

# Analysis of Fruit Juices Adulterated with Medium Invert Sugar from Beets

Thermo Fisher Scientific Inc.

## Introduction

Fruit juice adulteration presents an economic and regulatory problem. The United States orange juice industry estimates that orange juice sales gross more than one billion dollars annually.<sup>1</sup> The most common forms of adulteration include simple dilution and blending of inexpensive and synthetically produced juices into the more expensive ones. The source of sweetener can be other juices or sugar derived from fruits or vegetables. One adulterant currently in use is partially inverted sucrose, wherein about one-half of the sucrose has been hydrolyzed to glucose and fructose. This ratio of approximately 1:1:2 (glucose: fructose: sucrose) closely matches the ratio found in orange juice. Figures 1 and 2 show chromatograms of pure orange juice and medium invert sugar samples, respectively. When cane sugar is the source of inverted sucrose, Stable Isotope Ratio Analysis (SIRA) can be used to identify adulterated juices because the ratio of  $^{13}\text{C}$  to  $^{12}\text{C}$  is different for sugars in orange juice and cane sugar.<sup>2</sup> Beets, on the other hand, produce sugar via a metabolic pathway different from cane and similar to that of many fruits, so that the ratio of  $^{13}\text{C}$  to  $^{12}\text{C}$  is about the same for sugars in orange juice and beet sugar. This fact renders SIRA inadequate for detecting adulteration by beet sugar.

Recently, investigators using high performance anion exchange chromatography with pulsed amperometric detection (HPAE-PAD) have discovered several components in beet medium invert sugar (BMIS) that are not present in orange juice.<sup>1,3,4</sup> Swallow, Low, and Petrus have suggested that a pattern of late-eluting components appearing at about 60 minutes be used to identify adulteration (Method A herein). Tsang and coworkers have used raffinose—a trisaccharide of D-glucose, D-fructose, and D-galactose—as a marker for orange juice adulteration (Method B).<sup>3,4</sup> A third method (presented herein as Method C), similar to that of Swallow, et al., uses only one analytical column and also exhibits a pattern of late-eluting components indicative of adulteration by BMIS.



Conditions and illustrative chromatograms for each method are included in this application note. The selectivity of anion-exchange chromatography, especially for oligosaccharides, and the sensitivity and specificity of pulsed amperometric detection make HPAE-PAD uniquely suited to this analysis. For further information about HPAE-PAD, please refer to Thermo Scientific Technical Note 20: *Analysis of Carbohydrates by High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection*.<sup>5</sup>

## Equipment

Any Dionex chromatographic system\* consisting of:

- Advanced Gradient Pump (AGP)
- Liquid Chromatography Module
- Pulsed Electrochemical Detector or Pulsed Amperometric Detector
- Thermo Scientific Dionex AI-450 Chromatography Workstation\*\*

\*Equivalent or improved results can be achieved using the Thermo Scientific Dionex ICS-5000+ system.

\*\*Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System (CDS) Software, version 7.2 can be used.

**Conditions****Method A (As described by Swallow, et al.)**

Columns:	2 Thermo Scientific™ Dionex™ CarboPac™ PA1, 4 × 250 mm			
Eluent 1:	0.1 M Sodium hydroxide			
Eluent 2:	0.1 M Sodium hydroxide, 0.1 M Sodium acetate			
Eluent 3:	0.3 M Sodium hydroxide			
Gradient:	Time	%E1	%E2	%E3
	0–4 min	100	0	0
	4–20	100–97	0–3	0
	20–50	97–0	3–100	0
	50–60	0	100	0
	60	0	0	100
	All gradient steps are linear (AGP curve 5)			
Flow Rate:	0.70 mL/min			
Inj. Vol.:	100 µL			
Expected Pressure:	1400–2000 psi (10–14 MPa)			
Postcolumn Reagent:	0.3 M Sodium hydroxide			
Postcolumn Flow Rate:	0.8 mL/min			
Detection:	Pulsed amperometry, gold working electrode PAD Settings:			
	t(ms)	E(volt)*		
	120	0.05		
	120	0.80		
	420	–0.60		
Sample Prep.:	As described in ref. 1.			

