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WHITE PAPER

Using One Method for Screening Multiple Analytes of the Same Chemical Family: HPLC and Organic Acids

INTRODUCTION

Organic acids serve a variety of functions in food such as preservation, flavor, and nutrition. Some organic acids are naturally present in fruit and offer many health benefits. To evaluate organic acid content across fruit juices, the proper analytical tools for product investigation are necessary. Eurofins EAG Materials Science can identify and quantify the different organic acids using High Performance Liquid Chromatography (HPLC). This note demonstrates the abilities that Eurofins EAG has to quickly and accurately screen for common organic acids in fruit juices.

In this study, the objective was to screen four types of fruit juices (tomato, apple, orange, and coconut) for organic acids using high performance liquid chromatography with diode array detection (HPLC-DAD). A summary of test samples is presented in Table 1. This technique can utilize a variety of mobile phases, columns, and detectors to separate and identify specific components in simple to complex soluble sample matrices. The diode array detector is used to measure the sample's absorption of light at different wavelengths to identify a specific analyte.

Table 1: Description of fruit juice samples

Sample ID	Sample Description
S1	Tomato Juice
S5	Apple Juice
S6	Orange Juice
S17	Coconut Juice

ANALYSIS

For analysis, the samples were diluted in water and analyzed against organic acid reference standards. The standards include oxalic acid, tartaric acid, malic acid, citric acid, succinic acid, and ascorbic acid. A summary of potential therapeutic applications from these selected organic acids are presented in Table 2.

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Table 2: Description of screened organic acid and respective fruits and health problems/benefits

Organic Acid	Fruits Rich in Specified Acid	Health Problems/ Benefits
Oxalic Acid[1]	Apricot Figs Kiwi Plum	Reduces mineral absorption, can contribute to kidney stones
Tartaric Acid[2]	Grapes Apricots Banana Avocado	Antioxidant and anti-inflammatory, aids in digestion
Malic Acid[3]	Apple Watermelon Banana Blackberry	Treat kidney stones, prevent dry mouth, reduce ageing signs, treat acne, promote skin hydration
Citric Acid[4]	Lemon Lime Orange Grapefruit	Antioxidant, prevent kidney stones, clear skin
Succinic Acid[5]	Broccoli Rhubarb Beets Asparagus	Reduces arthritis and joint pain, symptoms related to menopause, reduces fatigue
Ascorbic Acid[6]	Cantaloupe Citrus fruits Kiwi Berries	Protection against immune system deficiencies, cardiovascular disease, prenatal health problems, eye disease, skin wrinkling

A summary of the organic acids screen is presented in Table 3 below. The data suggests the juices contain varying levels of oxalic acid, tartaric acid, malic acid, citric acid, succinic acid, and ascorbic acid. Citric acid and ascorbic acid are one of the most common organic acids in fruits and were quantitated.

Table 3: Detection of organic acids in fruit juice samples
Fruit Juice Samples: X = Present, ND = Not Detected

Organic Acid	S1 Tomato Juice	S5 Apple Juice	S6 Orange Juice	S17 Coconut Juice
Oxalic Acid	X	X	X	X
Tartaric Acid	ND	X	ND	ND
Malic Acid	ND	X	X	X
Citric Acid	X	X	X	X
Succinic Acid	X	ND	ND	ND
Ascorbic Acid	X	X	X	ND

Table 4 – Table 7 summarize the results of the quantitation of citric acid in tomato juice, apple juice, orange juice, and coconut juice. The quantitation was carried out using high performance liquid chromatography with diode array detection (HPLC-DAD). For quantitation, a five-point calibration curve was generated and observed to have an R2 of 0.999. The samples were fortified with a known amount of citric acid to assess accuracy and the spike recovery was observed to be 91% (tomato juice), 92% (apple juice), 99% (orange juice), and 98% (coconut juice). See Figure 1 for a representative chromatogram from the citric acid quantitation analysis of the samples.

