TECHNICAL NOTE

David S. Jackson,¹ B.S.; David F. Crockett,¹ B.S.; and Karen A. Wolnik,¹ B.S.

The Indirect Detection of Bleach (Sodium Hypochlorite) in Beverages as Evidence of Product Tampering

ABSTRACT: Bleach (sodium hypochlorite) has been identified as the adulterant in a relatively large number of product tamperings that have been investigated by the Forensic Chemistry Center (FCC) of the U.S. Food and Drug Administration. In this work, household bleach was added to 23 different beverages at each of three levels. The impact of sodium hypochlorite on these beverages over a 13-day study period was evaluated using the following techniques: diphenylamine spot test for oxidizing agents, potassium iodide-starch test paper for oxidizing agents, pH, iodometric titration for quantitating hypochlorite, ion chromatography for chloride and chlorate quantitation, automated headspace sampling with gas chromatography–flame ionization detection (GC–FID) for determination of chloroform, and visual and organoleptic observations. This study has shown that hypochlorite is fragile when added to most common beverages and typically breaks down either partially or completely over time. In cases where a beverage is suspected of being adulterated with bleach but tests for hypochlorite are negative, it is still possible to characterize the product to demonstrate that the results are consistent with the addition of bleach. An adulterated product will give a positive test for oxidizing agents using the diphenylamine spot test. It is likely that the pH of the adulterated product will be higher than a control of that product. Ion chromatographic analysis shows elevated chloride and chlorate as compared with a control. And, chloroform may also be detected by GC–FID especially if the beverage that was adulterated contains citric acid.

KEYWORDS: forensic science, product tampering, adulteration, bleach, sodium hypochlorite, ion chromatography, suppressed conductivity

From 1992 to 2004, 25 documented cases of product tampering involving bleach have been investigated by the U.S. Food and Drug Administration's Forensic Chemistry Center (FCC). The majority of these cases involved bleach being added to various beverages and in some cases being completely substituted for the beverage in its original container. Some other products which have been adulterated with bleach include: infant formula, soup, raw meat, syrup, and eye drops. Household bleach contains 4–6% (w/v) sodium hypochlorite, a caustic and strong oxidizer that is harmful if swallowed and can irritate and damage eyes and skin on contact. Additionally, bleach is inexpensive and readily available.

In December 2003, Italian officials received reports from over 10 cities in Italy alleging that bottles of mineral water had been adulterated with bleach and/or acetone (1). Thousands of mineral water bottles were removed from grocery shelves for examination. Several people were treated at hospitals for stomach discomfort causing panic and concern throughout Italy.

It is probable that there will be future incidents of product tampering that will involve bleach. Determining whether sodium hypochlorite has been added to beverages can be difficult because it can decay rapidly in certain matrices. Performing studies that allow quick response to alleged tamperings is a primary function of the FCC. This report provides methodology that can be used to rapidly determine whether or not household bleach has been used to adulterate a beverage.

Materials and Methods

Commercial Products Fortified with Bleach

Table 1 shows the 23 beverages selected for this study. Beverages were spiked with 4–6% sodium hypochlorite solution (bleach) in duplicate at the following levels (v/v): 0% (20 mL beverage+0 mL bleach) used as the control for this study, 50% (10 mL beverage+10 mL bleach), 25% (15 mL beverage+5 mL bleach), and 10% (18 mL beverage+2 mL bleach). The test mixtures were kept in closed containers at room temperature for the duration of the study.

Standards and Reagents

The purified deionized water used for sample dilutions and preparation of eluents was obtained using a Milli-Q water purification system (Millipore, Milford, MA). Potassium iodide (reagent grade), sodium hypochlorite (4–6% NaOCl) (purified grade), glacial acetic acid (HPLC grade), sodium thiosulfate (reagent grade), starch solution (stabilized), 0.01 N sodium hydroxide (volumetric standard solution), and potassium iodide-starch test paper were obtained from Fisher Scientific (Pittsburgh, PA). The chlorate and chloride stock standards used for ion chromatography (IC) analysis were obtained from Inorganic Ventures Inc. (Lakewood, NJ). Sodium carbonate (reagent grade), diphenylamine (reagent grade), citric acid (reagent grade), and

¹Forensic Chemistry Center, U.S. Food and Drug Administration, Cincinnati, OH, 45237.

