Fast determinations of brominated compounds in carbonated beverages using oxidative pyrolytic combustion and ion chromatography

Authors

Terri Christison, Sachin P. Patil, and Jeff Rohrer

Thermo Fisher Scientific, Sunnyvale, CA, USA

Keywords

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Goal

Demonstrate a fast determination of brominated compounds in carbonated beverages using combustion IC

Introduction

There are numerous functional additives to processed foods and beverages, such as acidulants, emulsifiers, artificial flavoring, and sweeteners, and they are commonly regulated under labeling requirements, U.S. Federal Register, 21 CFR part 101.[1] Brominated vegetable oil (BVO), one of these additives, is frequently used to emulsify citrus flavoring and provide a stable mixture in carbonated and other beverages.[2-5] BVO is a heterogenous triglyceride composed of three plant-based fatty acids (colloquially called oils), which are brominated at the previously unsaturated bonds. The BVO chemical composition varies due to the types of fatty acids, plant source, and local and seasonal variations of the crop. As a result, three compositions of BVO have been reported.[2] Figure 1 shows one of the possible chemical structures of BVO.[3] In the U.S., the most common plant source is soybean oil[2] although corn, flax (linseed), sesame, cottonseed, and olive oils[4] have been reported. The composition of fatty acids from soybean and corn are similar: polyunsaturated fatty acids >50%, 15 to 20% monounsaturated, and 15 to 20% saturated fatty acids (Tables 1 and 2).[3] The saturated fatty acids are not brominated to produce BVO because of the absence of unsaturated bonds.
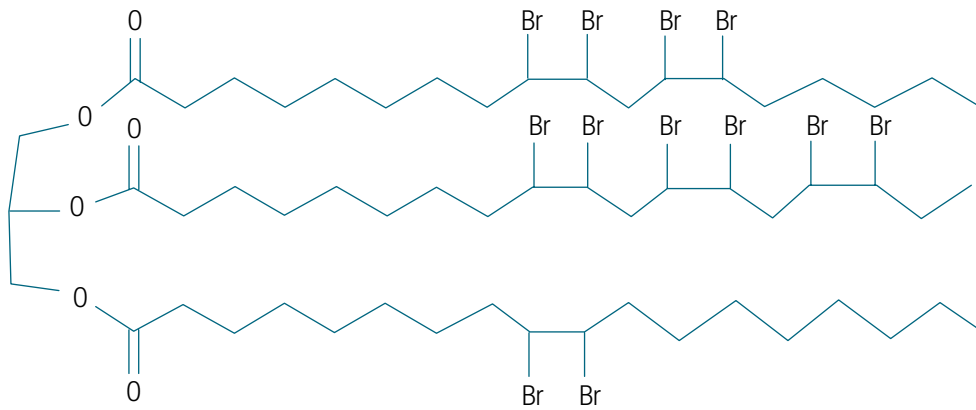
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Figure 1. A chemical structure of BVO showing oleic (bottom), linolenic (middle), and linoleic fatty acids (top)[3]

Table 1. Chemical structures of the fatty acids found in soybean and corn and reported in BVO[3,6]

Compound	Saturation	Structure
Oleic acid (C18:1)	Monounsaturated	
α-Linolenic (C18:3)	Polyunsaturated	
Linoleic (C18:2)	Polyunsaturated	

*Chemspider[6]

Table 2 summarizes the average amounts of palmitic, oleic, a-linolenic, and linoleic fatty acids typically found in soybean or corn oils. However, the fatty acid content is variable due to plant species and growth conditions.

To estimate the amount of bromination, the author assumed the following:

1) 100% bromination (C-Br) occurred when converting the C=C double bond to a C-Br single bond, and

2) soybean or corn oil samples were representative of the average composition.

The calculation for estimated bromination is shown for the soybean oil example that includes 22.6% monosaturated fatty acid, 51% di-unsaturated fatty acid, and 7% tri-unsaturated fatty acid.

Estimated average bromination = $(0.226 \times 2$ (one unsaturated bond))+ $(0.51 \times 4$ (2 unsaturated bonds))+ $(0.07 \times 6$ (3 unsaturated bonds)) = 2.91

The percent bromine in the triglyceride was calculated based on the percent of fatty acid and the estimated number of bromines.

The acceptability of BVO as a food additive is controversial. In the U.S. in 1958, it was rated as "generally regarded as safe (GRAS)".[7] In 1969, animal toxicology studies reported lipid storage myopathy and accumulation of bromine compounds in fatty tissues. Brominated aromatic fire retardants, sometimes confused with BVO, have also shown accumulation effects.[4,8-10] As a result, BVO was banned in many Asian and European countries. In the U.S., BVO was removed from the GRAS category and revised to an interim additive with a 15 mg/L limit in the finished beverage.[7] More recently, acute bromine intoxication in humans from over-consumption (liters) of BVO-containing beverages have also been reported.[11-13] As a result, there is increasing consumer pressure to remove BVO from beverages, syrups, and cocktails.

Analytical methods are needed for BVO determinations to maintain the beverage formulation and to meet labeling requirements. However, BVO determinations are challenging because of its nature as a non-polar suspension in an ionic sample matrix. As with other challenging matrices, pyrolytic combustion ion chromatography (CIC) is an ideal approach to eliminate the sample matrix and increase sample homogeneity. Pyrolytic CIC has been previously demonstrated for halide determinations in other challenging liquid matrices, such as tea[14], wastewater[15], and fuels[16–20] including crude oil and aromatic hydrocarbons; however, pyrolytic CIC has not been demonstrated on oil-based food additives.