**Method B (Raffinose as Adulteration Marker)**

Column:	Dionex CarboPac PA1, 4 × 250 mm			
Eluent:	0.10 M Sodium hydroxide			
Flow Rate:	1.0 mL/min			
Inj. Vol.:	50 µL			
Expected Pressure:	700–1000 psi (5–7 MPa)			
Detection:	Pulsed amperometry, gold working electrode PED program 1, or PAD Settings:			
	t(ms)	E(volt)*		
	480	0.05		
	120	0.60		
	60	–0.60		
	*Potentials are referenced to Ag/Ag(I).			
Sample Prep.:	Centrifuge at 16,000 G for 15 min. Dilute supernatant to 1/100 original concentration with deionized water. Filter through a 0.2 µm filter.			

**Method C (One-Column Alternative to Method A)**

Column:	Dionex CarboPac PA-100, 4 × 250 mm			
Eluent 1:	0.15 M Sodium hydroxide			
Eluent 2:	0.15 M Sodium hydroxide, 0.15 M Sodium acetate			
Gradient:	Time	%E1	%E2	Curve
	0–1 min	99	1	5
	1–20	99–0	1–100	9
	Equilibrate 10 minutes at starting conditions before each injection.			
Flow Rate:	1.0 mL/min			
Inj. Vol.:	25 µL			
Expected Pressure:	700–1000 psi (5–7 MPa)			
Detection:	Pulsed amperometry, gold working electrode PED program 1, or PAD Settings:			
	t(ms)	E(volt)*		
	480	0.05		
	120	0.60		
	60	–0.60		
	*Potentials are referenced to Ag/Ag(I).			
Sample Prep.:	Dilute sample to 1/10 original concentration with deionized water. Filter through a 0.2 µm filter.			

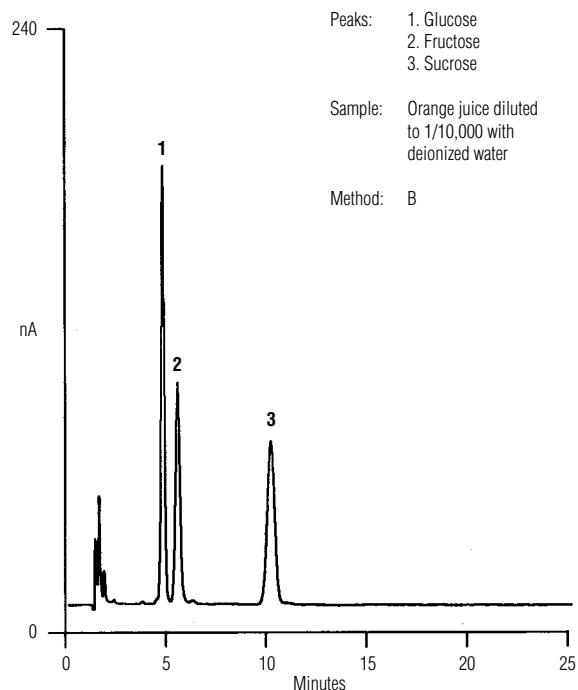


Figure 1. Orange juice analyzed by Method B.