Table 4: Quantitation of citric acid in S1, tomato juice

Sample Descrip.	Prep. No.	Injection No.	Citric Acid Concen. (wt%)[1]	Avg. Citric Acid Concen. (wt%)[1]	% RSD[2]
S1 Tomato Juice	1	1	1.56%	1.61%	2.6%
		2	1.66%		
	2	1	1.60%		
		2	1.62%		

[1]wt % = weight percent; [2]% RSD = Relative Percent Standard Deviation

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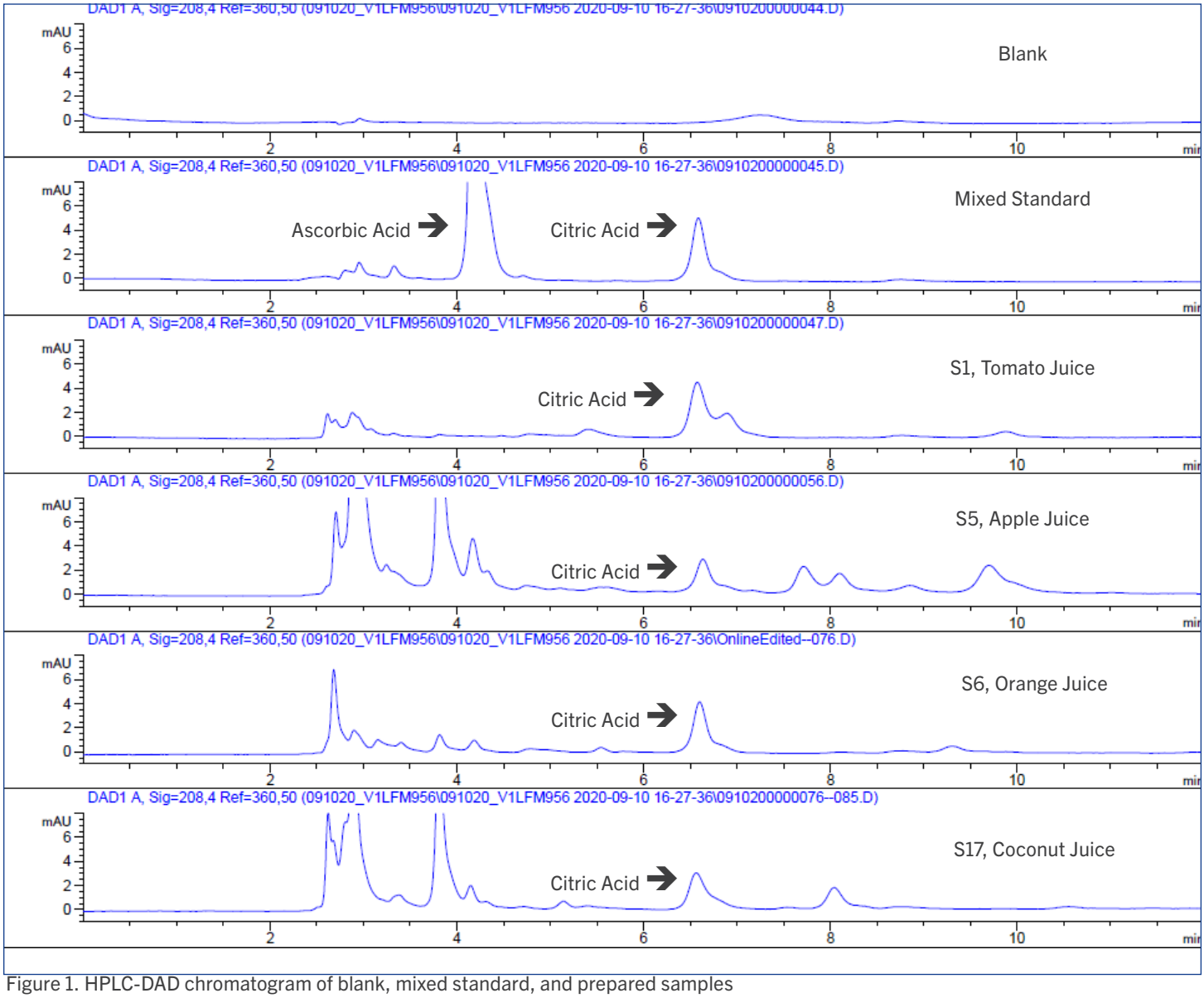