Table 2. Fatty acids in soybean and corn oils used to estimate bromination

	Palmitic (C18:0) (%)	Oleic (C18:0) (%)	α-Linolenic (C18:3) (%)	Linoleic (C18:2) (%)	Estimated bromination in triglyceride	
					(#)*	(%)**
Soybean	15.6	22.6	7	51	2.91	21.2
Corn	12.9	27.3	1	58	2.93	20.8

*Estimated bromination for soybean oil = $(0.226 \times 2) + (0.07 \times 6) + (0.51 \times 4)$
*Estimated bromination for corn oil = $(0.273 \times 2) + (0.01 \times 6) + (0.58 \times 4)$
** The estimated percent bromine is calculated on the estimated number of bromines included in the triglyceride formula weight adjusted for the percentage of fatty acid type.

In this application, BVO was determined in beverage samples by pyrolyzing a 50 µL aliquot at 1000 °C under an inert atmosphere to HX (gas), combusted with oxygen and water vapor at 900 °C, and collected in 30 mg/L peroxide to obtain bromide. Bromide and other anions from the 100 µL aliquot of the collected combusted sample are separated within 9 min by anion-exchange chromatography on a 4 × 150 mm column using electrolytically generated 23 mM KOH at 1.0 mL/min and detected by suppressed conductivity. Using overlap mode for the 5 min combustion, the total analysis time is 13 min after the first sample, which requires 18 min. BVO was determined in three carbonated beverage and two ginger beer samples. Bromide from total BVO was found at expected levels in one of the carbonated beverages and unexpectedly found at trace levels in another carbonated beverage. The method had good accuracy with recoveries within the 90–102% and good reproducibility with <3% RSDs.

Experimental
Equipment
- Mitsubishi Chemical Analytech™ Automatic Combustion Unit Model AQF-2100H system, including:
  – Automatic Boat Controller Model ABC-210
  – Liquid Sample Changer Model ASC-250L
  – Horizontal Furnace Model HF-210
  – Gas Absorption Unit GA-210
  – External Solution Selector ES-210
- Thermo Scientific™ Dionex™ Integrion™ HPIC™ system, RFIC model, including:
  – Dionex Integrion HPIC System Pump
  – Detector Compartment Temperature Control
  – Eluent Generation
  – Integrion IC Conductivity Detector, P/N 079829

Table 3 lists the consumable products needed for the CIC system.

Software
- Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software, version 7.2 SR9
- Mitsubishi NSX-2100 version 2.1.6.0

Reagents
- ASTM Type 1 deionized water (DI water) with 18 MΩ·cm resistivity[23]
- Argon (gas), UHP grade, 99.999%
- Oxygen (gas), UHP grade, 99.999%
- Acetone, certified ACS (Fisher Scientific P/N A18-1) used as the diluent to prepare BVO
- Thermo Scientific™ Dionex™ Seven Anion Standard II (P/N 057590)
- Brominated vegetable oil, food grade (Fisher Scientific P/N NC0415602), S.G. 1.325-1.335
- 30 mg/L hydrogen peroxide collection solution prepared from (w = 50%) hydrogen peroxide, stabilized (Fisher Scientific P/N AC302865000). Add 120 µL of hydrogen peroxide in a 2 L volumetric flask and fill to volume with DI water.
- Ammonium bromide, ACS grade (Fisher Scientific P/N P205-500 or A650-500)

Reagents used only for method evaluation:
- 1000 mg/L Bromate Ion Chromatography Standard (Fisher Scientific P/N BRO392Y) or Sodium bromate, certified (Fisher Chemical P/N S253-500)
- 1000 mg/L Phosphate Ion Chromatography Standard (Fisher Scientific P/N ASPO49-2Y)

Table 3. Consumables list for the Mitsubishi Combustion system coupled to the Dionex Integrion HPIC system*

Product Name	Description	P/N
Thermo Scientific™ Dionex™ IC PEEK Viper™ fitting tubing assembly kit	Dionex IC Viper fitting assembly kit for the Dionex Integrion RFIC system with CD: Includes one each of P/Ns: 088805–088811	088798
Thermo Scientific™ Dionex™ EGC 500 KOH Eluent Generator cartridge	Eluent generator cartridge for the Integrion system	075778
Thermo Scientific™ Dionex™ CR-ATC 600 Electrolytic trap column	Continuously regenerated trap column used with Dionex EGC KOH 500 cartridge	088662
HP EG Degasser Module	Degasser installed after Dionex CR-TC trap column and before the injection valve, used with eluent generation, included with installation	075522
Thermo Scientific™ Dionex™ ADRS 600 suppressor	Suppressor for 4 mm columns	088666
Thermo Scientific™ Dionex™ IonPac™ AG18-4µm Guard Column	Anion guard column, 4 × 30 mm	076035
Thermo Scientific™ Dionex™ IonPac™ AS18-4µm Analytical Column	Anion analytical column, 4 × 150 mm	076034
4 L eluent bottle	Two 4 L eluent bottles are needed for the CIC system	066019
ASC-250L autosampler vials	4 mL glass vials and caps with for liquid combustion autosampler	MC18445*
Quartz wool	Quartz wool for the combustion boats and the combustion tube	MC06175*
Extra combustion tube	AQF-2100H pyrolysis tube set	MC28002*
Extra absorption tube	Absorption tube, 10 mL	MC25000*
Ceramic boats	Ceramic combustion boats	MC17060*

* COSA Xentaur P/N[22]

Conditions

Pyrolysis/combustion conditions

AQF-2100H

Sample size combusted:	50 µL
Sample boat:	Quartz with quartz wool
Pyrolysis tube:	Quartz tube with ceramic insert and quartz wool at exit
Combustion run time:	5 min in overlap mode
Calculated dilution factor	425
Absorption solution:	30 mg/L hydrogen peroxide in DI water
Mode:	Constant volume
Argon and oxygen gas tank regulator setting, secondary gauge:	40 psi

Detailed combustion conditions*

HF-210 module conditions

Furnace inlet temperature:	900 °C
Furnace outlet temperature:	1000 °C
Argon flow (pyrolysis carrier gas):	200 mL/min
Oxygen flow (combustion):	400 mL/min