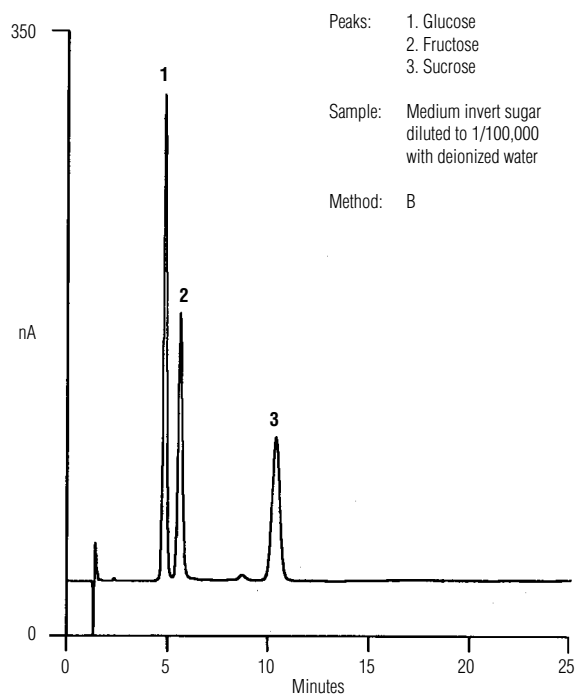


Figure 2. Medium invert sugar analyzed by Method B. This profile looks similar to the profile for pure orange juice in Figure 1.

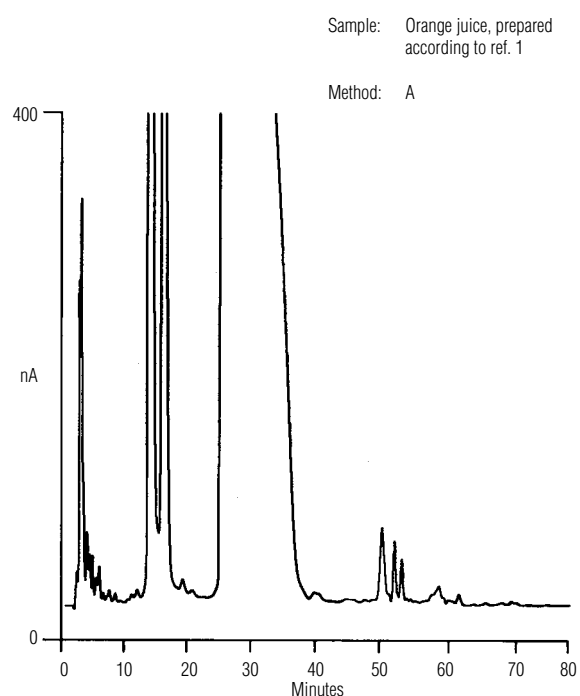


Figure 3. Orange juice analyzed by Method A.

## Discussion

Methods A (Figures 3 and 4) and C (Figures 5 and 6) rely on the analyst's ability to discern normal concentrations of these late-eluting components from elevated concentrations caused by adulteration. Raffinose is not found in pure orange juice (Figure 7), so its presence indicates BMIS adulteration, though not necessarily an exact measure of the extent of adulteration (as determined by Method B). The chromatogram in Figure 8 shows the presence of raffinose in BMIS.<sup>†</sup> A sample of pure orange juice which had been 12% adulterated with BMIS (Figure 9), was determined to contain 220 ng/mL of raffinose.

Each lot of BMIS may vary slightly in raffinose content and in the content of the unidentified late-eluting components. These facts make the precise determination of the extent of adulteration difficult, but any of these methods can be used to estimate adulteration levels above about 5%.

Method A requires extensive sample preparation. The elapsed time for preparing a sample is 3 to 5 days. In contrast, Methods B and C require less than 30 minutes per sample. In each case, sample throughput can be improved by preparing several samples in parallel.

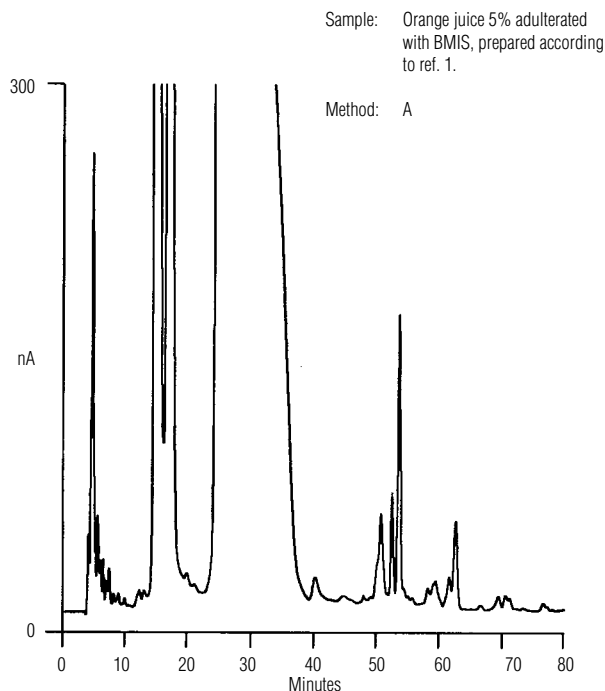


Figure 4. Orange juice adulterated with medium invert sugar, analyzed by Method A. Note the late-eluting fingerprint between 50 and 60 minutes.

