DIONEX

Application Note 193

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Determination of Additives in Carbonated Beverages

INTRODUCTION

The soft drink industry is one of the largest in the world, with revenue from sales of carbonated soft drinks totaling billions of dollars annually. While the recipes for these beverages remain proprietary, critical components used as preservatives and sweeteners are subject to analysis for quality control, and must be identified on the beverage's label.

Additives such as benzoate and sorbate function as preservatives. Citrate is used as both a preservative and a flavor agent. Caffeine is an integral part of the overall profile of most colas. Aspartame, acesulfame, and saccharin are three common artificial sweeteners which function as sugar substitutes. Many methods have been developed for determining sweeteners and additives in drink formulations.[1-4]

In this Application Note, we separate caffeine, sorbate, benzoate, citrate, aspartame, acesulfame and saccharin (structures are shown in Figure 1) in carbonated drinks in a single run using the Acclaim® Mixed-Mode WAX-1 column. This column features a new mixed-mode silica-based packing material that incorporates both hydrophobic and weak anion-exchange properties.[5] Unlike traditional reversed-phase substrates, the new packing features an alkyl longchain with an ionizable terminus. The column demonstrates great potential for separating a wide variety of samples that contain a mixture of anionic and neutral compounds, including food, beverage, pharmaceutical, and chemical samples.

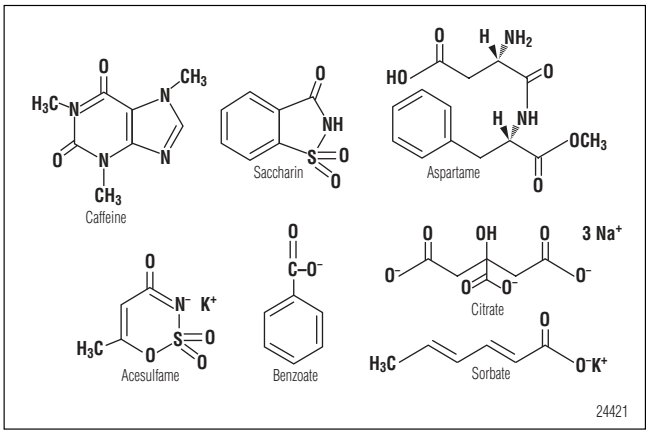

Figure 1. Structures of the seven analytes.

The Acclaim column's mixed-mode capability provides determination of the compounds of interest in many samples in a single injection, rather than two separate injections on two different types of columns.

Table 1. Preparation of Mixed Stock Standard Solution				
Analyte	**Concentration of Stock Standard (mg/L)**	**Volume of Added Stock Standard (mL)**	**Volume of Mixed Stock Standard (mL)**	**Concentration of Each Standard (mg/L)**
Caffeine	1400	1.43	100 (diluted with water)	20
Aspartame	500	12.00		60
Acesulfame	1000	20.00		200
Saccharin	1000	6.00		60
Sorbate	1000	10.00		100
Benzoate	1000	10.00		100
Citrate	1500	40.00		600

EQUIPMENT

UltiMate® 3000 HPLC system

HPG 3400A pump with SRD 3400 Solvent Rack w/degasser

TCC 3000 Thermostatted Column Compartment

WPS 3000TSL Autosampler

VWD 3400 UV/Vis detector

Chromeleon® 6.80 SP1 Chromatography Workstation

REAGENTS AND STANDARDS

Water, Milli-Q® Gradient A10

Methanol (CH_3OH) and acetonitrile (CH_3CN), HPLC grade, Fisher

Methanesulfonic acid (MSA), > 99.5%, Aldrich

Potassium dihydrogen phosphate (KH_2PO_4), analytical grade, SCRC, China

Caffeine, analytical grade, SCRC, China

Sorbate, analytical grade, SCRC, China

Benzoate, analytical grade, SCRC, China

Citrate, analytical grade, SCRC, China

Aspartame, analytical grade, Niutang Chemical Co. Ltd, China

Acesulfame, analytical grade, Supelco/Sigma Aldrich United States

Saccharin, analytical grade, Alfa Aesar China

PREPARATION OF STANDARDS

Stock standard solutions

The concentrations of stock standard solutions were 500 mg/L for aspartame, 1000 mg/L for acesulfame, saccharin, sorbate and benzoate, 1400 mg/L for caffeine, and 1500 mg/L for citrate.