GA-210 module conditions

Absorption tube:	10 mL
Sample loop:	100 µL
Final absorption volume:	10 mL
Absorption solution volume:	3.5 mL (initial collection vol.)
Water supply scale:	2
Argon flow for humidification:	100 mL/min

GA-210 washing conditions

Water injection time:	15 s
Drain time:	15 s
Washing time:	2
Gas line washing time:	0.5 s
Gas line washing interval:	3 s
Gas line washing times:	3
Washing time of sample absorption line:	5 s
Syringe washing times:	3

Detailed combustion conditions* (continued)

GA-210 gas line collection parameters

Collection time:	0.5 s
Collection interval:	3 s
Times:	3

GA-210 injection parameters

Washing time for injection start:	0 s
Sample purge time:	10 s
Sample absorption time:	10 s

ABC-210/ASC-250L

Syringe type:	100 µL
Injection position:	Horizontal
Liquid type:	Water
Argon time:	10 s
Oxygen time:	600 s

Position (mm)	Wait time (s)	Speed (mm/s)
65	10	20
100	30	0.4
130	30	0.4
End	60	20
Cool	60	40
Home	120	40

*CIC Program 1 for liquid samples

Ion chromatography conditions

Columns:	Dionex IonPac AG18-4µm guard (4 × 30 mm) and Dionex IonPac AS18-4µm separation (4 × 150 mm)
Eluent:	23 mM KOH
Eluent source:	Dionex EGC 500 KOH eluent cartridge, Dionex CR-ATC 600 trap column and high pressure degas module
Flow rate:	1 mL/min
Injection volume:	100 µL aliquot from collected, combusted sample in GA-210 Gas Absorption Unit
Column temperature:	30 °C
Detection/suppressor compartment:	15 °C
Detection:	Suppressed conductivity, Dionex DRS 600 suppressor, 4 mm, 57 mA, constant current mode and recycle mode
Conductance background:	< 1 µS/cm
Conductance noise:	< 1 nS/cm
System backpressure:	~ 2200 psi
Run time:	IC: 9 min, total run time: 13 min

Standard and sample preparation
BVO solutions
The preparation of BVO stock and working standards are described here, but because of heterogeneity the standards should be treated as samples. The exception would be if the BVO used in the beverage formulation is available.

Prepare 1000 mg/L (w/v) brominated vegetable oil (BVO) stock standard by dissolving 0.1000 g of BVO in 100 mL of acetone. Mix thoroughly and ensure that the BVO is thoroughly dissolved. (Note: BVO is not soluble in water.) The solution should not exhibit cloudiness, precipitation, or visible particulates (surface, bottom, or in acetone), which is evidence of insolubility. Dilute the 1000 mg/L BVO stock standard to 100 mg/L with 2000 µL of the stock standard and 18.0 mL of DI water. Visually examine solution as described above.

Prepare individual BVO working standards (5, 10, 15, and 20 mg/L) by adding 0.5, 1.0, 1.5, and 2.0 mL of the 100 mg/L BVO stock solution in a 10 mL flask and filling up to volume with DI water. The solutions should not exhibit cloudiness, precipitation or visible particulates (surface, bottom, or in acetone).

Stock standard solution
Prepare a 1000 mg/L bromide stock standard, dissolve 1.226 ± 0.001 g of ammonium bromide (NH_4Br) in 1 L DI water. (Ammonium bromide is volatile and therefore more suitable than potassium bromide as a CIC reagent.) Swirl to ensure dissolution and to thoroughly mix. Dilute 10-fold with DI water to 100 mg/L bromide intermediate standard.

Working standard solutions
Prepare individual bromide working standards (0.5, 1.0, 5.0, 10.0, 15.0, 20.0, and 25.0 mg/L) by diluting 0.5, 1.0, 5.0, 10.0, 15.0, 20.0, and 25.0 mL of 100 mg/L bromide stock standard into individual 125 mL bottles. Dilute to 100 mL with DI water. Swirl to mix thoroughly.

Prepare a 450-fold dilution of 5 mg/L bromide, using 220 µL of the 5 mg/L standard in 99.78 g total DI water. This standard is used as an external standard to compare the bromide concentration with and without combustion (simulates the combustion of 50 µL of 100 mg/L bromide collected in 10 mL of collection solution).

Create calibration standards for external calibration in the same way as the 450-fold dilution. Store all standards at 4 °C.

Method evaluation standards
1000 mg/L phosphate standard used without further dilution.

Prepare a 100 mg/L bromate solution by adding 10 mL of 1000 mg/L bromate standard to 90 mL of DI water.

Samples
Three brands of carbonated citrus beverages were analyzed: Sample A, Sample B, and Sample C. The samples were directly analyzed, or diluted as necessary. It is not advisable to use vacuum filtration for degassing or filtration of any type as the process removes the BVO. Carbonated samples were directly analyzed without issue.

Sample C reports brominated vegetable oil in the ingredients list. The presence of brominated vegetable oil or BVO was not reported on the ingredient labels for Samples A and B.

Instrument setup and installation
This application uses inline sample preparation by pyrolytic combustion followed by sample collection, mixing, and dilution. The combustion system triggers the IC to analyze the samples.

- Sample introduction:

  - The Mitsubishi ASC-250L Automatic Liquid Sampler transfers a 50 µL aliquot of the carbonated beverage sample from the sample vial to the combustion sample boat docked in the ABC-210 Automatic Boat Controller module.

  - The Mitsubishi ABC-210 Automatic Boat Controller module transports the combustion boat containing the liquid sample through the ceramic sleeve in the combustion tube in the Mitsubishi AQF-2100H Automatic Quick Furnace unit. The combustion boat passes through the two heating zones of the AQF-2100H and then is returned to dock in the ABC-210 module. The total cycle is 5 min.

