Pulsed Amperometric Detection of Carbohydrates in Fruit Juices Following High Performance Anion Exchange Chromatography

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Pulsed amperometric detection following liquid chromatography was used for the determination of carbohydrates in fruit juices. Carbohydrates were separated isocratically using 100 mM NaOH on an alkaline-tolerant anion-exchange column. Pulsed waveforms were generated using ChromGraph® software with the BAS DA-5 interface and LC-4C electrochemical detector. Juice samples were diluted, injected, and detected directly at a gold electrode following separation. Three sugars emerged as prominent and common to fruit juices bottled from concentrate: glucose, fructose, and sucrose. Detection of these compounds yielded detection limits of 0.7 ng (0.2 µM), 1.4 ng (0.4 µM), and 4.1 ng (0.6 µM) for glucose, fructose, and sucrose respectively. This applications paper describes quantitation of these sugars in fruit juice samples.

In the past 15 years, liquid chromatography followed by pulsed amperometric detection (LC-PAD) has become an important method for the separation and detection of polar aliphatic compounds. These compounds typically have poor detection properties, and their detection by optical methods requires derivatization. Also, dc amperometric detection, when possible, leads to diminishing response due to electrode fouling. PAD combines amperometric detection at a noble metal electrode with positive and negative potential excursions for "on-line" cleaning and reactivation of the electrode. The result is direct detection of compounds containing amine, alcohol, or sulfur moieties. PAD and its applications have been reviewed [1-3].

Perhaps the application most responsible for the popularity of PAD is that of carbohydrate analysis [4,5]. Carbohydrates are naturally occurring in many biological systems, and they are often used as food additives. Their determination often requires separation prior to detection. With alkaline-tolerant polymeric columns, separation of carbohydrates is possible using anion-exchange chromatography. Monosaccharides are eluted isocratically with hydroxide-containing eluent, while larger carbohydrates can be separated using an acetate gradient. Since PAD requires alkaline conditions for detection of carbohydrates at a gold electrode, the sugars can be directly detected following separation. This paper will examine the determination of common sugars, the figures of merit for such analyses, and application of this detection method to the determination of these sugars in fruit juices.

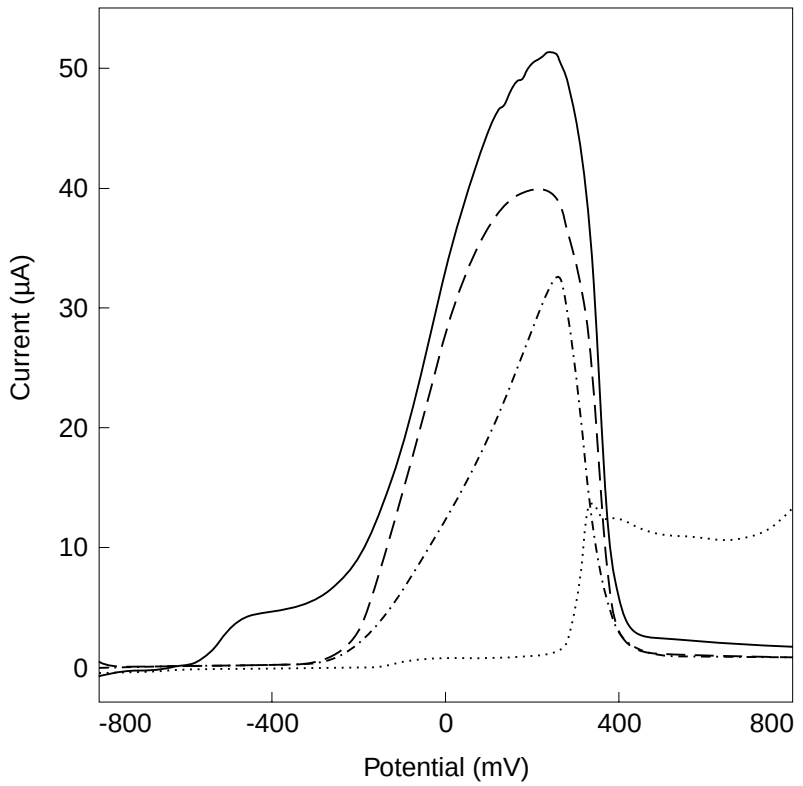
EXPERIMENTAL

Apparatus

Voltammetric data were obtained at a gold rotating disk electrode (RDE) using a Model AFMSRX rotator and a Model AFRDE4 potentiostat (Pine Instrument Co., Grove City, PA). Potentiostat control and data acquisition

