

Determination of Benzoate in Liquid Food Products by Reagent-Free™ Ion Chromatography

INTRODUCTION

Preservatives are commonly added to many food products, such as soda, fruit juice, soy sauce, jams and jellies, and other condiments, to inhibit decay. Since the early 1900s, benzoate has been widely used worldwide as a preservative due to its antimicrobial properties combined with its low toxicity and taste. Benzoate is most effective in an acidic environment ($\text{pH} \leq 4.5$) and is not recommended for use at higher pH .¹

Benzoic acid is an effective antimicrobial agent for the purpose of preservation. However, sodium benzoate is more effective and preferred because it is approximately 200 times more soluble than benzoic acid. The soft drink industry is the largest user of benzoate as a preservative due to the amount of high fructose corn syrup in many carbonated beverages. Soft drinks account for the largest human consumption of benzoate in the USA, Australia/New Zealand, France, and the United Kingdom.² Although soft drinks do not normally spoil due to their acidity and carbonation, preservatives are required to prevent changes during long-term storage.³

The Food and Drug Administration (FDA) regulates the uses of benzoate as a preservative in the USA. The FDA lists benzoate as a substance that is generally recognized as safe (GRAS) with a maximum permitted concentration of 0.1% in accordance with good manufacturing or feeding practices.⁴ Similarly, benzoate is regulated in Europe by the European Union Legislation (Directive 95/2/EC) with a limit of 0.015% in soft drinks and up to 0.2% in other food products.⁵ If higher concentrations of benzoate are used ($\sim 0.1\%$), then alterations in taste may occur in soft drinks.¹

On the other hand, concentrations less than 0.010% will have little inhibitory effect.^{2,6} Therefore, a reliable testing method is required to assure that the concentration of benzoate is within product and regulatory specifications.

Methods used to determine benzoic acid or its corresponding salt in foods, beverages, and other matrices include titrimetry, ion-selective electrodes, gas chromatography (GC), thin-layer chromatography, and high-performance liquid chromatography (HPLC). Many of these methods have significant disadvantages and are therefore not preferred for use in a quality control environment if a large number of samples are to be analyzed. For example, the GC method proposed by the Association of Official Analytical Chemists for the determination of benzoic acid and sorbic acid requires solvent extractions and derivatization techniques. This process involves complex procedures and is exceptionally time-consuming.⁷ From the previously listed techniques, HPLC (including reversed phase, ion exchange, and ion exclusion) is used most often for the determination of benzoic acid. With this technique, many samples can be simply diluted and injected directly into the chromatography system without any complex sample preparation.

In this application note, we describe a simple ion chromatography method for the direct determination of benzoate in liquid food products. This method incorporates a Reagent-Free Ion Chromatography (RFIC™) System, requiring only deionized water to electrolytically produce a potassium hydroxide eluent, thus further simplifying user operation.

EQUIPMENT

A Dionex ICS-2000 RFIC System was used in this work. The ICS-2000 is an integrated ion chromatograph that includes:

- Eluent generator
- Column heater
- Pump degas
- EluGen[®] EGC II KOH Cartridge
(Dionex P/N 058900)
- CR-ATC (P/N 060477)

AS50 Autosampler

Chromeleon[®] Chromatography Workstation

REAGENTS AND STANDARDS

Deionized water, Type I reagent-grade, 18 M Ω -cm resistivity or better

Sodium benzoate, 99% (Sigma-Aldrich P/N 10,916-9)

CONDITIONS

Columns: IonPac[®] AS18 Analytical, 4 × 250 mm (P/N 060549)
IonPac AG18 Guard, 4 × 50 mm (P/N 060551)

Eluent: 35 mM KOH from 0–10 min,
35–40 mM from 10–12 min

Eluent Source: ICS-2000 EG with CR-ATC

Flow Rate: 1.0 mL/min

Temperature: 30 °C

Injection: 25 μ L

Detection: Suppressed conductivity, ASRS[®]
ULTRA II, 4 mm (P/N 061561)
AutoSuppression[®] recycle mode
112 mA current

Background

Conductance: 1 μ S

System

Backpressure: ~2400 psi

Run Time: 20 min

PREPARATION OF SOLUTIONS AND REAGENTS

1000 mg/L Benzoate Standard Solution

Dissolve 0.119 g sodium benzoate in 100 mL of deionized water. Working standards were prepared by serial dilutions from the 1000-mg/L concentrate.