Figure 1. HPLC-DAD chromatogram of blank, mixed standard, and prepared samples

Table 5: Quantitation of citric acid in S5, apple juice

Sample Descrip.	Prep. No.	Injection No.	Citric Acid Concen. (wt%)[1]	Avg. Citric Acid Concen. (wt%)[1]	% RSD[2]
S5 Apple Juice	1	1	0.05%	0.05%	2.7%
		2	0.05%		
	2	1	0.05%		
		2	0.05%		

[1]wt % = weight percent; [2]% RSD = Relative Percent Standard Deviation

Table 6: Quantitation of citric acid in S6, orange juice

Sample Descrip.	Prep. No.	Injection No.	Citric Acid Concen. (wt%)[1]	Avg. Citric Acid Concen. (wt%)[1]	% RSD[2]
S6 Orange Juice	1	1	0.82%	0.81%	1.2%
		2	0.80%		
	2	1	0.82%		
		2	0.80%		

[1]wt % = weight percent; [2]% RSD = Relative Percent Standard Deviation

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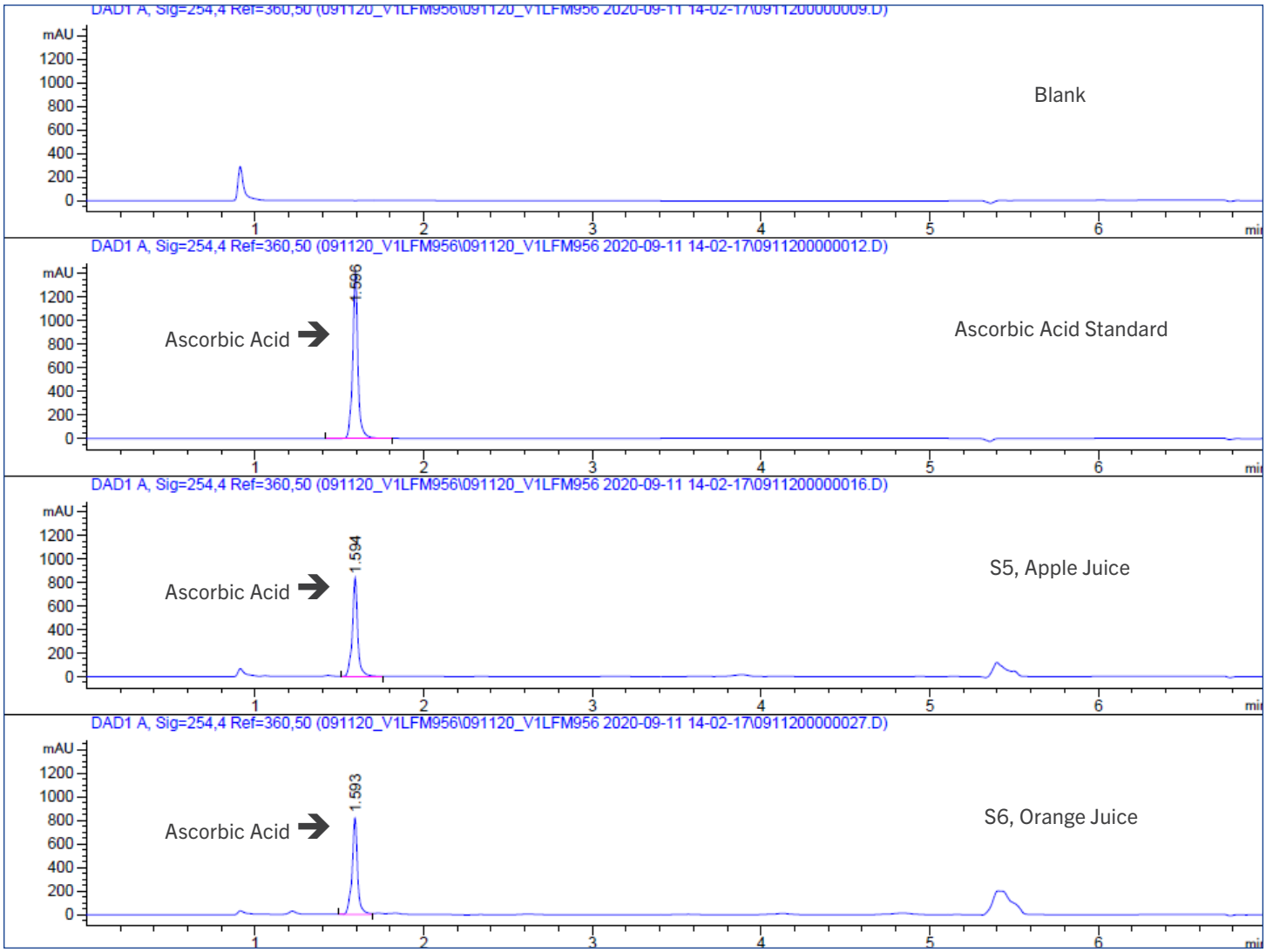