Mixed stock standard solution

The mixed stock standard solution was prepared according to the procedure specified in Table 1.

Mixed working standard solutions

The mixed stock standard solution was diluted with mobile phase solution to prepare the mixed working standard solutions used for calibration. The concentrations of each analyte in the mixed working standard solutions are shown in Table 2.

Table 2. Concentrations of Mixed Working Standard Solutions									
Analyte	Concentration (mg/L)								
	# 1	# 2	# 3	# 4	# 5	# 6	# 7	# 8	# 9*
Caffeine	2	2.5	3.33	4	5	6.67	10	20	70
Aspartame	6	7.5	10	12	15	20	30	60	—
Acesulfame	20	25	33.3	40	50	66.67	100	200	—
Saccharin	6	7.5	10	12	15	20	30	60	—
Sorbate	10	12.5	16.7	20	25	33.3	50	100	—
Benzoate	10	12.5	16.7	20	25	33.3	50	100	—
Citrate	60	75	100	120	150	200	300	600	—

* This additional standard is prepared by adding 50 µL of the 1400 mg/L caffeine stock standard to 950 µL of mobile phase.

SAMPLE PREPARATION

Six bottled carbonated drinks were purchased from a local supermarket. Prior to injection, the samples were filtered though a 0.45-µm filter and diluted with mobile phase.

CHROMATOGRAPHIC CONDITIONS

Column:	Acclaim Mixed-Mode WAX-1, 5 µm, 4.6 × 150 mm (P/N = 064984)
Column Temp.:	30 °C
Mobile Phase:	120 mM KH_2PO_4/CH_3CN (45% : 55%, v/v, pH 3.0, adjusted with MSA)
Flow rate	1.5 mL/min
Inj. Volume:	5 µL
Detection:	Absorbance at 210 nm

RESULTS AND DISCUSSION

Optimized chromatographic conditions

Separations with the Acclaim Mixed-Mode WAX-1 column can be optimized by changing the following conditions of the mobile phase: 1) concentration of organic solvent, 2) ionic strength (buffer concentration), and 3) pH. The effects of changing these parameters are demonstrated by recording the retention time changes of a polar compound (4-hydroxybenozic acid) and nonpolar compound (butylbenzene) with controlled changes of the mobile phase.[6]

Retention time for the nonpolar compound was almost unchanged when the buffer concentration changed from 100 to 20 mM. It altered only slightly when pH was changed from 6 to 2.6, but increased significantly when the organic solvent concentration was decreased from 50% to 45%.

Retention time of the polar compound increased significantly when the buffer concentration was changed from 100 to 20 mM. Retention time decreased significantly when pH was changed from 6 to 2.6, and decreased slightly when the organic solvent concentration was decreased from 50% to 45%. Using these guidelines, the chromatographic conditions were optimized

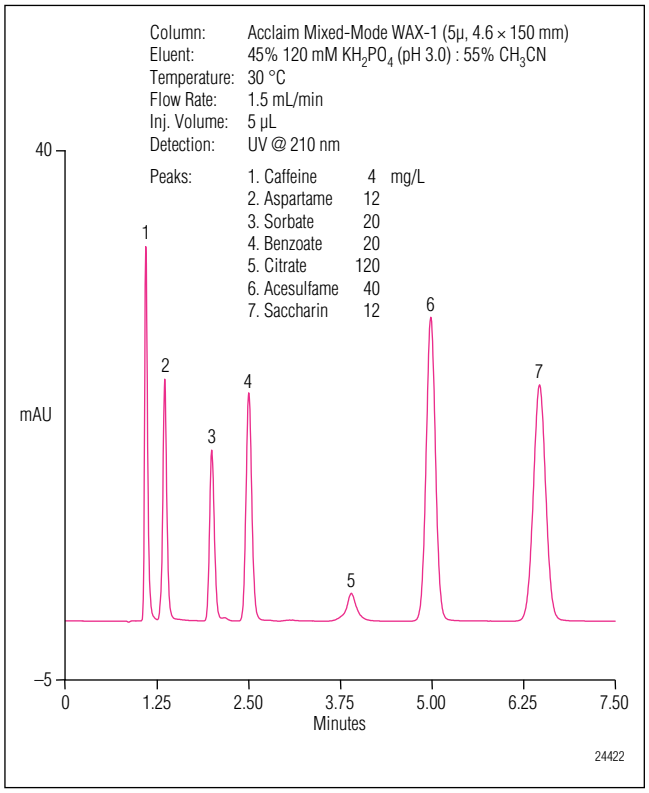

Figure 2. Chromatogram of mixed stock standard.

to obtain separation and baseline resolution of seven analytes in carbonated beverages (Figure 2).