Received 1 May 2005; and in revised form 13 Aug. 2005, 3 Nov. 2005; accepted 4 Feb. 2006; published 14 June 2006.

TABLE 1—Average pH of spiked beverages measured on days 3 or 4.

	% Bleach Added to Beverage (v/v						
Product	0	50	25	10			
Cola 1	2.4	3.1	3.1	3.2			
Cola 2	2.4	3.1	3.1	3.3			
Diet cola 1	3.2	7.3	6.7	6.2			
Diet cola 2	2.8	8.7	6.7	6.3			
Ginger ale	3.0	3.7	4.3	6.3			
Diet ginger ale	3.3	8.0	7.0	6.6			
Citrus soft drink	3.2	3.5	4.2	6.1			
Diet citrus soft drink	3.3	8.1	7.4	6.8			
Caffeinated citrus soft drink	3.2	3.6	4.3	6.3			
Root beer	4.5	3.2	3.5	4.0			
Red soda	3.6	3.5	3.9	5.5			
Diet red soda	4.0	9.2	7.7	7.5			
Grape drink	3.0	4.4	5.8	7.2			
Orange drink	2.9	4.6	6.5	7.8			
Carbonated water	7.2	8.5	8.3	8.7			
Spring water	7.4	12.2	11.8	10.9			
Skim milk	5.6	6.7	6.7	6.8			
Infant formula (milk based)	5.5	6.3	6.6	6.8			
Orange juice (unsweetened)	3.7	6.5	5.2	4.0			
Apple juice (unsweetened)	3.5	4.0	4.6	5.9			
Grape juice (unsweetened)	3.2	3.7	4.1	3.3			
Beer	4.3	8.1	7.1	6.5			
White wine	3.0	6.1	5.6	3.8			

n=2.

N,*N*-dimethylacetamide (reagent grade) were obtained from Sigma-Aldrich (St. Louis, MO). Chloroform (reagent grade) and concentrated sulfuric acid (reagent grade) were obtained from J.T. Baker Inc. (Phillipsburg, NJ). Buffers for measuring pH were obtained from LaMotte Company (Chestertown, MD).

Diphenylamine Spot Test for Oxidizing Agents

The diphenylamine spot test (2) is used to indicate the presence of oxidizing agents like hypochlorite and chlorate, which are found in commercial bleach. One milliliter of a 1% diphenylamine solution in 60% sulfuric acid in water is placed on a porcelain spot plate. A 100 μ L volume of sample is added to the diphenylamine solution. An immediate deep blue color indicates the presence of an oxidizing agent such as hypochlorite, chlorate, bromate, nitrite, and/or nitrate. The lowest concentration of sodium hypochlorite in water that could be detected by visual observation was determined by incrementally testing standards at low concentrations and found to be 2 μ g/mL.

Potassium Iodide-Starch Test Paper

Potassium iodide-starch test paper (3) is used to test for the presence of oxidizers like hypochlorite and hydrogen peroxide. One milliliter of a liquid sample is placed in a small beaker. The end of the potassium iodide-starch test paper is placed in the solution, and the solution is allowed to migrate up the test paper. A blue color indicates the presence of an oxidizing agent. The lowest concentration of sodium hypochlorite in water which could be detected by visual observation was determined by incrementally testing standards at low concentrations and was found to be $1 \mu g/mL$.

pH Measurement

The pH of the spiked products and controls was measured using a pH meter, Orion 610 pH meter (Orion Research Inc., Boston, MA) with Orion solid state pHuture probe LM. The meter was calibrated using buffer solutions over the pH range of 2.0–12.0.