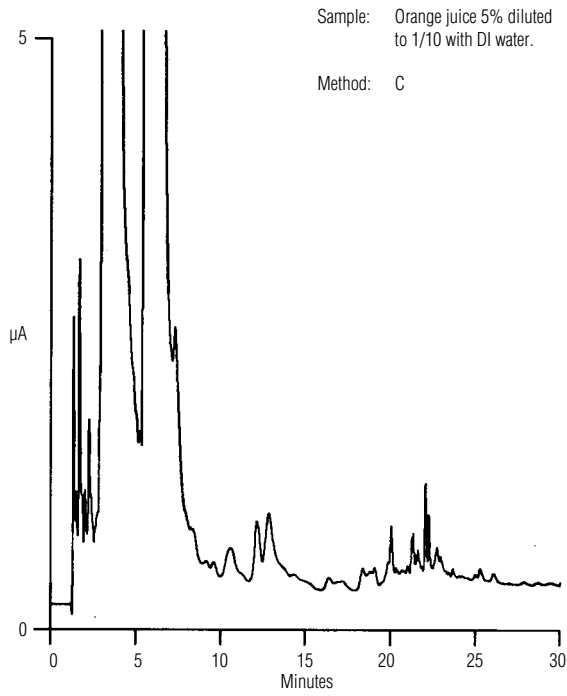


Figure 5. Orange juice analyzed by Method C.

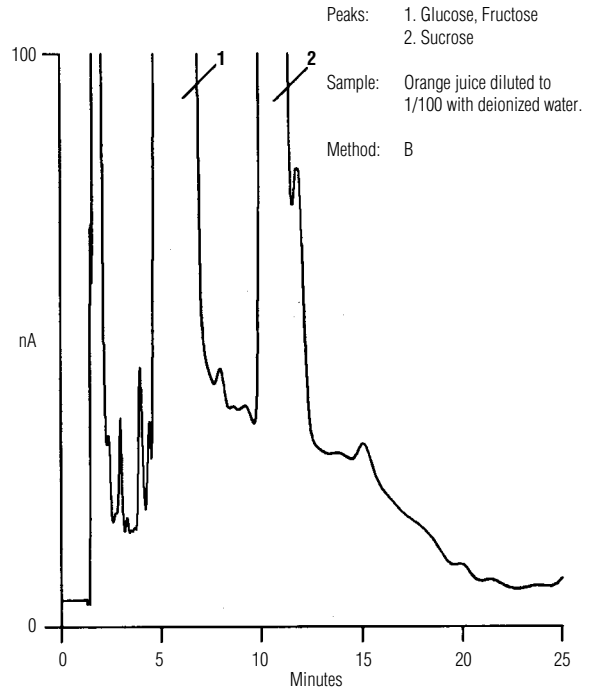


Figure 7. Orange juice analyzed by Method B. Note the lack of any peaks eluting at 20 minutes.