F1

Pulsed voltammetric response as a function of E_{det} for glucose, fructose, and sucrose at a Au RDE in 100 mM NaOH. Rotation speed: 900 rpm. Residual response (···) shown for reference. Background subtracted responses shown for 0.2 mM solutions of glucose (—), fructose (– –), and sucrose (– · –).



were accomplished with a 286/16 MHz IBM™ compatible computer interfaced using a DAS-20 AD/DA expansion board (Keithley Data Acquisition, Taunton, MA). Pulsed voltammetric waveforms were generated using ASYST scientific software (Asyst Software Technologies, Inc., Rochester, NY).

The Au RDE used was 3.0 mm in diameter (Pine), and the auxiliary electrode used was Pt wire. All electrode potentials are reported versus a Ag/AgCl reference electrode (Model 13-620-45; Fisher Scientific, Pittsburgh, PA). The electrochemical cell (ca. 125 mL) was constructed of Pyrex glass with two side arms separated from the cell body with fine glass frits.

Chromatographic instrumentation consisted of an Advanced Gradient Pump (Dionex Corporation, Sunnyvale, CA). The injection valve (Rheodyne, Cotati, CA) was fitted with a 20 µL injection loop. The column was a CarboPac PA1 anion-exchange column (Dionex). The flow rate was 1.00 mL/min.

PAD was accomplished using an LC-4C Amperometric Detector (Bioanalytical Systems, Inc. (BAS), West Lafayette, IN). Output of waveform and data collection were controlled by ChromGraph® software (BAS) interfaced using a DA-5 ChromGraph Interface (BAS) to a 486 IBM™ compatible computer. The detection cell was comprised of a 1.6 mm Au working electrode, a Ag/AgCl reference electrode (MF-2021, BAS) and a stainless steel auxiliary electrode. The cell was housed in a grounded CC-5 liquid chromatography column and cell compartment (BAS).

Reagents

Carbohydrates were reagent grade (Sigma Chemical Co., St. Louis, MO). Mobile phase was prepared from 50% w/w NaOH (Fisher Scientific), and filtered with 0.2 µm filters (Rainin Corp., Woburn, MA) and a solvent filtration apparatus (Microfiltration Systems, Rainin). Mobile phase was deaerated with dispersed N_2. Water was purified using a reverse-osmosis system coupled with multi-tank/ultraviolet/ultrafiltration (U.S. Filter/IONPURE, Lowell, MA).

Procedure

Pulsed voltammetry (PV) scans were generated by using the PAD waveform and incrementing the detection potential over a defined range. This technique has been described in optimizing the PAD waveform for use with carbohydrates [6]. PV scans were first run on a blank solution and repeated after addition of analyte. Background corrected PVs were generated by subtracting residual response from analyte response.