Iodometric Titration

Iodometric titration (4) was used to quantitate the hypochlorite content in the spiked beverage solutions. One milliliter of sample solution is diluted with 50 mL of purified, deionized water in a glass-stoppered flask. One gram of potassium iodide is added to the solution followed with 5 mL of 6 N acetic acid. The liberated iodine is titrated with standardized 0.1 N sodium thiosulfate solution. As the end point is approached, the color of the solution changes from brownish orange to light yellow. At this point, 1 mL of starch solution is added to the sample solution, which produces a deep blue color. Titration with sodium thiosulfate is continued until dissipation of all of the blue color which is the end point. Each mL of 0.1 N sodium thiosulfate titrant is equivalent to 3.722 mg of sodium hypochlorite. Because sodium thiosulfate degrades with time, it is necessary to standardize it before use.

Ion Chromatography (IC)

Chloride and chlorate are degradation products of sodium hypochlorite: $3OCl^- \rightarrow ClO_3^- + 2Cl^-$ (5). Anion exchange IC with suppressed conductivity was used to determine the chloride and chlorate concentrations in the spiked beverages. The instrument used for anion analysis consisted of a Dionex (Sunnyvale, CA) DX500 IC system with a GP40 gradient pump, an ED40 conductivity detector, ThermoSeparations AS3500 autosampler (25 µL injected) with Peaknet Version 5.1 software for instrument control, data acquisition and calculation, Dionex Anion Self Regenerating Suppressor used in recycle mode with an SRS controller current setting of 100 mA. A Dionex IonPAC AG9-HC guard and AS9-HC analytical column were used with a mobile phase of 9 mM sodium carbonate (1.3 mL/min) to achieve separation. Spiked samples were diluted 1:1000 with deionized water before analysis and the controls were analyzed undiluted. The orange juice, skim milk, and infant formula were filtered through a 0.45 µm nylon filter before analysis. Figure 1 shows a diagram of an IC system using suppressed conductivity detection. Applying a current to the electrolytic suppressor causes a reduction in the background conductivity of the eluent before the detector by exchanging the sodium ions for hydronium ions. The cation exchange membrane in the suppressor is continuously flushed with water or recycled eluent from the detector to remove the sodium ions. In order to show the stability of the hypochlorite anion in the IC system, eluent was collected at different locations (A, B, and C) while making repeated 100-µL injections of 4-6% hypochlorite, and the hypochlorite concentration determined by iodometric titration.



FIG. 1—Schematic of ion chromatography system using suppressed conductivity detection. The letters A, B, and C show the location where eluent was collected and hypochlorite concentration was determined.

Product Days tested	% Bleach Added to Beverage (v/v)											
	0		50		25		10					
	1	5–6	13	1	5–6	13	1	5–6	13	1	5–6	13
Cola 1	_	_	_	+	_	_	+	+	_	+	+	_
Cola 2	_	_	_	+	+	_	+	+	_	+	+	+
Diet cola 1	_	_	_	+	+	+	+	+	+	+	+	+
Diet cola 2	_	_	_	+	+	+	+	+	+	+	+	+
Ginger ale	-	-	-	-	-	-	-	-	-	-	_	-
Diet ginger ale	_	_	_	+	+	+	+	+	+	+	_	_
Citrus soft drink	_	_	_	-	_	_	_	_	_	_	_	_
Diet citrus soft drink	_	_	_	+	+	+	+	+	+	+	_	_
Caffeinated citrus soft drink	_	_	_	+	+	_	+	+	_	+	+	_
Root beer	_	_	_	+	_	_	+	+	_	+	+	+
Red soda	-	-	_	-	-	_	-	-	_	-	-	_
Diet red soda	_	_	_	+	+	+	+	+	+	+	+	_
Grape drink	_	_	_	_	_	_	_	_	_	_	_	_
Orange drink	_	_	_	_	_	_	_	_	_	_	_	_
Carbonated water	_	_	_	+	+	+	+	+	+	+	+	+
Spring water	_	_	_	+	+	+	+	+	+	+	+	+
Skim milk	_	_	_	+	+	+	+	+	_	+	_	_
Infant formula (milk based)	_	_	_	+	+	+	+	+	_	+	+	_
Orange juice (unsweetened)	_	_	_	+	+	_	+	+	_	+	_	_
Apple juice (unsweetened)	_	_	_	_	_	_	_	_	_	_	_	_
Grape juice (unsweetened)	_	_	_	+	+	+	+	+	_	_	_	_
Beer	_	_	_	+	_	_	+	_	_	+	+	+
White wine	_	-	—	+	—	_	+	_	_	+	+	-

TABLE 2—Spot test results for oxidizing agents using potassium iodide-starch test paper.