Figure 2. HPLC-DAD chromatogram of blank, ascorbic acid standard, and prepared samples

Table 7: Quantitation of citric acid in S17, coconut juice

Sample Descrip.	Prep. No.	Injection No.	Citric Acid Concen. (wt%)[1]	Avg. Citric Acid Concen. (wt%)[1]	% RSD[2]
S17 Coconut Juice	1	1	0.07%	0.07%	5.5%
		2	0.07%		
	2	1	0.07%		
		2	0.07%		

[1]wt % = weight percent; [2]% RSD = Relative Percent Standard Deviation

Table 8 and Table 9 summarize the results of the quantitation of ascorbic acid in apple juice and orange juice. The quantitation was carried out using high performance liquid chromatography with diode array detection (HPLC-DAD). For quantitation, a five-point calibration curve was generated and observed to have an R2 of 0.998. Note, ascorbic acid degrades when exposed to light and warm temperatures. As a result, all preparations were performed with cold diluent and in salinized vials. The samples were fortified with a known amount of ascorbic acid to assess accuracy and the spike recovery was observed to be 117% (apple juice) and 122% (orange juice). See Figure 2 for a representative chromatogram from the ascorbic acid quantitation analysis of the samples.

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Table 8: Quantitation of ascorbic acid in S5, apple juice

Sample Descrip.	Prep. No.	Injection No.	Ascorbic Acid Concen. (wt%)[1]	Avg. Ascorbic Acid Concen. (wt%)[1]	% RSD[2]
S5 Apple Juice	1	1	0.05%	0.05%	2.7%
		2	0.05%		
	2	1	0.05%		
		2	0.05%		

[1]wt % = weight percent; [2]% RSD = Relative Percent Standard Deviation

Table 9: Quantitation of ascorbic acid in S6, orange juice

Sample Descrip.	Prep. No.	Injection No.	Citric Acid Concen. (wt%)[1]	Avg. Citric Acid Concen. (wt%)[1]	% RSD[2]
S6 Orange Juice	1	1	0.82%	0.81%	1.2%
		2	0.80%		
	2	1	0.82%		
		2	0.80%		

[1]wt % = weight percent; [2]% RSD = Relative Percent Standard Deviation

SUMMARY

In summary, high performance liquid chromatography (HPLC) is a chromatographic technique that can separate different components across various samples matrices. This allows for screening for multiple specific analytes of the same chemical family, such as organic acids, in the same method. It also offers specificity, which enables distinction of one organic acid from another and means and quantifying specific organic acids in the presence of others. This approach can be useful for quality control, assessing label claims, reverse engineering studies, and comparison of different consumer products.

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