SAMPLE PREPARATION

Carbonated samples should be degassed in an ultrasonic bath prior to dilution. All samples were diluted with deionized water by 1:100 prior to analysis, except the diet soda that was diluted 1:20.

RESULTS AND DISCUSSION

If a product contains a preservative, such as benzoate, then the chemical must be declared on the label according to U.S. FDA regulation. The U.S. FDA permits the use of up to 0.1% benzoate. In this study, four samples were analyzed for the presence of benzoate. Each product declared the use of benzoate on their respective labels. In addition to benzoate, many diet sodas and other types of soft drinks contain appreciable amounts of citrate. Citrate is commonly added to soft drinks as a food acidulant and flavor enhancer.³

In this application note, the IonPac AS18 was found to be the most suitable column for the separation of benzoate in food products. The AS18 is a high-capacity, hydroxide-selective, anion-exchange column, enabling it to tolerate the high-ionic-strength samples sometimes encountered in the food and beverage industry. In addition, the column provides an optimum selectivity for benzoate, resulting in excellent resolution between anions present at higher concentrations—such as chloride and phosphate—while still eluting anions with a higher affinity for the anion-exchange resin—such as citrate—within a reasonable time period (<20 min).

The system was calibrated from 1–20 mg/L to cover the expected range of benzoate in the diluted samples. In this application note, citrate was not of interest and was therefore not included in the calibration. Table 1 summarizes the calibration data and limit of detection for benzoate. The response for benzoate was linear over the concentration range investigated with an r^2 value of 0.9998. The method detection limit (MDL) was determined by performing seven replicate injections of a 20- $\mu\text{g/L}$ benzoate standard and calculating the MDL based on the standard deviation of the mean multiplied by 3.143 (Student's t value for a 99% confidence level for $n = 7$). The calculated MDL, based on the replicate injections, was 4.9 $\mu\text{g/L}$.

Table 1. Linearity and Method Detection Limits for Benzoate

Analyte	Range (mg/L)	Linearity (r^2)	Calculated MDL ($\mu\text{g/L}$)	MDL standard ($\mu\text{g/L}$)
Benzoate	1–20	0.9998	4.9	20

Table 2 summarizes the data obtained from the analysis of four samples for benzoate. As shown, most samples contained approximately 0.05% (500 ppm) of benzoate as a preservative, which is well below the 0.1% regulation specified by the FDA. However, the diet soda contained about half the benzoate (~0.02%) compared to the other samples analyzed. Figures 1–4 show chromatograms of benzoate determinations for flavored soda, diet soda, soy sauce, and lemon juice, respectively.

Table 2. Concentrations and Retention Time and Peak Area Precisions of Benzoate in Food Products

Sample	Concentration Found (%) ^a	Retention Time Precision (%RSD ^a)	Peak Area Precision (%RSD)
Flavored soda	0.043	0.020	0.49
Diet soda	0.019	0.021	0.44
Soy sauce	0.051	0.055	0.47
Lemon juice	0.048	0.019	0.40

^a The concentrations and relative standard deviations (RSDs) were calculated from 10 replicate injections ($n = 10$)