+, positive result for hypochlorite using KI-starch test paper; –, negative result for hypochlorite using KI-starch test paper.

Headspace Gas Chromatography–Flame Ionization Detection (GC–FID)

Results and Discussion

Visual and Organoleptic

Chloroform is known to occur in waters that have been chlorinated using sodium hypochlorite (6). Citric acid in the presence of sodium hypochlorite has been shown to contribute to the formation of chloroform (7). Many of the soft drinks in this study were labeled as containing declared citric acid. A series of citric acid standard solutions (4, 10, 20, 40, 100, 200, 500, and 1000 µg/ mL) were spiked at 10% (v/v) with 4–6% sodium hypochlorite and analyzed for chloroform after 7 days to determine the effects that the citric acid concentration had on chloroform formation. The chloroform levels in the soft drinks and the citric acid spikes were determined by Headspace GC-FID using a Tekmar model 7000/7050 Automated Equilibrium Headspace Sampler, HewlettPackard 5890 Series II GC/FID with a Restek Rtx-1301 capillary column (60 m \times 0.53 mm id \times 3 μm film thickness). The column flow rate was set at 12 mL/min at 45°C. The purge valve was turned off at injection and then on at 0.4 min. The injection port temperature on the GC was set to 220°C. The GC Oven was set to: 45°C for 1 min and ramped to 115°C at 5°C/min and to 235°C at 30°C/min. The detector temperature was 250°C. The vial was maintained at 85°C for 10 min before headspace sampling. The limit of quantitation was estimated to be 0.5 µg/mL. Samples were prepared by placing 250 µL of sample solution and 250 µL of N,N-dimethylacetamide into a 20 mL glass headspace vial with Teflon-lined butyl rubber septa and crimp cap. The chloroform standards were prepared in N,N-dimethylacetamide. In order to matrix match the aqueous beverage matrices and chloroform standards, an equal ratio of N.N-dimethylacetamide and beverage were used for samples and an equal volume of water and N,Ndimethylacetamide was used for the chloroform standard. This made the ratio of N,N-dimethylacetamide to water 1:1 in both the samples and standards.

The majority of beverages that were spiked with bleach exhibited a fading of the beverage's original color as compared with the control. The milk and the infant formula exhibited a slight darkening in color as compared with the control. The clear and colorless beverages remained clear and colorless throughout the 13-day study.

Many of the beverages spiked with bleach retained a hint of the beverage's original odor along with a sweet, chlorine, and/or organic odor, which was similar to that of chloroform. Three of the diet soft drinks and two of the colas exhibited a noxious odor after 13 days.

Detection, Stability, and Quantitation of Hypochlorite

Table 2 shows the results for the potassium iodide starch test paper spot tests. The diphenylamine spot test gave a positive result for oxidizing agents in all of the beverages spiked with bleach throughout the course of the 13-day study and negative results for all controls. The potassium iodide-starch test paper gave a positive result only when hypochlorite remained. After 24 h, the following beverages tested negative for hypochlorite at all spiking levels using the potassium iodide-starch test paper: apple juice, orange and grape drinks, red soda, citrus soft drink, and ginger ale. Additionally, after 13 days, the following beverage spikes tested negative for hypochlorite at all spiking levels: wine, orange juice, caffeinated citrus soft drink, and cola 1. Diet colas 1 and 2, carbonated water, and spring water were the only beverages that tested positive at all spiking levels over the 13-day study.

Table 1 shows the pH of the various spikes and controls taken on days 3 or 4 of this study. Because sodium hypochlorite solution is basic (pH 12), it was expected that the pH of the samples would have become basic when spiked with commercial bleach; however, only the beer, spring water, carbonated water, orange and

n = 2.



