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ARTICLE

Trace Analysis of Bromate in Potato Snacks Using High-Performance Liquid Chromatography−Tandem Mass Spectrometry

Francisco Arias,* Lijuan Li, Thomas G. Huggins, Paul R. Keller, Paula M. Suchanek, and Kenneth R. Wehmeyer

The Procter and Gamble Company, Global Analytical Capability, 8700 Mason-Montgomery Road, Mason, Ohio 45040

A simple, sensitive, and selective high-performance liquid chromatography−tandem mass spectrometry (HPLC−MS/MS) method in the negative-ion electrospray ionization (ESI^-) mode was validated for the quantitation of bromate (BrO_3^-) in potato snacks. Ground snack specimens (~0.5 g/sample) are spiked with $Br^{18}O_3^-$, stable-isotope labeled bromate internal standard (IS), and vortexed with a mixture of distilled/deionized water (dd water) and heptane. Subsequently, the specimens are centrifuged, and a small portion of the aqueous extract is isolated, diluted with dd water (1:4), and analyzed by HPLC−MS/MS. The methodology has a quantitation range of 10−1000 ppb, an accuracy of 1.5−7.5%, and a precision of 5.2−13.4% across the concentration range.

KEYWORDS: Bromate; potato snacks; HPLC−MS/MS; stable isotope-labeled internal standard; HILIC; extraction; precision; accuracy; stability; specificity; recovery

INTRODUCTION

Bromate (BrO_3^-) has been used in low levels to enhance the maturing process and baking of flour products, which results in consumer-appealing properties, such as increased bread volume and a crumbly structure (*1*). Nonetheless, the International Agency for Research on Cancer, a capability of the World Health Organization, has classified bromate as a Group 2B or "possible human carcinogen" and suggested, as a general principle, that it should not be present in food (*2*, *3*). The U.S. Food and Drug Administration and the U.S. Environmental Protection Agency mandated a feasible maximum bromate contamination level of 10 parts per billion (ppb) in consumable materials (*4*). This resolution generated a need for reliable and sensitive analytical methodology to quantitate bromate in bakery and a wide range of consumer goods. Several assays have been reported for the analysis of bromate in water and bread products, but no methodology has been reported for potato snacks (*1*, *5*−*8*). The previously reported methods were laborious, time-consuming, and expensive and involve multi-step sample preparation steps using solid-phase extractions to remove lipids (*8*), cation-exchange resin filtrations to remove undesirable ions (*5*, *6*, *8*), and in some cases, chemical derivatization of the analyte (*1*, *7*, *8*). Furthermore, photospectrometric detection techniques employed in many of these assays do not allow for high analyte specificity (*1*, *7*, *8*).

The aim of this paper was to develop a rapid and simple sample preparation approach coupled with highly selective high-performance liquid chromatography−tandem mass spectrometry (HPLC−MS/MS) for the analysis of bromate in potato snacks.

*To whom correspondence should be addressed. Telephone: 513-627-8463. Fax: 513-627-4401. E-mail: arias.f@pg.com.

MATERIALS AND METHODS

Reagents. Potassium bromate (99.8% purity), potassium metal (99.95%), bromine (99.99%), ^{18}O-labeled water ($H_2{}^{18}O$; 99% atom), and triethylamine (TEA; 99.5% purity) were purchased from Sigma-Aldrich (St. Louis, MO). Acetonitrile (ACN; HPLC grade), heptane (HPLC grade), isopropanol (IPA; HPLC grade), methanol (MeOH; HPLC grade), and formic acid (FA; 85%, ACS reagent) were acquired from EMD (Madison, WI). Distilled/deionized water (dd water) was obtained using an in-house distillation apparatus combined with a Milli-Q system (Millipore, Bedford, MA). Potato snacks (unseasoned or seasoned with barbecue, sour cream and onion, and cheese flavors) were provided by the Procter and Gamble Company (Cincinnati, OH). The analytical 96-well plates (1.3 mL/well, catalog number 07-3400) were purchased from MicroLiter Analytical Supplies, Inc. (Suwanee, GA). Silicone sealing mat caps for 96-well plates (catalog number 521-01-201) were from AXYMAT (Union City, CA).

Synthesis of $KBr^{18}O_3$. Stable-isotope labeled internal standard (IS) ($KBr^{18}O_3$) was prepared in-house according to a modified synthetic procedure described below (*9*). Briefly, under nitrogen in a glovebag, slowly add 1.0 mL of $H_2{}^{18}O$, a drop at a time, into a vial already containing ~0.2 g of potassium metal to generate a solution of $K^{18}OH$ in $H_2{}^{18}O$. Extreme care should be used because of the extremely exothermic reaction of potassium with water. Allow the mixture to cool and then cap with a Teflon-lined cap. Once cooled, add 120 μL of bromine, a drop at a time, with stirring to $K^{18}OH$ in $H_2{}^{18}O$ solution prepared above. Cap the resulting mixture with a Teflon-lined cap and heat at 60 °C for 2 h. After heating, place the vial in a refrigerator overnight, and the following day, isolate the solid material by filtration, rinse the isolated solid material with ~1 mL of methanol, and dry the isolated solid under vacuum. The reaction

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yielded ~16 mg of $KBr^{18}O_3$. Subsequent to our completion of this paper, a commercial source of $KBr^{18}O_3$ became available from Cambridge Isotope Laboratories (Andover, MA).