- Pyrolysis and combustion:

  – Initially, the BVO and beverage matrix are pyrolyzed under argon inert atmosphere at 900 °C and then oxidized in the presence of oxygen and water vapor to HX and HSOx gases at 1000 °C.

- Sample collection:

  – The argon gas carries and aerates the sample into a collection solution (30 mg/L hydrogen peroxide in DI water) in the GA-210 Gas Absorption unit.

  – The aeration continues which provides mixing while the sample is diluted to 10 mL total volume.

- Sample analysis:

  – The GA-210 unit loads a 100 µL aliquot of the sample and triggers the IC injection via the TTL_Input_3 cable and programming instructions created in the Chromeleon CDS configuration.

To install the CIC system, follow the installation instructions as described in Thermo Scientific Technical Note TN72211 - Combustion ion chromatography with a Dionex Integrion HPIC System[24] and Figures 2 and 3. Connect the RS232 cables from the GA-210 module and the USB cable from the Dionex Integrion IC to the computer. Connect the RS232 cables from the ES-210, ABC-210, and ASC-250L modules to the GA-210 module. Install the power cords and power-up the modules. Install the twisted pair cable from the Integrion IC to the Mitsubishi GA-210 module according to the instructions in TN72211.

Configure the IC modules in the Chromeleon CDS software as described in TN72211, including the instructions for TTL_Input_3 and the Device Name as InjectValve_CIC. Add the AGF-2100H combustion system as the Remote Inject Device module according to the instructions. This application is configured as described
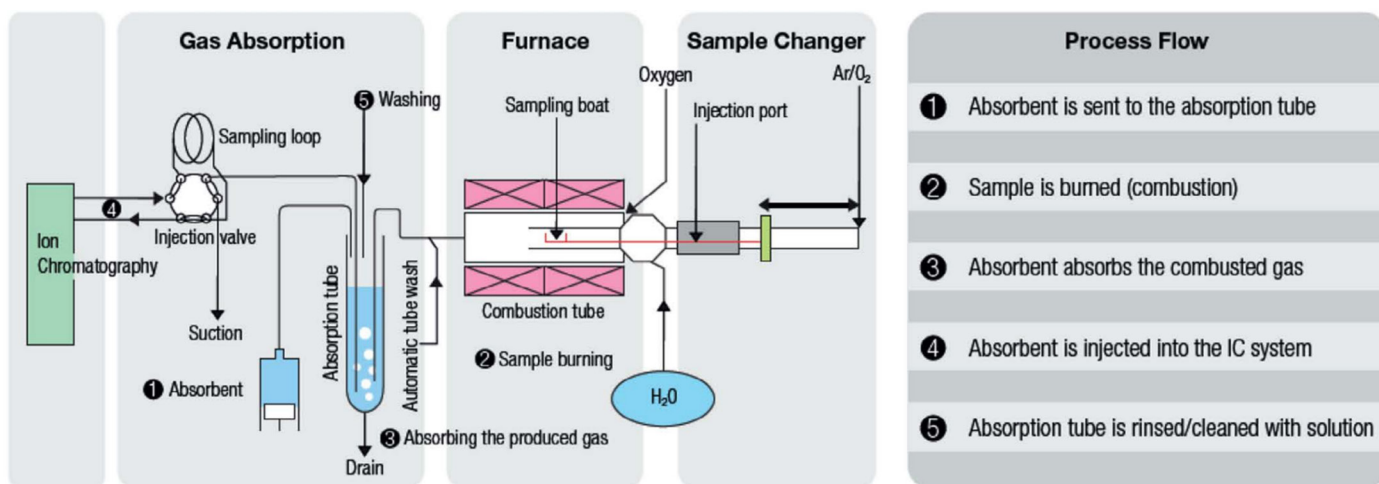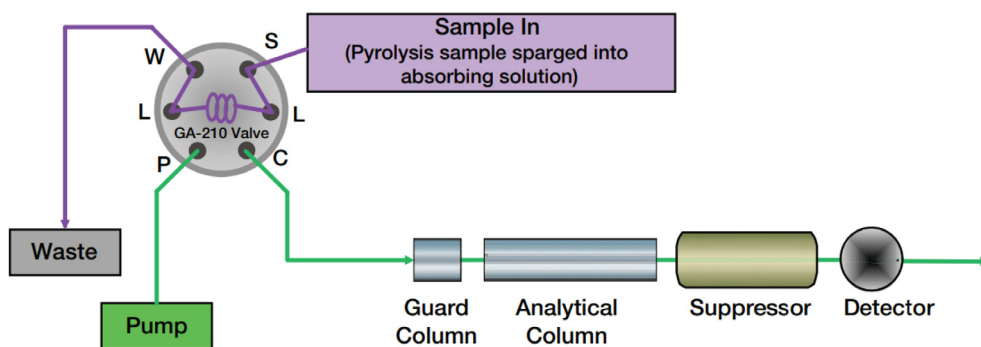

Figure 2. Diagram of combustion IC system[25]

Figure 3. IC schematic[27]

under "Direct injection using the injection valve in the Mitsubishi GA-210 Module" in TN72211. Additional information on the combustion system can be found in the product manual.[26]

Plumbing the Integrion IC

Plumb the Integrion IC as a standard Reagent-Free™ IC (RFIC™) system shown in Figure 3 and shown on the schematics on the inside doors of the Integrion IC system. Yellow (0.003 in i.d., 0.0762 mm i.d.) PEEK tubing was added after the degas module and before the IC injection valve to bring the IC system pressure to ~ 2200 psi.

Conditioning electrolytic devices and columns

Important: Do not remove consumable tracking tags on the columns and consumable devices. These tags are required for consumables monitoring functionality.