FIG. 2—Hypochlorite concentrations at different times for the beverages spiked at the 50% level that had remaining hypochlorite after 13 days.

grape drinks, and the diet beverages had spikes with a pH above 7. All of the spiked beverages exhibited a higher pH than the corresponding controls with the exception of root beer and red soda. It was expected that the 50% spikes would have had a higher pH than the 10% spikes; however, all of the beverages sweetened with sugars had the highest pH in the 10% spikes and the lowest pH in the 50% spikes. The shaded rows on Table 1 show the beverages that had the highest pH at the 10% or 25% spiking level. The diet beverages, spring water, orange juice, beer, and wine had the highest pH at the 50% spiking level and the lowest pH at the 10% spiking level. A 0.01 N sodium hydroxide solution was spiked into ginger ale, which contains sugar at the same spiking levels as used for the sodium hypochlorite. After 24 h, the highest pH was observed in the highest spiking level of sodium hydroxide (50%) and the lowest pH at the lowest spiking level (10%) as expected. This was opposite to the pH pattern observed for hypochlorite added to sugar containing beverages. The presence of hypochlorite appears to be causing this unexpected pH result in beverages with sugars. Hypochlorite is known to oxidize sugars in solution forming sugar acids, organic acids, and giving off carbon dioxide (8-10). It is believed that because the 50% spikes had the highest ratio of sodium hypochlorite to the sugars in the beverage a more thorough oxidation of sugars occurred. It is believed that the formation of sugar acids and organic acids caused the lower pH in the 50% spiked solution relative to the 10% spikes.

Figure 2 shows the hypochlorite concentrations measured using iodometric titration over time for the seven beverages spiked at 50% that had quantifiable hypochlorite remaining after 13 days. With the exception of spring water, all of the 50% spikes showed significant breakdown of hypochlorite after 5–6 days. The common factors among these beverages with remaining hypochlorite are their lack of sugar and that all the pH's of the beverages were above 7.0.

Figure 1 shows the locations where eluent was collected to quantitate the hypochlorite concentration in the IC system. The hypochlorite concentration diminished as it traveled through the IC system with a significant decrease observed at the interface between the suppressor and detector (C). The fraction corresponding to chloride by IC was collected and tested for hypochlorite using the potassium iodide-starch test paper. The results were positive for hypochlorite indicating that hypochlorite that did not break down in the suppressor coeluted with chloride.

Because of the loss of hypochlorite in the suppressor and its coelution with chloride, the hypochlorite anion cannot be accurately quantitated using suppressed IC. It is anticipated that a more accurate chloride value would be obtained if all of the hypochlorite in a sample solution has broken down before IC analysis. Ion



FIG. 3—Overlay chromatogram of cola 1 spiked with hypochlorite solution at 0%, 10%, 25%, and 50% (v/v) measured within 24 h. Peak 1 = chloride, 2 = chlorate.

chromatographic analysis of the spiked beverages showed elevated levels of chloride and chlorate as compared with the controls. Figure 3 shows an overlay chromatogram of cola 1 and its spikes. The peaks corresponding to chloride and chlorate increased proportionally in size as the concentration of hypochlorite added increased. The chloride concentrations at the various spiking levels were more consistent for all of the beverages at a given spiking level than the chlorate. The chloride concentrations and standard deviations for all of the 50%, 25%, and 10% beverage spikes after 13 days were: 27.8 ± 3.3 , 13.7 ± 1.3 , and 5.2 ± 0.7 mg/mL, respectively. None of the control samples had a chloride concentration greater than 1.0 mg/mL. Figure 4 shows the chlorate concentrations for the 23 beverages at the three different spiking levels after 13 days. No chlorate was detected in any of the control samples. Six of the 50% spikes have a chlorate level that is significantly higher than the others. The four factors that distinguish these six beverages from the others are: all were carbonated beverages, they contained no added sugars, the pH after spiking was above 7, and after 13 days they had measurable hypochlorite present.