Reproducibility, linearity and detection limits

Prior to sample analysis method reproducibility was demonstrated by making seven replicate injections of mixed stock standard solution. Table 3 summarizes the retention time and peak area precision data.

Table 3. Reproducibility of Retention Times and Peak Areas

Analyte	RT RSD (%)	A_{peak} RSD (%)
Caffeine	0.000	0.169
Aspartame	0.000	0.257
Sorbate	0.000	0.223
Benzoate	0.130	0.594
Citrate	0.091	1.221
Acesulfame	0.065	0.294
Saccharin	0.098	0.299

This table shows seven injections of mixed standard #4 (Table 2)

Calibration linearity for each of the seven compounds was determined by making replicate injections of a mixed standard prepared at eight different concentrations. The external standard method was used to calculate the calibration curve and to quantify each of the compounds in the six samples tested. Table 4 shows the calibration data. The single-sided Student's *t*-distribution was used to estimate method detection limits (MDL). These data are also reported in Table 4.

Sample analysis

Six different soft drinks were tested: one lemon-lime soda, one orange soda, two colas, and two diet colas. The diet colas contained no added sugar. Figures 3-8 show chromatograms of each sample and the same sample spiked with a mixed standard. Results, amounts

Table 4. Calibration Data and MDLs

Analyte	Equations	r	RSD (%)	MDL (mg/L)
Caffeine	A = 0.3700C + 0.0181	0.9999	1.6036	0.6
Aspartame	A = 0.1043C + 0.0735	0.9998	1.2287	0.2
Sorbate	A = 0.0520C + 0.0417	0.9995	1.8813	1.1
Benzoate	A = 0.0829C + 0.1198	0.9992	2.3464	1.2
Citrate	A = 0.0028C – 0.0774	0.9988	2.6191	8.5
Acesulfame	A = 0.0917C + 0.1352	0.9998	1.2345	1.2
Saccharin	A = 0.3143C + 0.1499	0.9999	1.6036	0.4

Note: The single-sided Student's *t* test method (at the 99% confidence limit) was used to determine MDL, where the standard deviation (SD) of the peak area of seven injections is multiplied by 3.14 (at n = 7) to yield the MDL

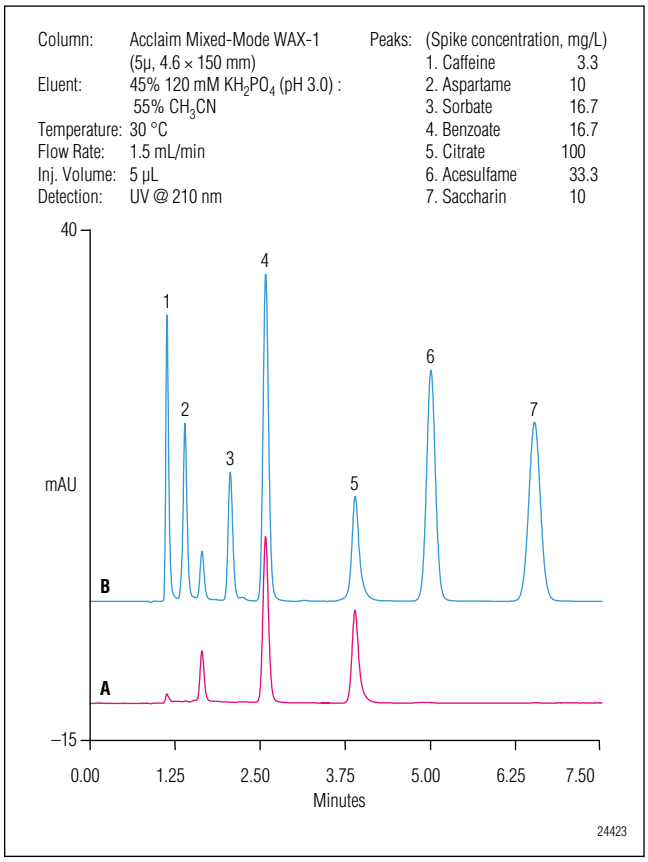

Figure 3. Overlay of chromatograms of a lemon-lime carbonated beverage, diluted ten-fold (A) and the same sample spiked (B).