Hydrate and condition the Dionex EGC 500 KOH eluent generator cartridge and Dionex CR-ATC 600 Continuously Regenerated Trap column according to Thermo Scientific Technical Note TN175-Configuring the Dionex Integrion HPIC System for High-Pressure Reagent-Free Ion Chromatography, product manuals, or the instructions in the drop-down menu (Select Consumables, and Conditioning on the Chromeleon Console panel).[28-30] Condition the columns as described in the product manual and Consumables Conditioning instructions.[31] Install the conditioned columns according to Figure 3.

Hydrate the Dionex ADRS 600 suppressor for 20 min as described in TN175 for the Dionex electrolytic suppressor and in the product manual.[28,32] Install the suppressor in recycle mode according to Figure 3.

Combustion system

Preparing the pyrolysis tube and the ceramic boat

Unscrew the knob on the ASC-250L module to release the autosampler table and gently push the table to the back. Add a small amount of quartz wool to a new ceramic boat, barely covering the bottom of the boat. Install the boat inside ABC-210 module so that the hook is attached the boat handle.

Remove the plug of quartz wool from the pyrolysis tube with the hooking tool. Install a new plug of quartz wool inside the pyrolysis tube at the exit. Carefully install the ceramic sleeve and install the pyrolysis tube inside the HF-210 furnace. Complete the installation by completing the glass fittings. Important: Pull the autosampler table to the front and secure the table by turning the knob clockwise. If the knob is not tightly closed, the table will not be secured which will subsequently damage the injection needle.

Starting the CIC system

Power up each module. Open the Ar and O_2 gas tank regulators and set the secondary gauges of the gas regulators to 40 psi. Open the gas flow to the tubes feeding the CIC system.

Open the AQF-2100H software program. Turn on the gas by selecting the first flame icon. Wait a few seconds for the gas flows to reach the set points (200, 400, 100 mL/min for argon carrier, oxygen, and argon carrier to collection tube, respectively). To heat up the furnace, select the second flame icon. The heat up time to the set temperatures will be ~1-2 h.

Creating sequences

Create a new combustion sequence using the Mitsubishi software by copying a previous sequence and pasting without results. (For the first sequence, copy one of preprogrammed sequences.) Rename the copy of the sequence to a new name. Open the new sequence by double clicking on the sequence name. Select the Step List tab. In the Type section (blue) enter the sample name in the ID and vial locations. In the Volume section (green) enter the sample injection volume (0 for system blanks or 50 µL for samples). In the GA Constant Volume section (Enable is highlighted pink) enter 13 (min) for the total IC and combustion run time. Select the Prepare button to save the sequence.

Open the Chromeleon instrument console program and create a sequence duplicating the list of samples in the combustion sequence. Create a 9 min IC instrument method using the Chromeleon Wizard and the parameters listed in the Conditions section.

Consumables device tracking and starting the sequences

Equilibrate the IC at the application conditions until the total conductivity is <1 µS/cm.

Approve the consumables in the Consumables Tracking panel located on the Chromeleon console (Figure 4). Start Chromeleon sequence.

Verify that the furnace temperatures and gas flows of the combustion system are at the set points. Select the "Prepare" button to save the sequence and select start (blinking blue arrow). The data will be stored in Chromeleon.

Results and discussion

Much of the initial method development was focused on experimentation to further understand the chemistry and mechanisms of the pyrolysis/combustion system and evaluating the characteristics of BVO.

Method development and method evaluation
Evaluating the pyrolysis and oxidative combustion process

Combustion IC is used to determine halogens and sulfur species in various samples; however, the intended samples, carbonated beverages, contain up to percent concentrations of citrate and phosphate, which could be problematic to achieve fast sample runs. In a combustion method, it is expected that citrate would oxidize to carbon dioxide; however, the fate of phosphate was less clear. Additionally, it was important to determine whether bromide is partially or fully oxidized to bromate in the combustion process. To confirm that phosphate would not be detected by CIC, 50 µL of 10-fold diluted Dionex Combined Seven Anion Standard was analyzed by CIC. Figure 5 shows that phosphate, which would typically elute around 16–18 min, was not detected by CIC.

Figure 4. Consumables tracking

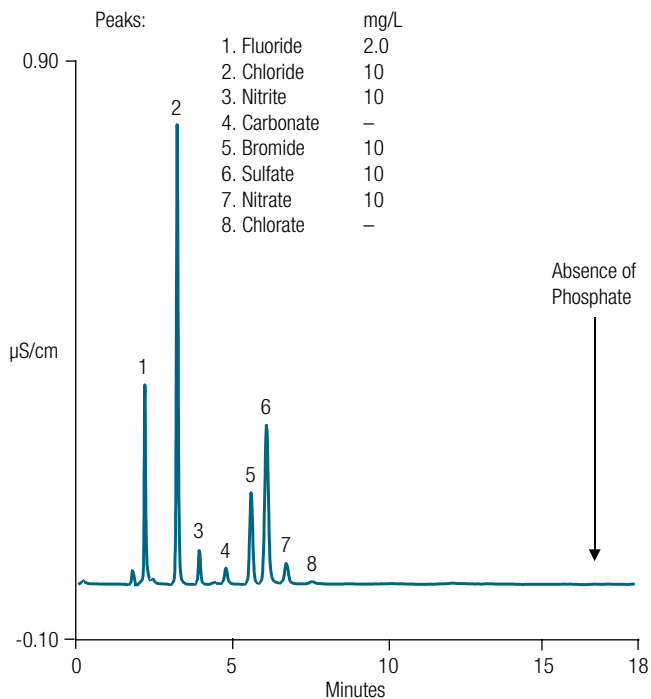|	
Column:	Dionex IonPac AG18-4µm, 4 × 30 mm
	Dionex IonPac AS18-4µm, 4 × 150 mm
Eluent:	23 mM KOH
Eluent Source:	Dionex EGC-500 KOH cartridge, Dionex CR-ATC 600
	trap column, Dionex high pressure degasser
Flow Rate:	1.0 mL/min
Injection Vol.:	100 µL aliquot of collected solution
Column Temp.:	30 °C
Detection:	Suppressed conductivity, Dionex ADRS 600, 4 mm,
	constant current mode, 57 mA, recycle mode
Sample Prep.:	Pyrolysis and combustion of 50 µL standard
Pyrolysis:	900 °C, 10 s, Ar, 200 mL/min
Combustion:	1000 °C, 600 s, O_2, 400 mL/min
Collection Sol.:	30 ppm hydrogen peroxide, 10 mL
Collect. gas:	Ar carrier, 100 mL/min, DI water
Standard:	10-fold diluted Dionex Combined Seven Anion Standard