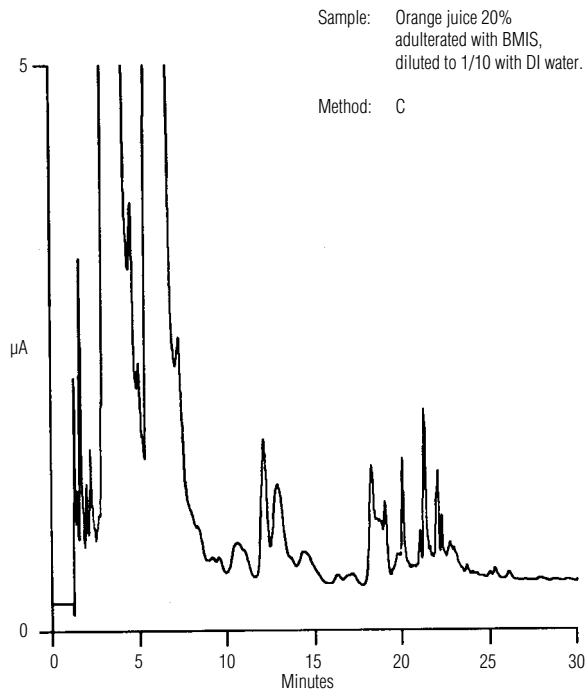


Figure 6. Orange juice adulterated with medium invert sugar, analyzed by Method C. Note the late-eluting fingerprint between 18 and 24 minutes.

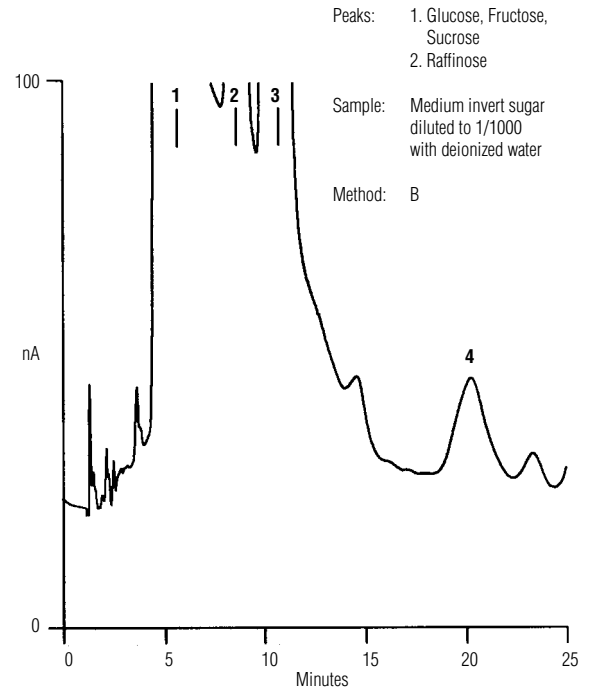
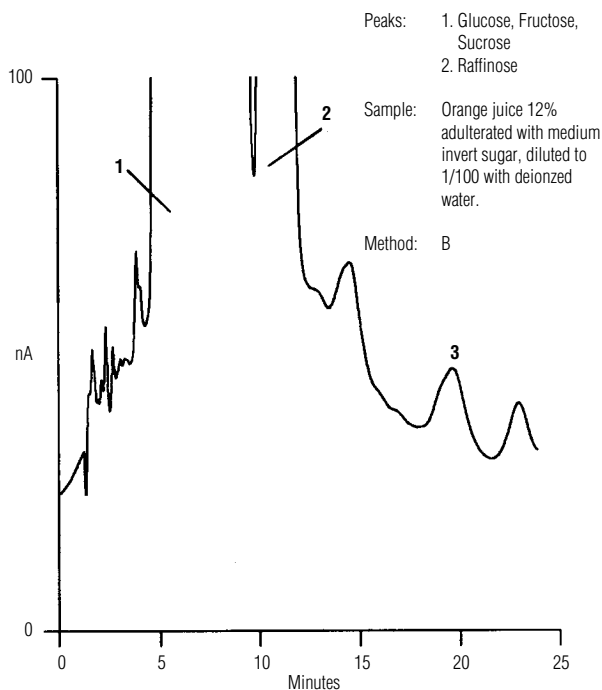


Figure 8. Medium invert sugar analyzed by Method B. Note the raffinose peak eluting at approximately 20 minutes.



## References

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- Tsang, W.S.C.; Cargel, G.L.R.; Clarke, M.A. *Proceedings of the 1990 Sugar Processing Research Conference* **1991**, 368.
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Figure 9. Orange juice adulterated 12% with medium invert sugar, analyzed by Method B. Adulteration can be detected by the presence of raffinose.

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