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Selective and sensitive determination of bromate in bread by IC-MS

Gemma Ellison, Manali Aggrawal, Paul Dewsbury, and Jeff Rohrer, Thermo Fisher Scientific, Sunnyvale, CA

ABSTRACT

Purpose: To develop a method for determination of bromate in flour and flour products using Ion Chromatography (IC) coupled with single quadrupole MS detection.

Methods: Flour samples were extracted with high-purity water and subjected to a series of simple clean up steps before they were analyzed on the IC-MS system. A high-capacity anion-exchange column was used to separate bromate from matrix anions.

Results: Six commercial flour and flour products including homemade bread baked using flour containing potassium bromate were analyzed. The addition of an MS detector to the IC system provided high detection specificity, and quantification of those samples with an unresolved bromate peak.

INTRODUCTION

Potassium bromate is a food additive used as “flour improver” in the baking industry. Bromate is considered a carcinogenic[1] and nephrotoxic substance. Due to its carcinogenic potential, many countries have banned this use of potassium bromate. The US FDA restricts its use and allows up to 50 mg of potassium bromate to one kg of flour[2], with the belief that the baking process converts potassium bromate to non-carcinogenic bromide. However, if baking is incomplete, there may be significant residual bromate. It is thus important to monitor bromate concentration in finished flour products.

We developed a method for a selective and sensitive determination of bromate in flour products that uses IC coupled with single quadrupole MS (IC -MS). Flour samples were extracted with de-ionized (DI) water and subjected to a series of simple clean up steps before they were analyzed on the IC-MS system. Thermo Scientific™ Dionex™ IonPac™ AS31 column was used to separate bromate from matrix anions. It is a high capacity column, which allows relatively large injection volumes, thus facilitating the determination of low bromate concentrations. In this work, six commercial flour and flour products, including homemade bread baked using bromated flour, were analyzed for their bromate content using IC-MS.

MATERIALS AND METHODS

Table 1. Chromatographic conditions

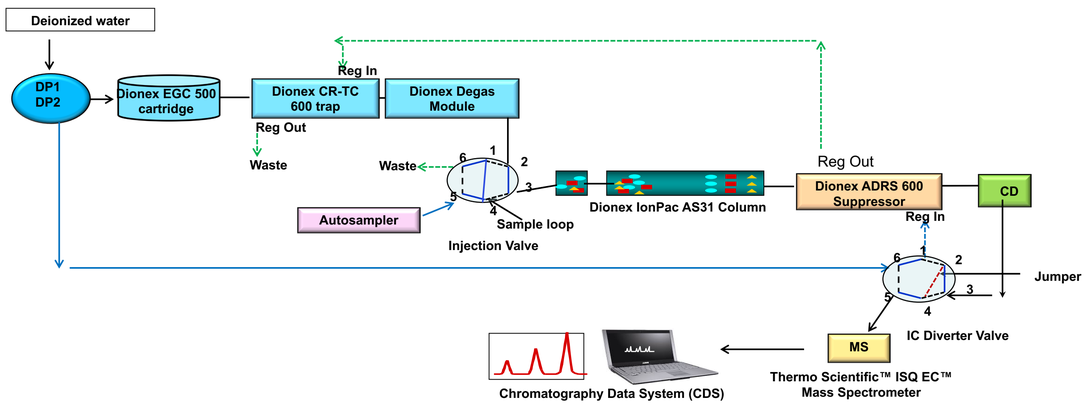
System	Thermo Scientific™ Dionex™ ICS-6000 Capillary HPIC™ System
Column	Dionex IonPac AG31 Guard, 2 × 50 mm (P/N 303147) Dionex IonPac AS31 Analytical, 2 × 250 mm (P/N 303148)
Eluent	0 – 20 min: 5 mM KOH 20-25 min: 70 mM KOH 25-30 min: 5 mM KOH
Flow Rate	0.3 mL/min
Injection Vol.	62.5 µL
Column Temp.	15 ℃
Detection 1	Suppressed Conductivity
Suppressor	Thermo Scientific™ Dionex™ DRS 600 Dynamically Regenerated Suppressor (2 mm), external water mode
Suppressor current	52 mA
Detection 2	Thermo Scientific™ ISQ™ EC Single Quadrupole Mass
Ionization interface	ESI, negative mode
Sheath gas pressure	45 psi
Aux gas pressure	4.5 psi
Sweep gas pressure	1 psi
Source Voltage	-2500 V
Vaporizer Temp.	450 ℃
Ion Transfer tube temp.	200 ℃

Data Analysis

Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software was used for all data acquisition and processing.