Peaks:		mg/L
	1. Fluoride	2.0
	2. Chloride	10
	3. Nitrite	10
	4. Carbonate	–
	5. Bromide	10
	6. Sulfate	10
	7. Nitrate	10
	8. Chlorate	–

Figure 5. Phosphate is not detected by CIC.

Column:	Dionex IonPac AG18-4µm, 4 × 30 mm
	Dionex IonPac AS18-4µm, 4 × 150 mm
Eluent:	23 mM KOH
Eluent Source:	Dionex EGC-500 KOH cartridge, Dionex CR-ATC 600
	trap column, Dionex high pressure degasser
Flow Rate:	1.0 mL/min
Injection Vol.:	100 µL aliquot of collected solution
Column Temp.:	30 °C
Detection:	Suppressed conductivity, Dionex ADRS 600, 4 mm,
	constant current mode, 57 mA, recycle mode
Sample Prep.:	Pyrolysis and combustion of 50 µL standard
Pyrolysis:	900 °C, 10 s, Ar, 200 mL/min
Combustion:	1000 °C, 600 s, O_2, 400 mL/min
Collection Sol.:	30 ppm hydrogen peroxide, 10 mL
Collect. gas:	Ar carrier, 100 mL/min, DI water

Peaks:	Bromide	
	A. System blank	– (mg/L)
	B. 100 mg/L Bromate	58.8*
	C. 100 mg/L Bromide	99.9

*94.1% yield from 62.5% theoretic bromine content
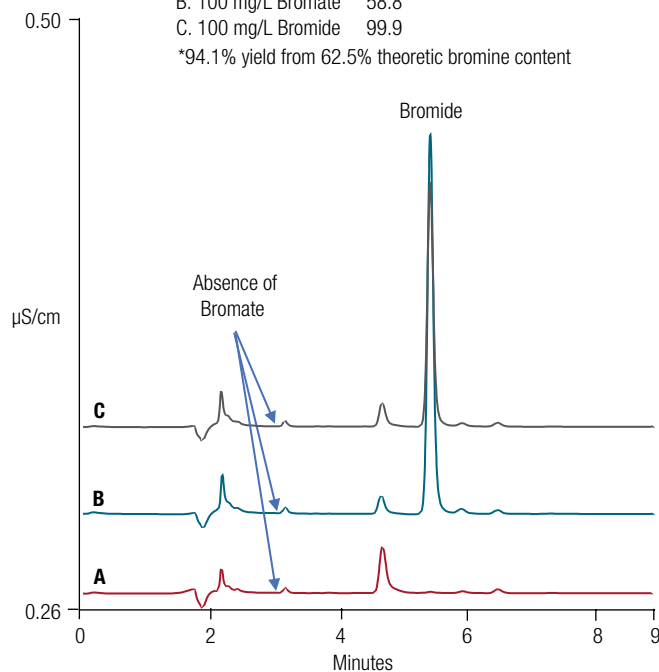
Figure 6. Comparison of A) system blank, B) 100 mg/L bromate, and C) 100 mg/L bromide

Similarly, 100 mg/L bromide and bromate were analyzed separately by CIC to confirm that bromate was converted to bromide and to determine the pyrolysis/combustion efficiency. Figure 6 shows the confirming results, that bromate was converted to bromide with efficient recovery (94.1% recovery of 52.5% theoretical bromine). No bromate was detected. (Bromate elutes near arrow before small peak in Figure 6.) This agrees with the CIC flow path and conditions in the HF-210 furnace: pyrolysis at a high temperature of 900 °C to HX (gas) under an inert atmosphere preventing oxidation followed by oxidation at 1000 °C where the HBr gas is dissolved to bromide in solution, rather than fully oxidized to bromate.

A dilution factor is needed to ascertain the volume of collection solution and water introduced by humidification. To determine this factor, the response of a 5 mg/L bromide injected as an external standard with the External Sampler module ES-210 is compared to the same standard used as the collection solution by the Gas Absorption GA-210 unit. The dilution factor was 9.5×.

Some analysts may prefer to use external calibration mode, which requires determining the response factor of combustion IC versus using direct injection by the external sampler module to the IC. The responses of a 5 mg/L bromide standard were compared using both methods with ~450x difference in response. To confirm the results, a 0.011 mg/L standard was prepared by diluting the 5 mg/L bromide standard 450-fold with DI water. The results shown in Figure 7 show that there was 425x response factor (including the dilution).