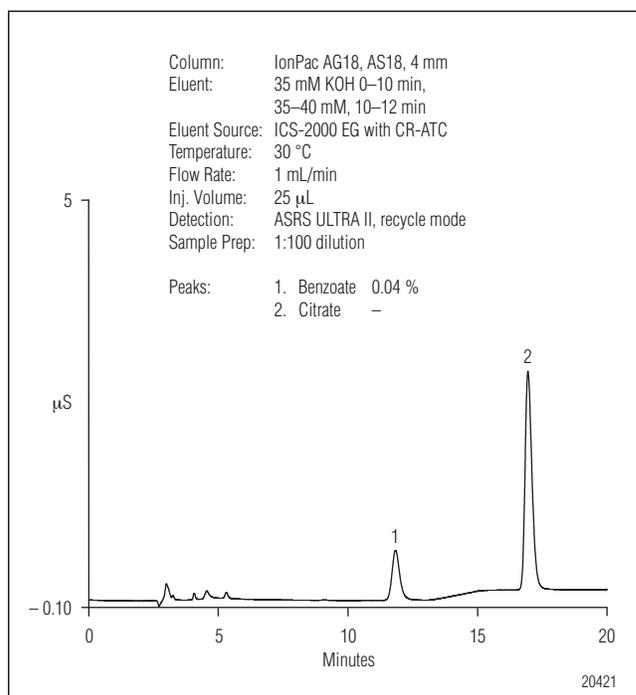


Figure 1. Determination of benzoate in flavored soda.

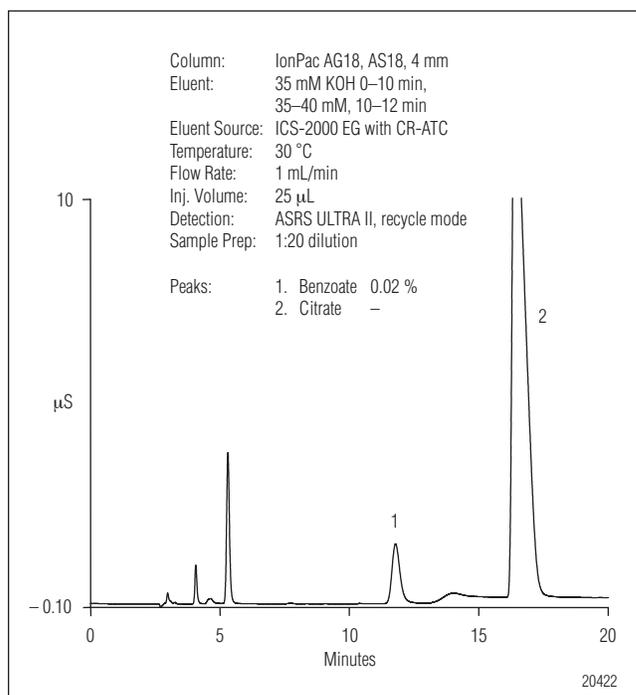


Figure 2. Determination of benzoate in diet soda.