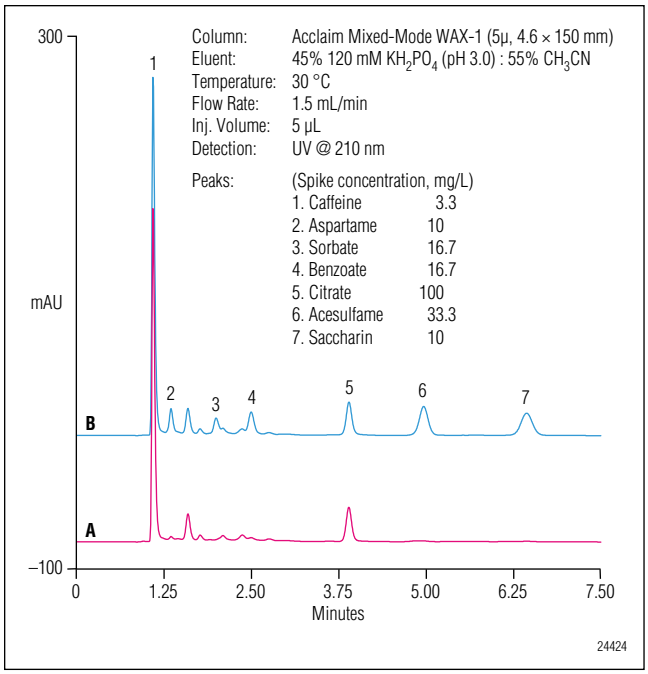

Figure 4. Overlay of chromatograms of cola #1, (A) diluted three-fold and (B) the same sample spiked.

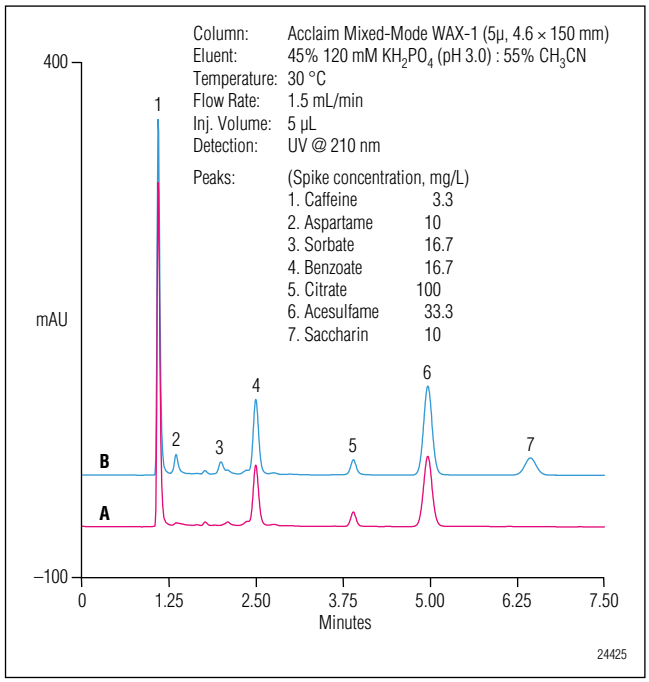

Figure 5. Overlay of chromatograms of diet cola #1, (A) diluted three-fold and (B) the same sample spiked.

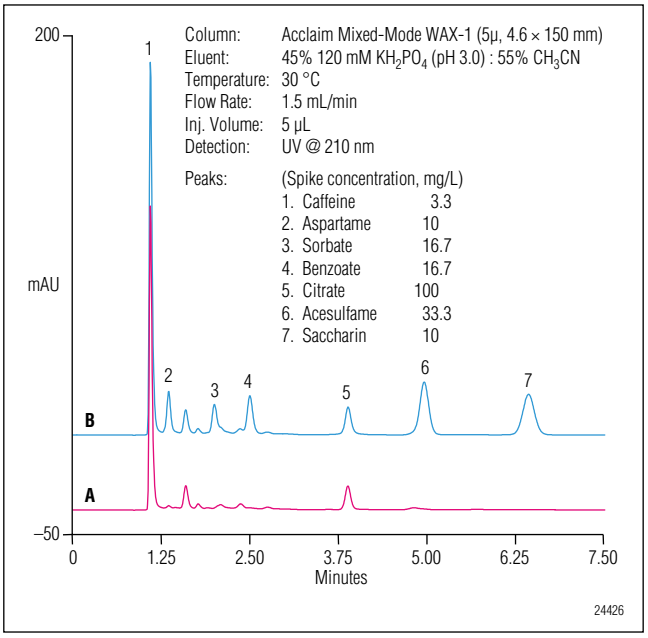

Figure 6. Overlay of chromatograms of cola #2, (A) diluted five-fold and (B) the same sample spiked.