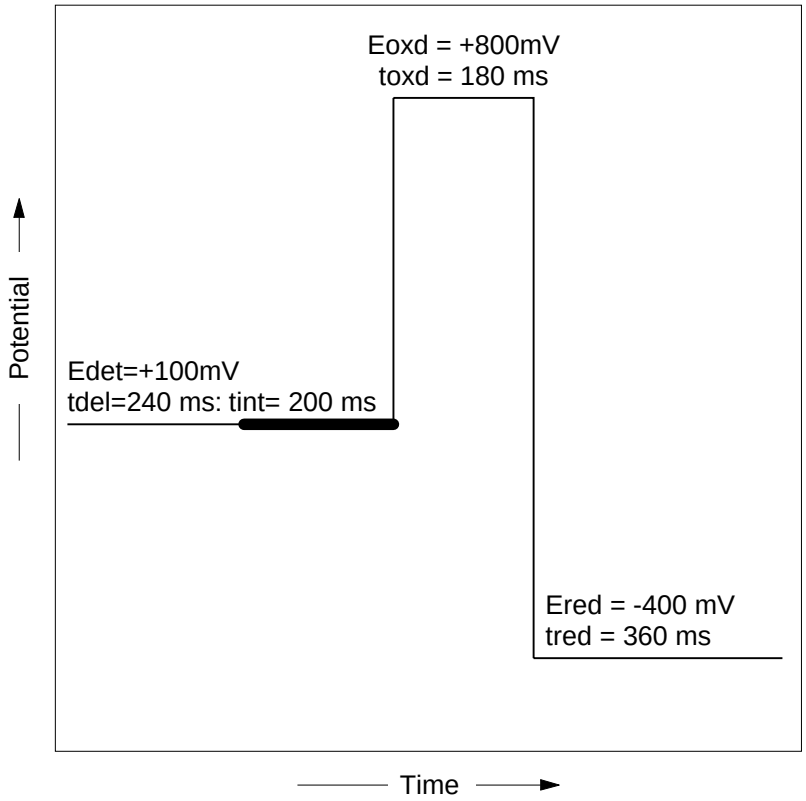
Results and Discussion

Pulsed Voltammetry

F1 shows the background-corrected current to potential (i-E) plot for glucose (—), fructose (– –), and sucrose (– · –) at a Au RDE in 100 mM NaOH. The residual response (····), shown for reference, shows an anodic response beginning at ca. +300 mV corresponding to the formation of surface gold oxide. Glucose, an aldohexose, shows anodic response commencing at ca. -600 mV due to the oxidation of the aldehydic group to the corresponding carboxylate group. The increased response at ca. -300 mV through ca. +400 mV corresponds to oxidation of hydroxyl groups. This response is also present for fructose, a ketohexose, and sucrose, a disaccharide. Response is attenuated beyond ca. +400 mV due to surface oxide formation inhibiting detection of carbohydrates. A detailed explanation of the mechanisms of detection in PAD can be found in several reviews [1,7]. Inspection of the background-subtracted PVs indicates that optimum potential for the detection of these sugars is ca. +200 mV. Although +200 mV is the optimal detection potential, +100 mV was chosen for this application in order to achieve selectivity of carbohydrates over any free amino acids (many of which are also detected using PAD at potentials greater than ca. +150 mV [8]) possibly found in the fruit juice. In addition, the selection of -400 mV for

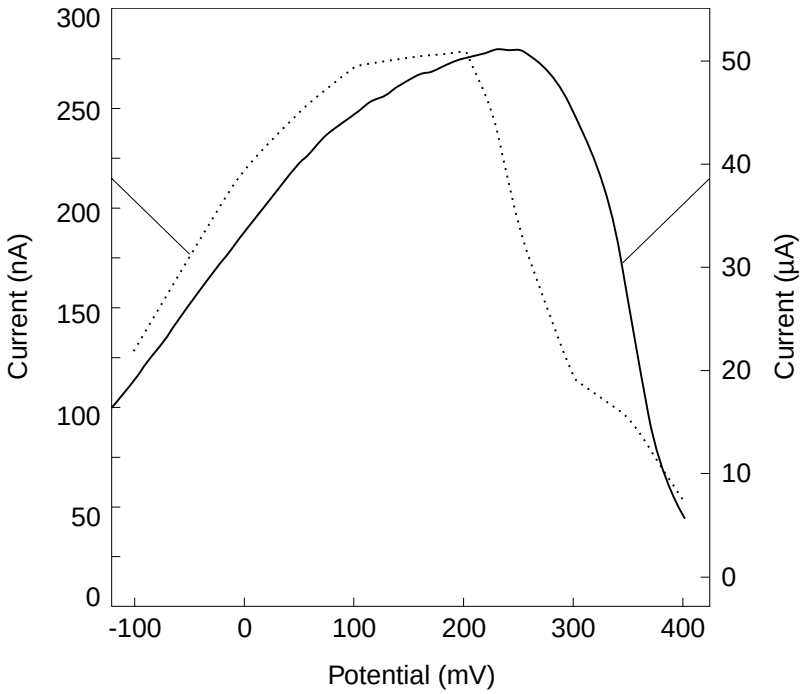
F2

PAD waveform used in this study. (det=detection, del=delay, int=integration, oxd=oxidation, red=reduction.)



F3

Voltammetric response of glucose. HDV (···) and PV (—). HDV conditions: 12 µM glucose, 100 mM NaOH at 1 mL/min. PV conditions: 200 µM glucose, 100 mM NaOH, 900 rpm.



T1

Response values at various DA-5 filter settings.

Filter Setting (Hz)	Peak Height (nA)	Baseline Noise (nA)	S/N
0.051	20.02 ± 0.47	1.08	18.5 ± 0.4
0.076	21.59 ± 0.08	1.25	17.3 ± 0.1
0.102	23.38 ± 0.41	1.60	14.6 ± 0.3
0.153	21.65 ± 0.31	1.52	16.2 ± 0.2
0.254	24.54 ± 0.66	4.00	6.1 ± 0.2

reduction potential has shown to give selectivity over amine-based compounds in general [9]. ***F2*** shows the PAD waveform with the values used for these studies. Optimization of all waveform parameters is discussed elsewhere [6,10].