SYSTEM SET UP

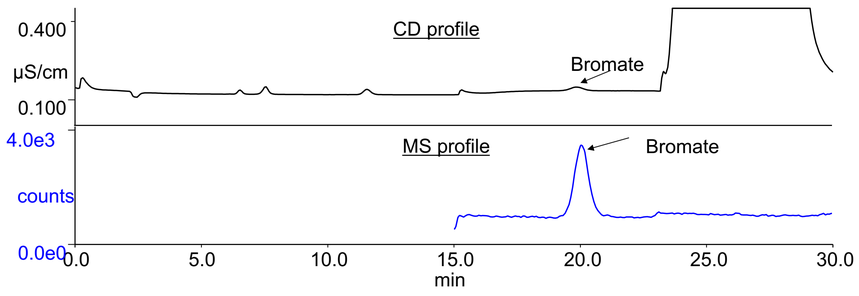
Figure 1. Flow Diagram for IC/MS set up



SEPARATION

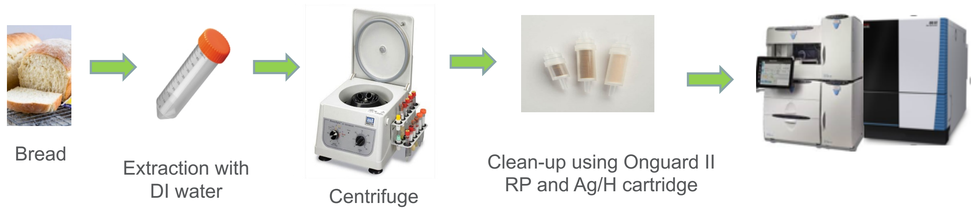
Bromate was separated on a 2 × 250 mm IonPac AS31 column at a 0.3 mL/min flow rate with 5 mM KOH produced by the Thermo Scientific™ Dionex™ EGC 500 KOH Eluent Generator Cartridge. A large volume injection (62.5 µL) was used to obtain the lowest detection limit without overloading the column. After the separation, bromate was detected in sequence by suppressed conductivity (CD) and MS. Figure 2 shows chromatography of a 1 µg/L bromate std.

Figure 2. Chromatogram of a 1 µg/L bromate standard



Bromate has two major isotopes of molecular weight of 126.92 g/mol and 128.91 g/mol, thus signal was detected at SIM channels of m/z 127 ($79BrO_3^-$) and 129 ($81BrO_3^-$).

SAMPLE PRETREATMENT



SAMPLE ANALYSIS

All flour samples were bought from Sunnyvale, California markets except bromated flour samples, which were bought online (https://www.bakersauthority.com/). Figure 3 shows the chromatograms of three flour samples along with a 10 µg/L bromate standard. Out of three flour samples, two are bromated flour samples and found to contain ~15 and ~9 mg/kg bromate, respectively. No bromate was detected in all-purpose flour sample (FS1).

Figure 3. Chromatography of flour samples

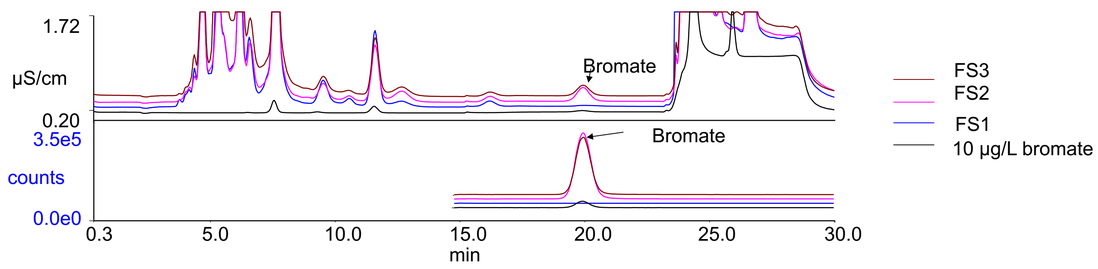
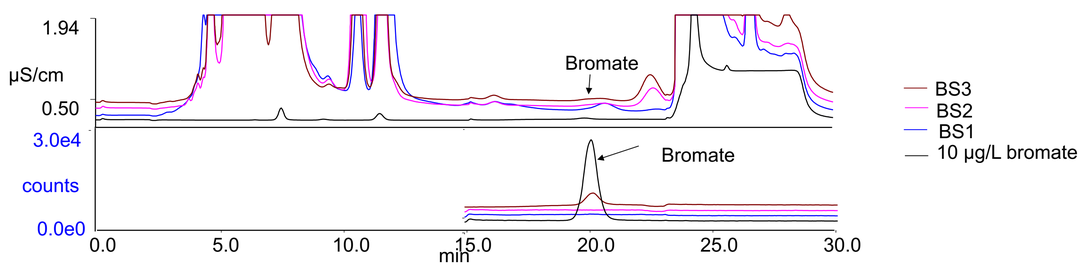


