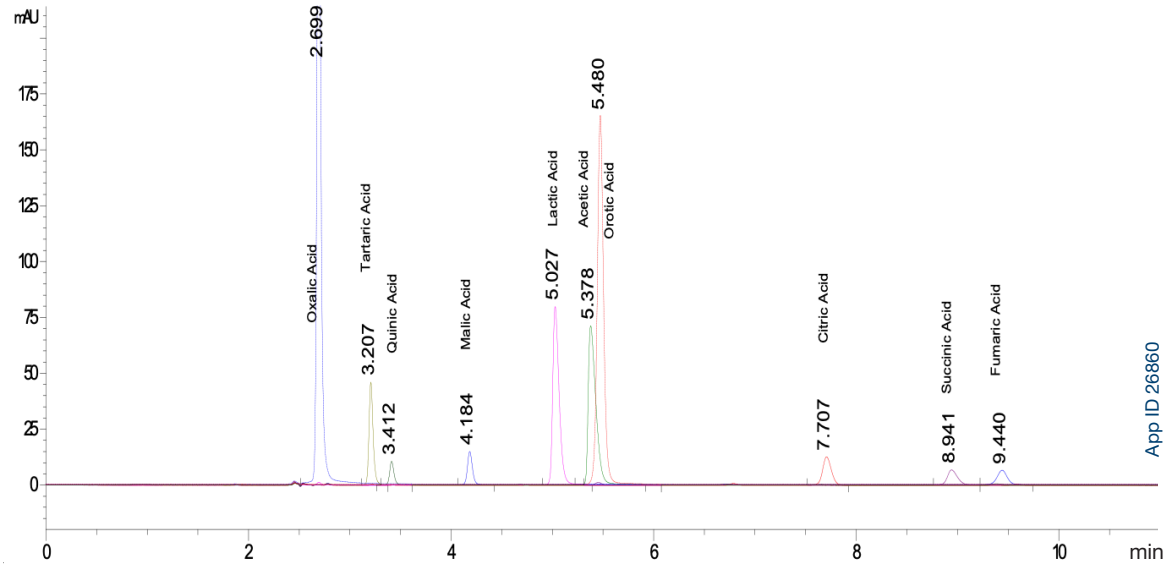


Figure 6.
Milk Panel

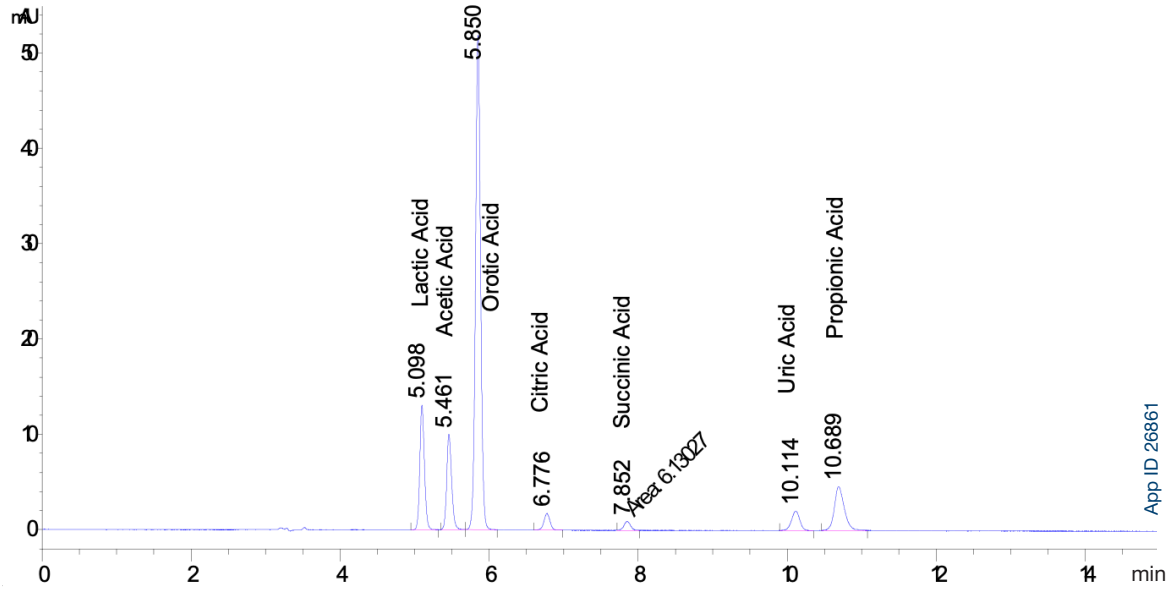


Figure 7.
Milk Sample Chromatogram – Overlay on Standards