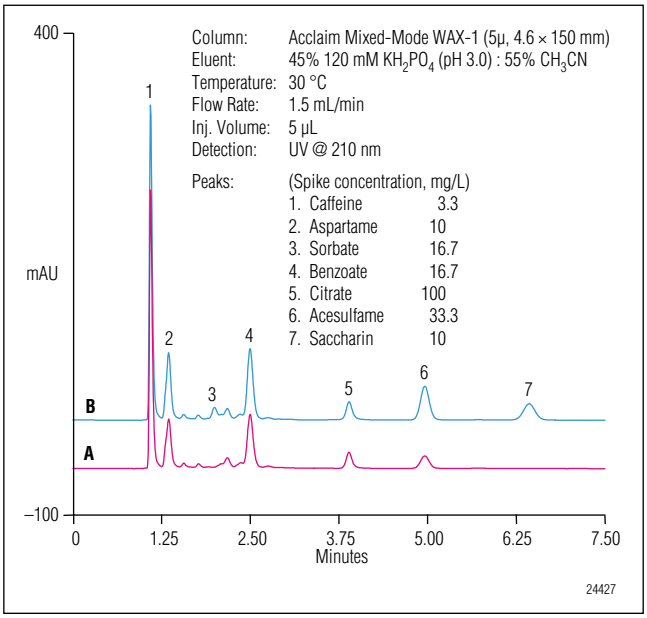

Figure 7. Overlay of chromatograms of carbonated beverage diet cola #2, (A) diluted three-fold and the same sample (B) spiked.

of each additive per beverage, and recovery data are summarized in Table 5.

Samples show an unidentified peak eluting at approximately 1.6 min, however, this peak was not present in either of the diet colas tested (samples 3 and 5). Tests showed the peak to correspond to fructose (Figure 9).

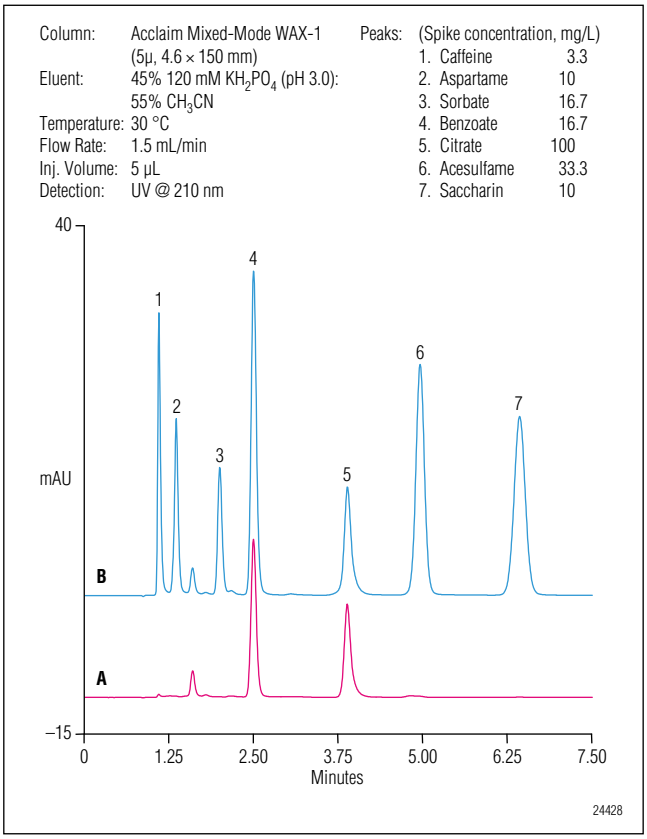

Figure 8. Overlay of chromatograms of (A) orange soda sample, diluted ten-fold and (B) the same sample spiked.