Figure 4 displays the chromatograms of three baked samples: two commercial baked goods (sliced white bread and burger bun) and one homemade bread baked with flour containing 65 mg/kg potassium bromate.

Figure 4: Chromatography of bread samples



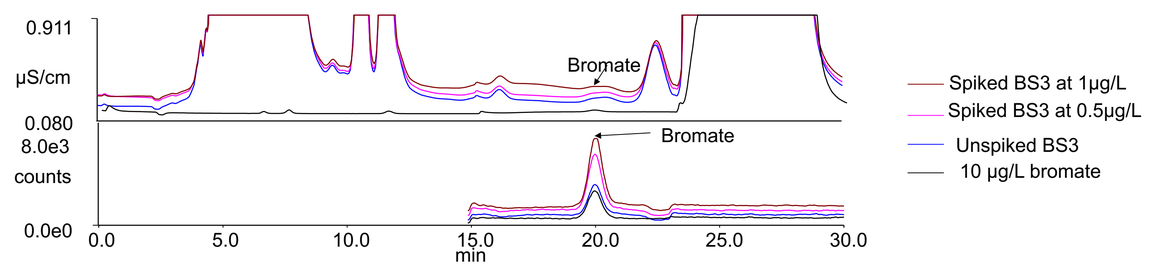
The CD profile shows that bromate is not very well resolved in any of the three bread samples. Because of the coelution, CD detection was not be used for the quantification of these samples. MS detection can resolve co-eluting peaks using their mass-to-charge ratios and is used for the quantification. No bromate was detected in bread samples BS1 and BS2. In homemade bread sample BS3, bromate was detected at 1.2 µg/L, corresponding to 60 µg/kg bromate in bread which is ~0.1% of the bromate originally added in the flour.

Table 3. Amount of bromate in flour and bread samples

#	Sample	Amount of bromate (mg/Kg)
FS1	All purpose flour	Not found
FS2	Gold medal Full strength bromated flour	15 mg/Kg(300ppb)
FS3	Pillsbury Hi gluten bromated flour	9 mg/Kg(180ppb)
BS1	White bread	Not found
BS2	McDonald Burger	Not found
BS3	Bread (baked using bromated flour)	0.06 mg/Kg

RECOVERY

Figure 5. Bromate recovery in a bread sample



Due to the interfering peaks in the bread sample, bromate could not be detected by CD (Figure 5), and thus only MS detection was used for the calculation of bromate recoveries in bread samples (Table 4).

Table 4. Recovery calculated by CD and MS detections

Sample#	CD detection			MS detection		
	Found (µg/L)	added (µg/L)	Recovery %	Found (µg/L)	added (µg/L)	Recovery %
FS1	Not found	10	85.6	Not found	10	92.0
		100	101		100	102
FS2	295	100	87.0	304	100	91.0
FS3	181	100	103	183	100	98.0
BS1	-*	*- not detected by CD		Not found	10	89.0
BS2	-*				100	92.6
BS3	-*			Not found	10	85.8
					100	104
				1.20	0.5	108
					1	110
					10	99.7
					100	90.5

CONCLUSIONS

- Using a high capacity anion exchange column and large volume injection, low concentrations of bromate were detected in flour and bread samples.
- The addition of a MS detector to the IC system provided high detection specificity, and quantification in samples with an unresolved bromate peak.
- The method showed good precision with RSDs <0.2%, and <5% (n=8), for RT and peak area respectively.
- The LOD and LOQ of bromate were 0.10 µg/L and 0.34 µg/L, respectively, which corresponded to 5 µg/kg and 17 µg/kg in bread.

REFERENCES

1. Potassium Bromate. IARC Monograph Evaluating Carcinogenic Risk to Humans, IARC. 1986, 40, 207-220
2. Bromated flour. CFR - Code of Federal Regulations Title 21 Section 137.155 - Bromated flour. [Online: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=137.155] accessed on April 2019

TRADEMARKS/LICENSING

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