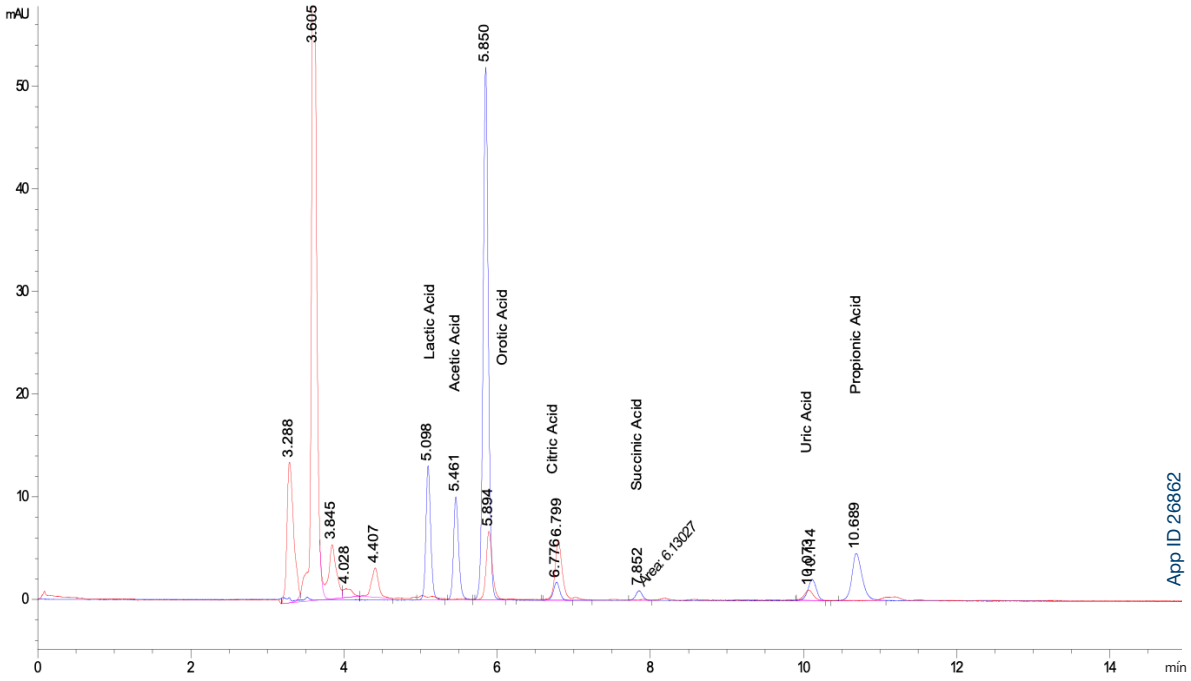
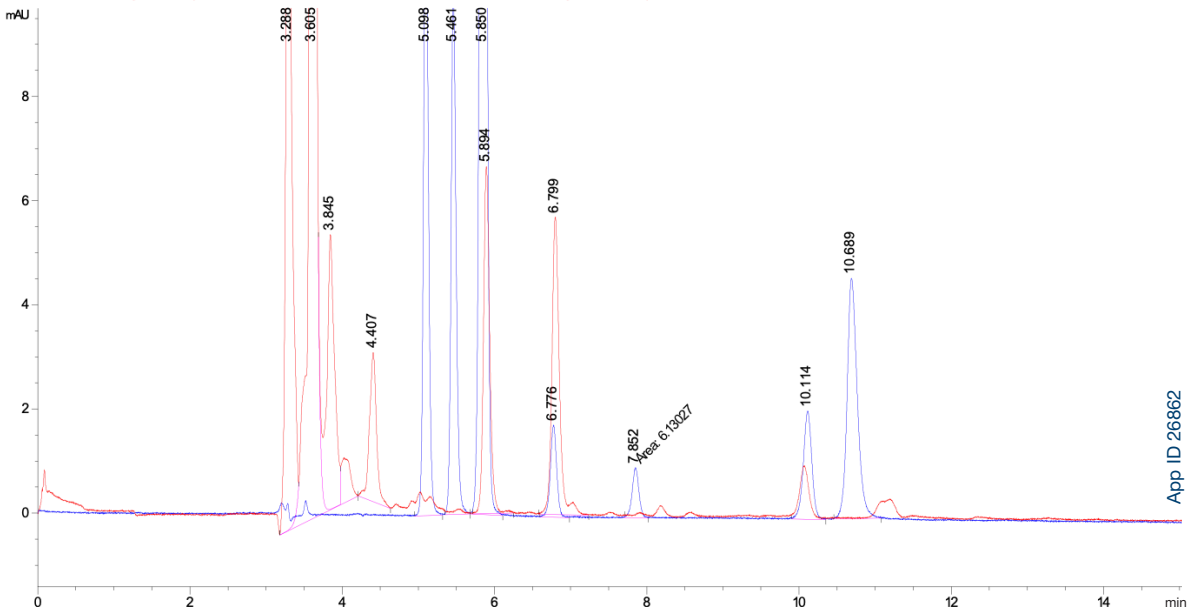


Figure 8.
Milk Sample Overlay Zoom-In



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