Column: Dionex IonPac AG18-4µm, 4 × 30 mm
Dionex IonPac AS18-4µm, 4 × 150 mm
Eluent: 23 mM KOH
Eluent Source: Dionex EGC-500 KOH cartridge, Dionex CR-ATC 600
trap column, Dionex high pressure degasser
Flow Rate: 1.0 mL/min
Injection Vol.: 100 µL aliquot of collected solution
Column Temp.: 30 °C
Detection: Suppressed conductivity, Dionex ADRS 600, 4 mm,
constant current mode, 57 mA, recycle mode
Standard: A: 5 mg/L bromide
B: 450-fold diluted "A" –0.011 mg/L
Sample Prep.: A: CIC
B: External injection (without pyrolytic and oxidative combustion)
For Standard A:
Sample Prep.: Pyrolysis and combustion of 50 µL standard
Pyrolysis: 900 °C, 10 s, Ar, 200 mL/min
Combustion: 1000 °C, 600 s, O_2, 400 mL/min
Collection Sol.: 30 ppm hydrogen peroxide, 10 mL
Collect. gas: Ar carrier, 100 mL/min, DI water

Peaks: A   B
1. Bromide   0.004317   0.004035   (µS*min)
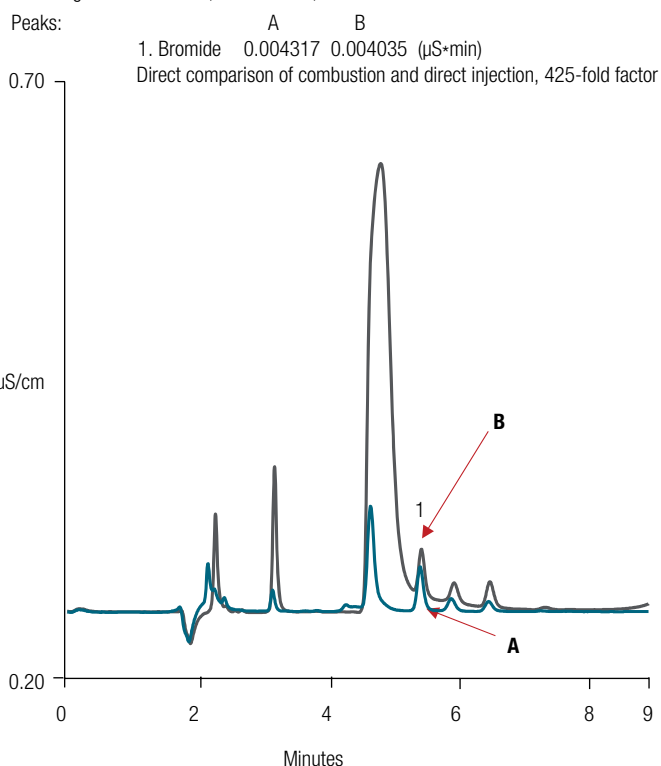Direct comparison of combustion and direct injection, 425-fold factor

Figure 7. Determining CIC to external mode factor: A) 5 mg/L bromide by combustion IC, and B) 0.011 mg/L bromide by external mode

Evaluating BVO characteristics

As a result of its non-polar characteristics, BVO was not soluble in DI water, a challenge not commonly encountered in IC methods. Previous researchers reported preparing the BVO stock standard in diethyl ether with subsequent dilutions using DI water as the diluent to 1000 mg/L intermediate standard and the further diluted working standards.[2] However, this approach was abandoned when the solution became cloudy and particulates were observed when diluting with DI water to the 100 mg/L intermediate standard. Acetone was found to be more suitable as the stock standard diluent, as further experiments found that BVO dissolved in acetone and stayed in solution in the subsequent dilutions with DI water to generate the intermediate and the working standards. As previously stated, BVO is a heterogenous material with variable fatty acid composition and unsaturation, and therefore with variable bromination. To estimate the bromination in the BVO reagent, a calibration response curve using CIC was created of 0.5, 1.0, 2.0, 5.0, and 10 mg/L bromide standards (n = 3). A 100 mg/L BVO standard was determined by CIC using this calibration curve (linear, coefficient of determination, $r^2 = 0.999$) and found to contain 2.3 ± 0.002 mg/L of bromide (Figure 8). The calculated bromine content is 2.3%, which is considerably lower content than shown estimated in Table 2 (2.91 bromines, 21.2%) and in Figure 1 (12 bromines). The supplier clarified in further discussions about the absence of a molecular weight, that the BVO reagent was a mixture from a natural source. Therefore, the vendor expected the BVO would have variable types and amounts of the fatty acids and subsequent variable bromination. The experimental results showed that the BVO reagent was stable and the results were reproducible, providing confidence in the method. However the determined percent bromine was too low as compared to the proposed theoretical composition (Table 2). A secondary reference standard is needed.

Linearity and limit of determinations

The limit of detection (LOD) and linearity of the bromide-calibration were determined. The LOD was determined using the 0.5 mg/L bromide standard (n = 7, 3× S/N) and found to be 0.34 mg/L. The linearity was determined by triplicate injections of standards from 0.5 mg/L to 25 mg/L bromide, the results showed a linear relationship with a coefficient of determination, $r^2 = 0.998$.

Sample analysis

The method was applied to three carbonated beverages: 1) Sample A, a clear carbonated beverage, 2) Sample B, opaque white carbonated beverage, and 3) Sample C, yellowish-green carbonated beverage. BVO was listed on the label of Sample C (Figure 9).

Column: Dionex IonPac AG18-4µm, 4 × 30 mm
Dionex IonPac AS18-4µm, 4 × 150 mm
Eluent: 23 mM KOH
Eluent Source: Dionex EGC-500 KOH cartridge, Dionex CR-ATC 600 trap column, Dionex high pressure degasser
Flow Rate: 1.0 mL/min
Injection Vol.: 100 µL aliquot of collected solution
Column Temp.: 30 °C
Detection: Suppressed conductivity, Dionex ADRS 600, 4 mm, constant current mode, 57 mA, recycle mode
Sample Prep.: Pyrolysis and combustion of 50 µL standard
Pyrolysis: 900 °C, 10 s, Ar, 200 mL/min
Combustion: 1000 °C, 600 s, O_2, 400 mL/min
Collection Sol.: 30 ppm hydrogen peroxide, 10 mL
Collect. gas: Ar carrier, 100 mL/min, DI water
Peaks: A. 100 mg/L BVO   3.3* (mg/L)
B. 5 mg/L Bromide   4.96