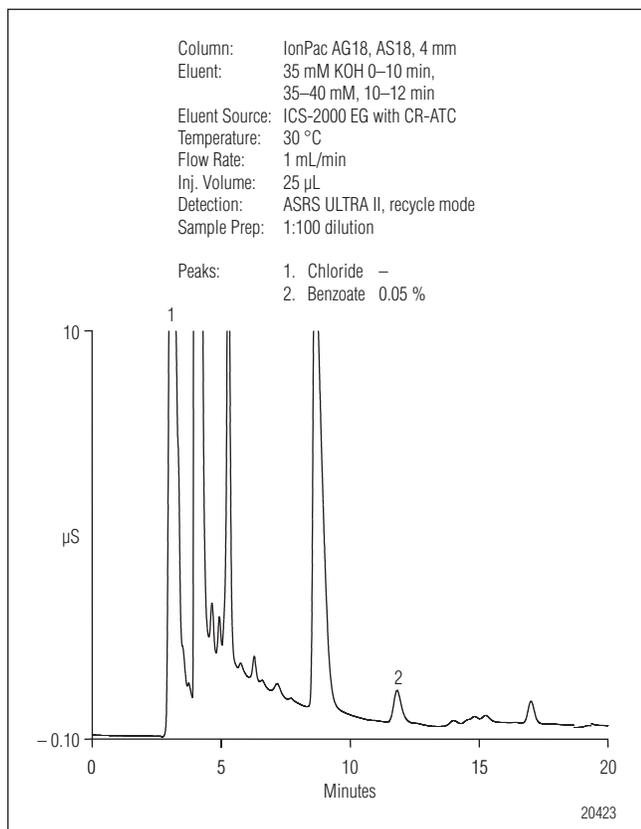


Figure 3. Determination of benzoate in soy sauce.

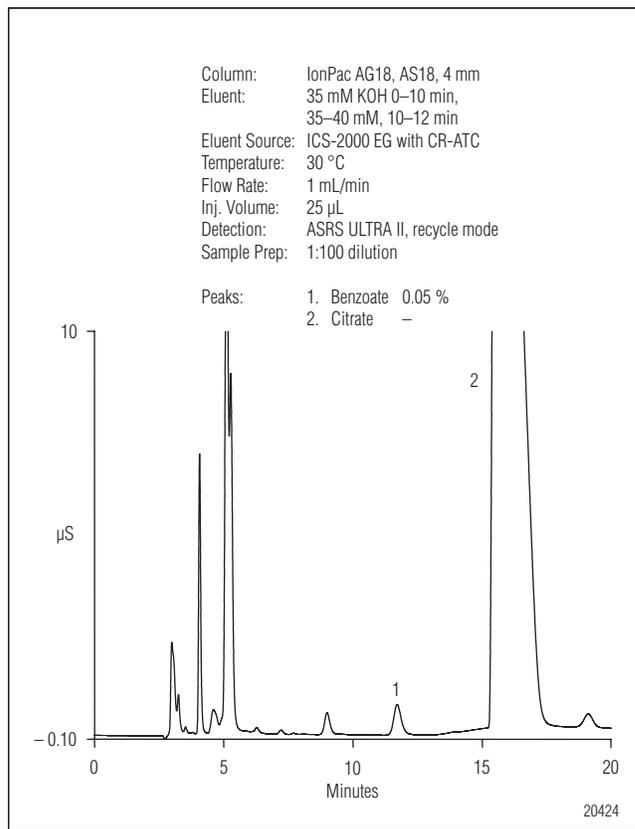


Figure 4. Determination of benzoate in lemon juice.

The high capacity of the AS18 column enabled it to tolerate the high amounts of chloride in soy sauce and citrate in lemon juice, while still providing a good selectivity for benzoate. The precision of ten replicate sample injections resulted in retention time and peak area RSD values of <0.06% and <0.50%, respectively. The high repeatability of the method reflects results typically found when using an RFIC system. Each sample was spiked with benzoate at approximately the same amount of benzoate found in the diluted samples. The average spiked recoveries, based on triplicate injections, yielded recoveries in a range of 90–101% (Table 3).

Sample	Amount Added (mg/L)	Recovery ^a (%)
Flavored soda	4.3	101.2
Diet soda	9.6	90.2
Soy sauce	4.8	94.5
Lemon juice	4.7	97.7

^aThe average recovery was calculated from triplicate injections.

CONCLUSION

This application note demonstrates a simple and reliable RFIC method for the determination of benzoate in food products using a high-capacity, hydroxide-selective, anion-exchange column. In addition to benzoate, the method can determine other anions that are often present in many food products, such as chloride, phosphate, and citrate. In comparison to previously reported methods for benzoate, RFIC provides added convenience and simplicity for the user by enabling full control of the hydroxide eluent concentration through Chromeleon software. In addition, samples only require a simple dilution prior to injection. Furthermore, the precision is significantly improved by avoiding manual preparation of eluents.

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SUPPLIER

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