LC-PAD

In order to validate the PV results done at the RDE, the response of glucose to the PAD waveform in the flow-through cell was examined using hydrodynamic voltammetry (HDV). Here glucose is injected and detected in consecutive runs in which the detection potential in PAD is changed incrementally. Peak height is plotted versus detection potential. The resulting hydrodynamic voltammogram yields information about analyte response under actual chromatographic conditions. ***F3*** shows that the response of glucose, determined by HDV, is predicted by the PV results. This profile supports the choice of +100 mV for detection potential, showing that little is lost in sensitivity in using a lower potential, which results in increased selectivity.

The DA-5 is equipped with digital filters which can be selected by the user. In order to use the system under optimized conditions, the filters were examined against resulting signal-to-noise. While background noise patterns are common in all settings, the likelihood of discriminating a narrow peak from background noise was higher with some filtering. ***T1*** shows the results for injecting glucose at each filter setting. In general as filtering frequency increased, so did the peak height for injection of 1.0 µM glucose, as well as the noise. The ratio of these values ultimately determines sensitivity of a method. A setting of 0.051 Hz was chosen, because it gave the highest S/N ratio.

Under alkaline conditions, weakly-acidic carbohydrates are present as anions, and are amenable to separation by anion-exchange chromatography. ***F4*** shows the elution of several carbohydrates. The first eluting peaks are sugar alco-

T2

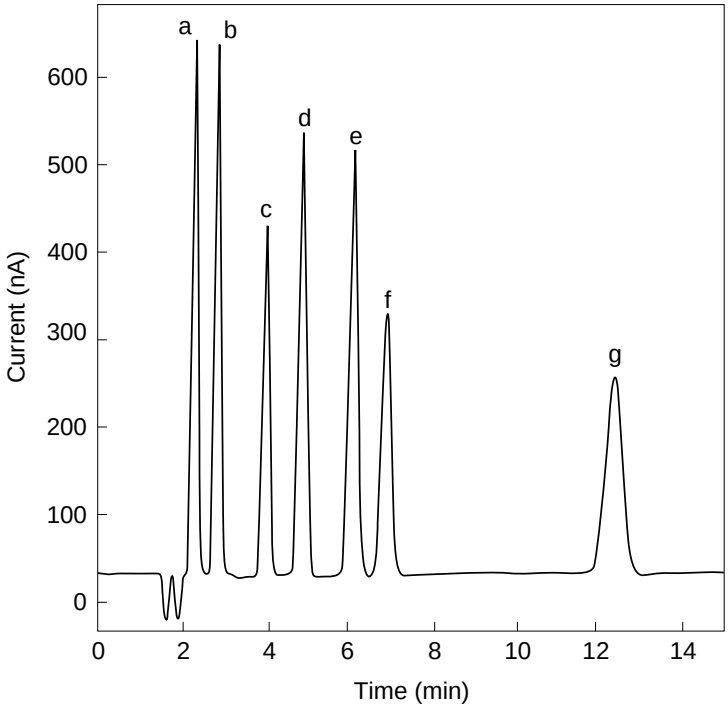
Analytical figures of merit for sugars found in fruit juices

Compound	Linear Range nA= *a*(μM)+*b*					Repeatability
	LOD* (ng, μM)	*a*	*b*	R^2	Deviation from Linearity	%RSD (μM, n)
Glucose	0.7, 0.2	16.4	19.8	0.9999	500 μM	0.7 (12, 6)
Fructose	1.4, 0.4	10	23.5	0.9999	500 μM	0.6 (12, 6)
Sucrose	4.1, 0.6	6.5	46.6	0.9998	100 μM	0.9 (34, 6)

* Calculated at S/N = 3 from injection within a S/N = 5.