*2.3% calculated bromine content

Figure 8. Comparison of A) 100 mg/L BVO, and B) 5 mg/L bromide

Column: Dionex IonPac AG18-4µm, 4 × 30 mm
Dionex IonPac AS18-4µm, 4 × 150 mm
Eluent: 23 mM KOH
Eluent Source: Dionex EGC-500 KOH cartridge, Dionex CR-ATC 600 trap column, Dionex high pressure degasser
Flow Rate: 1.0 mL/min
Injection Vol.: 100 µL aliquot of collected solution
Column Temp.: 30 °C
Detection: Suppressed conductivity, Dionex ADRS 600, 4 mm, constant current mode, 57 mA, recycle mode
Sample Prep.: Pyrolysis and combustion of 50 µL standard
Pyrolysis: 900 °C, 10 s, Ar, 200 mL/min
Combustion: 1000 °C, 600 s, O_2, 400 mL/min
Collection Sol.: 30 ppm hydrogen peroxide, 10 mL
Collect. gas: Ar carrier, 100 mL/min, DI water
Peaks: 1. Bromide
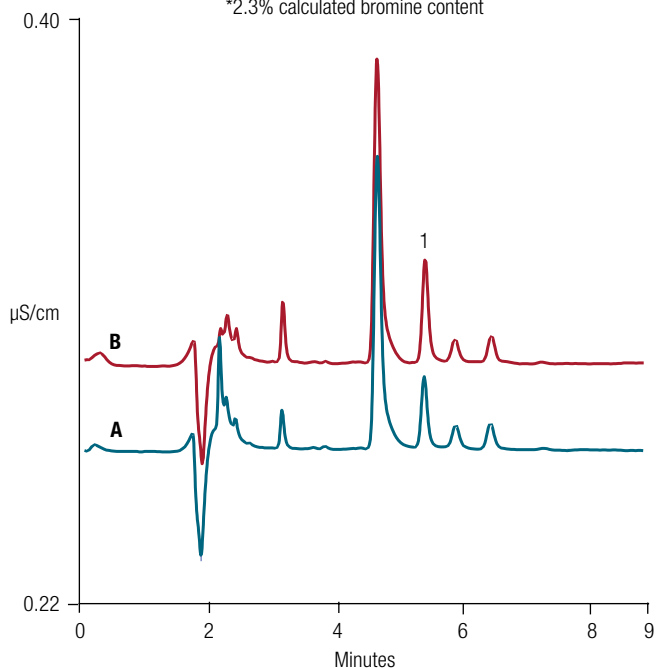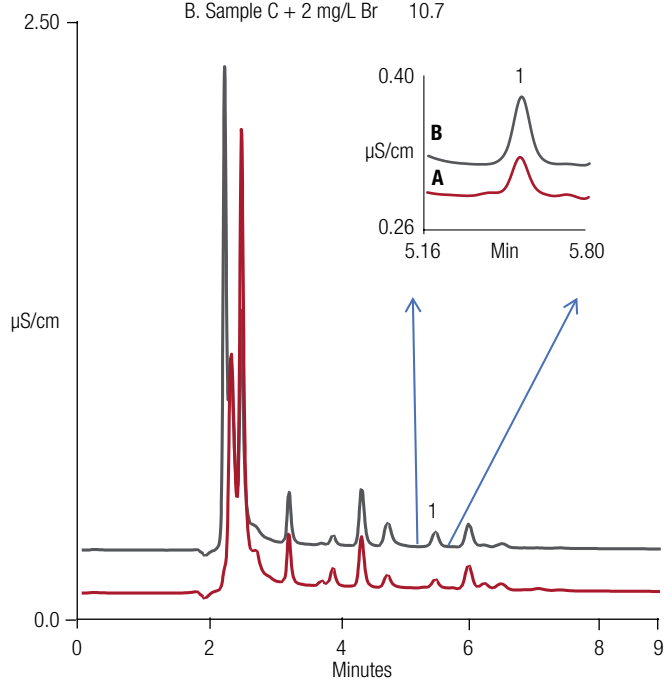A. Sample C   8.7 (mg/L)
B. Sample C + 2 mg/L Br   10.7

Figure 9. Comparison of A) Sample C, and B) Sample C plus 2 mg/L bromide

To ascertain that the total bromide by CIC is due to the BVO concentration, free bromide determinations were needed. As previously described, an external calibration was done using the ES-210 External Sampler module. Duplicate injections of 450-fold diluted 1.0, 5.0, and 10.0 mg/L bromide standards were used to generate a calibration plot for the free bromide. The response to concentration was linear with a coefficient of determination (r^2) of 0.999. The carbonated beverage samples were diluted similarly and analyzed in replicate for free bromide. Negligible free bromide concentrations were measured (Table 4).

Table 4 shows the total bromide concentrations in the samples measured by CIC, with significant amounts of total bromide (8.7 mg/L) in Sample C. The accuracy of the method was determined by adding in 0.5, 0.5, and 2 mg/L bromide to samples A, B, and C, respectively. The results in Table 4 show good recoveries, from 90 to 102%.

Table 4. Summary of results and recoveries of BVO in carbonated beverages

Sample	Free Bromide (mg/L)	Total Measured (mg/L)	RSD	Added (mg/L)	Recovery (mg/L)	RSD	%
Sample A	ND	ND	--	0.5	0.45	2.2	90
Sample B	0.007	< LOD	--	0.5	0.51	2.8	102
Sample C	0.049	8.7	2.5	2	10.7	1.9	100

Conclusion

Combustion IC (CIC) provides an automated and fast method to determine total and free halides in complex and challenging sample matrices, such as an oil-based food additive in carbonated beverages, by eliminating the sample matrix. In this application, mg/L determinations of bromide from BVO were applied to the analysis of three carbonated beverage samples.

This technique could be used to profile incoming lots of BVO for the bromination as well as to determine BVO in carbonated beverage formulations.

More information on CIC and CIC applications can be found on Thermo Scientific website and on the Thermo Fisher Scientific AppsLab Digital Library.[33,34]

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