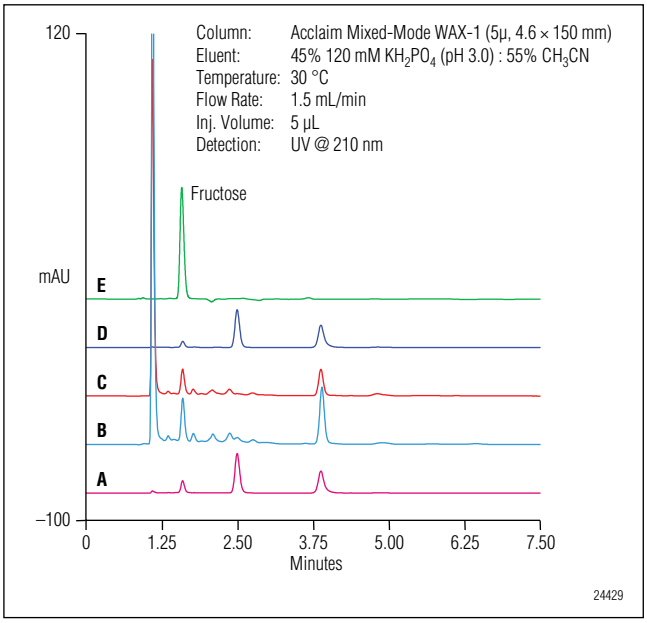

Figure 9. Overlay of chromatograms of (A) lemon-lime carbonated beverage diluted ten-fold, (B) cola #1, diluted three-fold , (C)cola #2, diluted five-fold, (D) orange soda, diluted ten-fold, and (E) single standard of fructose, 5%, w/w.

Table 5. Analysis Results for the Carbonated Drinks[1]								
Analyte	**Lemon-Lime Soda (Diluted 10-fold)**				**Cola #1 (Diluted 3-fold)**			
	Detected (mg/L)[3]	**Added (mg/L)**	**Found (mg/L)[4]**	**Recovery (%)**	**Detected (mg/L)**	**Added (mg/L)**	**Found (mg/L)**	**Recovery (%)**
Caffeine	ND[2]	3.3	3.4	103	94	3.3	3.6	109
Aspartame	ND	10	9.3	93	ND	10.0	11.3	113
Sorbate	ND	16.7	17.4	106	15.6	16.7	22.4	108
Benzoate	200	16.7	14.9	89	ND	16.7	20.7	103
Citrate	2767	100	89	89	2250	100	106	106
Acesulfame	ND	33.3	33.9	102	ND	33.3	34.2	103
Saccharin	ND	10.0	10.0	100	ND	10.0	10.0	100
Analyte	**Diet Cola #1 (Diluted 3-fold)**				**Cola #2 (Diluted 5-fold)**			
	Detected (mg/L	**Added (mg/L)**	**Found (mg/L)**	**Recovery (%)**	**Detected (mg/L**	**Added (mg/L)**	**Found (mg/L)**	**Recovery (%)**
Caffeine	131.4	3.3	3.1	94.0	99.3	3.3	3.1	94
Aspartame	ND	10.0	10.4	104	ND	10.0	9.4	94
Sorbate	15.6	16.7	17.3	104	18.9	16.7	16.9	101
Benzoate	104	16.7	15.5	93	ND	16.7	14.3	86
Citrate	1410	100	92	92	1852	100	106	106
Acesulfame	324	33.3	29	87	ND	33.3	36.1	108
Saccharin	ND	10.0	9.9	99	ND	10.0	9.9	99
Analyte	**Diet Cola #2 (Diluted 3-fold)**				**Orange Soda (Diluted 10-fold)**			
	Detected (mg/L	**Added (mg/L)**	**Found (mg/L)**	**Recovery (%)**	**Detected (mg/L**	**Added (mg/L)**	**Found (mg/L)**	**Recovery (%)**
Caffeine	113.4	3.3	3.5	106	ND	3.3	3.5	106
Aspartame	113.1	10.0	10.5	105	ND	10.0	9.9	99
Sorbate	ND	16.7	16.6	99	ND	16.7	15.2	91
Benzoate	183	16.7	14.8	89	180	16.7	18.4	110
Citrate	1770	100	111	111	3815	100	100	100
Acesulfame	56.4	33.3	35.3	106	ND	33.3	33.8	102
Saccharin	ND	10.0	10.0	100	ND	10.0	9.7	97

Notes: 1. One sample and one spiked sample were prepared, with 3 injections made for each.

2. ND="not detected"

3. Detected = measured value of sample × diluted fold

4. Found = measured value of spiked sample – measured value of sample

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