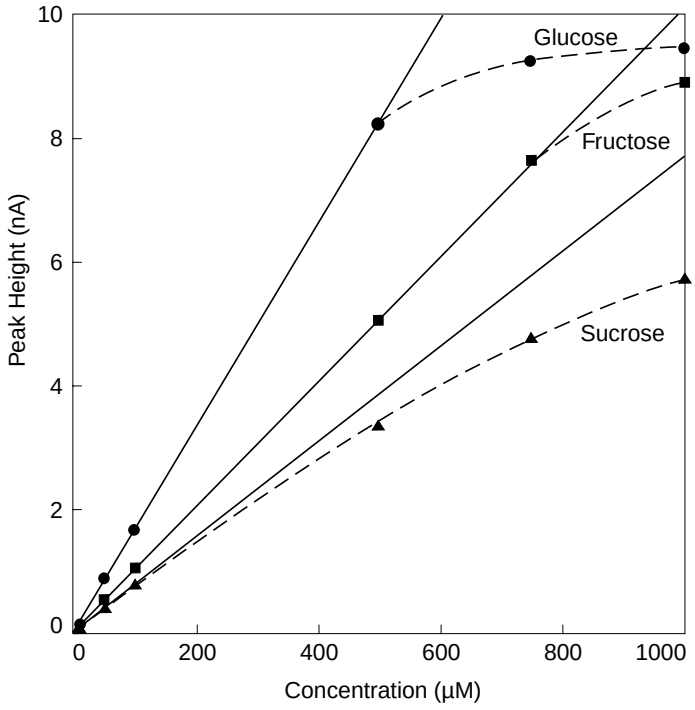
F4

Separation of common carbohydrates. Conditions: CarboPac PA1 Anion-Exchange column, isocratic elution with 100 mM NaOH at 1 mL/min. Detection as in Figure 2. Peak identification: (a) xylitol, (b) sorbitol, (c) rhamnose, (d) arabinose, (e) glucose, (f) fructose, (g) sucrose.



F5

Calibration curve for glucose, fructose, and sucrose. Conditions as in F4.

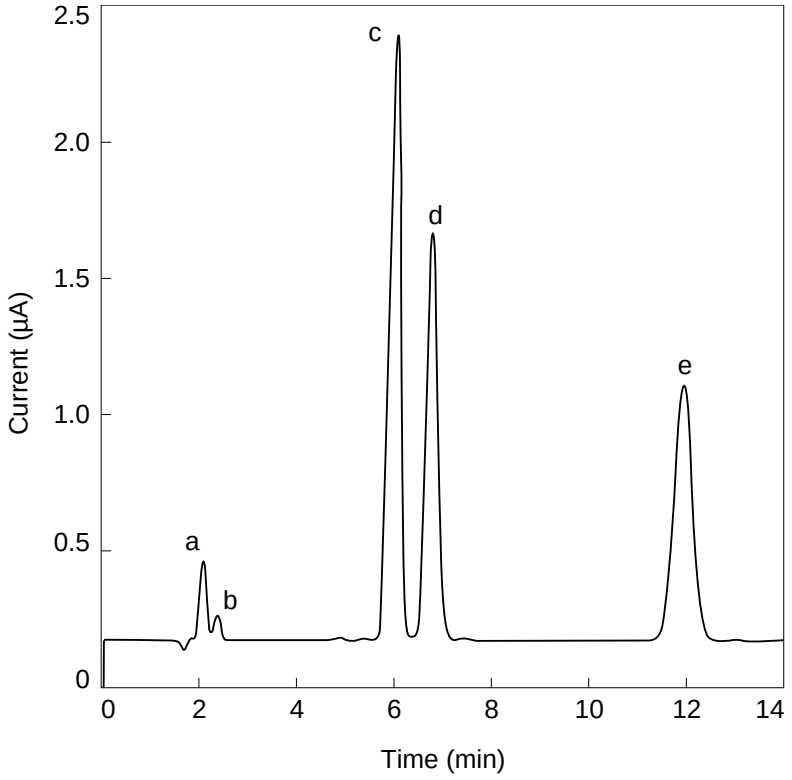


hols, followed by monosaccharides, and finally a disaccharide. These compounds show baseline resolution under isocratic conditions of 100 mM NaOH. The peaks of interest for this study are those which were prominent and common in most fruit juices: glucose, fructose, and sucrose. Calibration curves for these sugars are shown in ***F5***. As can be seen, the response for each is linear through 100 μM. Beyond this concentration, sucrose first deviates from linearity. Glucose and fructose depart from linearity at higher concentrations (***T2***). This phenomena has been discussed previously [10]. The analytical figures of merit for these three sugars are listed in ***T2***. Limits of detection were 0.7 ng (0.2 μM), 1.4 ng (0.4 μM), and 4.1 ng (0.6 μM) for glucose, fructose, and sucrose respectively. Repeatability for each compound was below 1% relative standard deviation.