Figure 5 shows a headspace GC-FID chromatogram of chloroform from ginger ale spiked at 25%. Chloroform was detected in all of the spiked soft drinks at concentrations ranging from 3 to 205 µg/mL. The diet citrus soda had the highest level of chloroform detected, and cola 1 (which was not labeled to contain citric acid) had the lowest level of chloroform detected. The sodium hypochlorite solution used for spiking was determined to contain 11 µg/mL chloroform. Some of the soft drinks had measured levels of chloroform below the level of chloroform found in the hypochlorite solution used for spiking. In these cases, the chloroform may be attributed to the spiking solution. For the soft drinks that had chloroform detected in excess of the amount found in the hypochlorite spiking solution, the chloroform can be attributed to interaction between components of the beverage and the hypochlorite. Figure 6 shows a graph of chloroform concentration versus citric acid concentration for a series of citric acid standards that were spiked at 10% (v/v) with 4-6% sodium hypochlorite. The graph illustrates that the level of chloroform generated by the interaction of hypochlorite with citric acid increases as the concentration of citric acid increases.

Summary

The studies described in this paper have shown that hypochlorite is fragile when added to most common beverages and typi-



FIG. 4—Graph of chlorate concentrations of beverages spiked with hypochlorite solution at 50%, 25%, and 10% (v/v) after 13 days.



FIG. 5—Gas chromatography–flame ionization detection chromatogram of chloroform in ginger ale 13 days after addition of 25% (v/v) hypochlorite solution. Peak 1 = chloroform (136 µg/mL), 2 = N,N-dimethylacetamide.

cally breaks down either partially or completely over time. In cases where it is suspected that a beverage is adulterated with bleach but no hypochlorite is detected, it is still possible to characterize the product and demonstrate results that are consistent with the addition of bleach. It is also helpful to have a control sample to make direct comparisons with the suspect sample and to spike a control sample with bleach to compare to the suspect sample. The diphenylamine spot test gives a positive result for the breakdown product, chlorate, whether or not hypochlorite is



FIG. 6—Chloroform concentration versus citric acid concentration of citric acid solutions 7 days after addition of 4-6% sodium hypochlorite at 10% (v/v).

present. It is likely that the pH of the adulterated product will be higher than the pH of the control. A chlorine or organic odor may be noted. Elevated chloride and chlorate may be observed by IC as compared with a control. Chloroform may also be detected by headspace GC–FID especially if the beverage has citric acid in it. These factors can be used to indicate adulteration with bleach.

Acknowledgments

The authors would like to thank Kevin J. Mulligan, Ph.D., and Douglas T. Heitkemper, Ph.D., for helpful discussions during the early stages of this work and during the preparation of this manuscript.

References

- Italian Poison Alert Over 'Aquabomber' The United Kingdom Times 2003 December 8; Overseas News;13.
- Clarke EGC. Isolation and identification of drugs. 2nd ed. London: The Parmaceutical Press, 1986:5.
- Clarke EGC. Isolation and identification of drugs. 2nd ed. London: The Parmaceutical Press, 1986:67.
- The Council of Experts. The United States pharmacopeia. 28th rev. ed. Rockville: The United States Pharmacopeial Convention, 2005:1786.
- Dionex Application Note 29R. Analysis of hypochlorite and chlorate in bleach using combined conductivity and electrochemical detection. Sunnyvale, CA: Dionex Corp, 1981.
- Aggazzotti G, Fantuzzi G, Righi E, Predieri G. Environmental and biological monitoring of chloroform in indoor swimming pools. J Chromatogr 1995;A710:181–90.
- Robinson D, Mead GC, Barnes KA. Detection of chloroform in the tissues of freshly eviscerated poultry carcasses exposed to water containing added chlorine or chlorine dioxide. Bull Environ Contam Toxicol 1981;27:145–50.
- Whistler RL, Schweiger R. Preparation of D-arabinose from D-glucose with hypochlorite. J Am Chem Soc 1959;81(19):5190–2.
- Henderson JT. The action of an aqueous chlorine system on methyl β-Dglucopyranoside. J Am Chem Soc 1957;79(19):5304–8.
- Whistler RL, Schweiger R. Oxidation of alginic acid with hypochlorite at different hydrogen ion concentrations. J Am Chem Soc 1958;80(21):5701–4.

Additional information and reprint requests: David S. Jackson, B.S. U.S. Food and Drug Administration Forensic Chemistry Center 6751 Steger Drive Cincinnati, OH 45237 E-mail: david.jackson@fda.hhs.gov