This method was applied to the determination of carbohydrates in fruit juices. Juice samples (Tropicana® and Everfresh® brands) were obtained from a local grocery store and diluted 1:1000 in water. The resulting solution was filtered using a syringe filter prior to injection in order to eliminate any fruit pulp from the chromatographic system. The resulting chromatograms were similar for each juice. Three major peaks were evident as common to most juices. ***F6*** is the chromatogram of diluted orange juice. The large peaks are identified by retention time as glucose, fructose, and sucrose. ***F7*** shows several smaller peaks which can be discerned from the baseline, conjectured to be other naturally occurring monosaccharides and/or free amino acids. While three sugars emerged as common to all tested juices, the ratios of these sugars is unique to each juice. ***T3*** lists the sugar concentrations for each juice tested. Grape juice contains a large amount of both glucose and fructose, but no detectable sucrose. Less diluted samples of grape juice also showed no sucrose peak. Each juice was la-

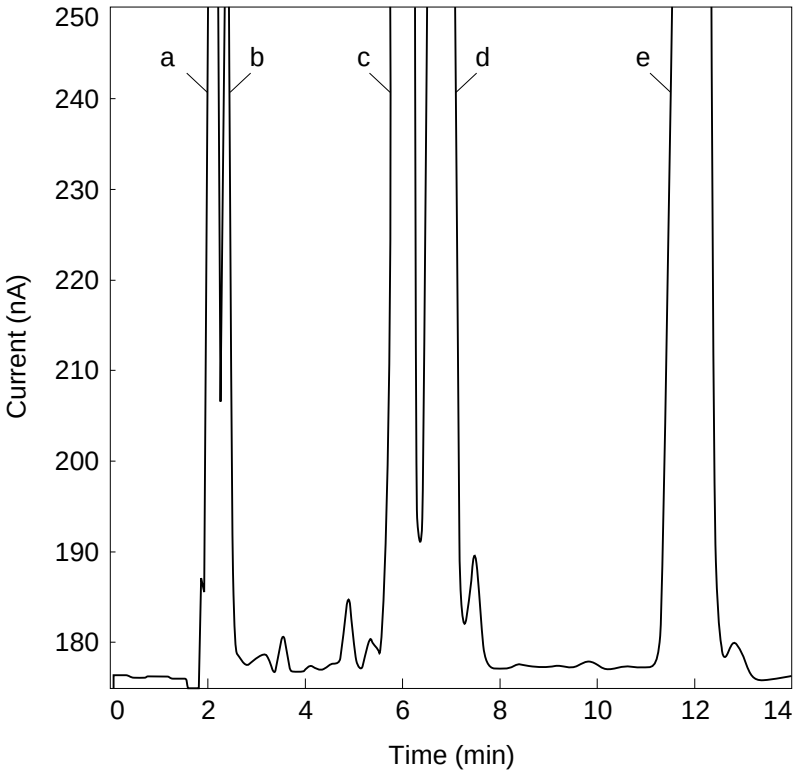
F6

Separation of carbohydrates found in orange juice. Peak identification: (A) xylitol, (B) sorbitol, (C) glucose, (D) fructose, and (E) sucrose. Conditions as in Figure 4.



F7

Expansion of baseline from Figure 6.



T3

Quantitative results of amounts of sugars in fruit juices.

Juice	Sugar Concentration (mM)		
	Glucose	Fructose	Sucrose
Apple	163 ± 1	343 ± 2	41.8 ± 0.2
Cranberry	410 ± 2	324 ± 2	9.55 ± 0.04
Grape	412 ± 10	436 ± 12	ND
Grapefruit	132 ± 2	140 ± 3	75.7 ± 1.3
Orange	130 ± 2	139 ± 2	135 ± 2

beled as juice from concentrate and had high fructose corn syrup listed as an ingredient. These results do not reflect direct quantitation of the high fructose corn syrup. Such quantitation is possible using PAD, but would require a gradient program in order to elute the highly retained oligosaccharides.

Conclusions

The separation and direct detection of carbohydrates found in fruit juices using liquid chromatography followed by pulsed amperometric detection has been presented. Calibration of glucose, fructose, and sucrose reveals limits of detection below 1 µM, linearity up to 100 µM, and reproducibility under 1% relative standard deviation. Chromatograms of diluted juices showed similarity in the prominence of glucose, fructose, and sucrose. Differences between juices arose in the particular ratios of the major sugars. This method is applicable to determination of food quality or authenticity.

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