Preparation of Blank Snack Extract. Blank snack extract was prepared by placing a few unseasoned potato snacks into a plastic bag and crushing them using a Pyrex beaker. Approximately 0.5 g amounts of the ground snacks were transferred into separate glass vials. Then, 4.5 mL of dd water and 4.5 mL of heptane were added to each vial, and the vials were capped with Teflon-lined caps and vortexed for 15 min. After vortexing, the vials were centrifuged at 3500 rpm for 30 min and the aqueous phase was isolated from each vial and combined to give the blank snack extract. The blank snack extract was stored refrigerated when not in use.

Preparation of Working Standards. Bromate stock solutions were prepared in dd water over a concentration range from 10 to 1000 ppb. Working standards were prepared at the 10, 15, 50, 100, 850, and 1000 ppb levels by aliquoting 20 μL of each stock solution into predefined positions of a 96-well plate already containing 160 μL of blank snack extract and 20 μL of IS solution (500 ng/mL $Br^{18}O_3^-$ in dd water). Then, 800 μL of dd water was added to each working standard well, and the plate was sealed with a mat cap, mixed by vortexing, and analyzed by HPLC−MS/MS, as described below.

Preparation of Quality-Control (QC) Samples. QC samples were prepared at low-QC (LQC; 20 ppb), mid-QC (MQC; 200 ppb), and high-QC (HQC; 800 ppb) levels. The LQC, MQC, and HQC levels were prepared by spiking 0.5 g samples of ground unseasoned snacks for each QC level (n = 6 at each QC level) with an aliquot (100 μL) of an appropriate aqueous bromate spiking solution prepared from a separate weighing of potassium bromate. The spiking aliquot was allowed to soak into the snacks for 5 min. Then, each QC sample was spiked with a 500 μL aliquot of IS solution (500 ng/mL $Br^{18}O_3^-$ in dd water), and the IS was allowed to soak into the snacks for 5 min. Then, 4.4 mL of dd water and 4.5 mL of heptane were added to each vial, and the vials were capped with Teflon-lined caps. The QC samples were vortexed for 15 min and then centrifuged for 30 min at 3500 rpm. A 200 μL aliquot of each QC sample was transferred to a predefined position of a 96-well plate already containing 800 μL of dd water, and the plate was sealed with a mat cap, vortexed, and analyzed by HPLC−MS/MS, as described below.

Preparation of Potato Snack Samples. A few snacks from a given lot were placed in a plastic bag and crushed as described above, and replicate 0.5 g amounts of each sample were transferred to separate glass vials. Each sample was spiked with a 500 μL aliquot of IS solution (500 ng/mL $Br^{18}O_3^-$ in dd water), which was allowed to soak into the snacks for 5 min. Then, 4.5 mL of dd water and 4.5 mL of hexane were added to each vial, and the vials were processed as described above and analyzed by HPLC−MS/MS, as described below.

HPLC−MS/MS Conditions. Bromate and the stable-isotope-labeled IS were analyzed by hydrophilic interaction chromatography (HILIC) and detected by MS/MS using a multiple reaction monitoring (MRM) scheme, as outlined in **Table 1**. The HPLC system was composed of a Waters (Milford, MA) X-Bridge HILIC column, Schimadzu (Columbia, MD) SCL-10 binary pumps, Shimadzu model SCL-10A vp system controller, CTC Analytics (Zwigen, Switerland) CTC PAL autosampler, and an Applied Biosystems (Foster City, CA) API 5000 triple quadrupole mass spectrometer.

Negative-Ion Electrospray ESI−MS/MS Data Acquisition. The product scans for bromate and the ^{18}O-labeled bromate IS were obtained as shown in **Figure 1**. The analyte and IS precursor ions chosen for fragmentation in the collision cell were m/z 126.8 (**Figure 1a**; $^{79}BrO_3^-$) and 132.9 (**Figure 1b**; $^{79}Br^{18}O_3^-$), respectively. The bromate species can lose one, two, or three oxygen atoms during fragmentation.

Method Validation Experiments. The specificity of the HPLC−MS/MS methodology was examined by extracting replicate (n = 3) samples from several lots of different types of potato snacks, including unseasoned (unflavored, n = 2 lots), barbecue (n = 3 lots), sour cream and onion (n = 3 lots), and cheese (n = 3 lots) snacks using the procedure described above. Blank samples were prepared by extracting the samples without adding IS. Standard zero samples (STD 0) were prepared by spiking the snacks with IS before extraction. LQC samples were prepared by spiking the snacks at the 20 ppb level. The blank, STD 0, and LQC samples were prepared in triplicate for each lot and prepared for HPLC−MS/MS analysis, as described above.

Table 1. HPLC−MS/MS Analysis Conditions[a]

column	X-Bridge HILIC (2.1 × 50 mm, 3.5 μm particles)					
flow rate (μL/min)	350					
needle rinse 1	ACN/IPA/MeOH/TEA (2:1:1:0.001, v/v/v/v); 3 rinses					
needle rinse 2	ACN/IPA/dd water/FA (4:1:1:0.001, v/v/v/v); 4 rinses					
mobile phase A	dd water with 0.1% FA					
mobile phase B	ACN with 0.1% FA					
gradient	time (min)	percentage of mobile phase A		percentage of mobile phase B		divert valve
	0.00	5		95		to waste
	0.10	5		95		to MS
	0.50	50		50		
	1.20	75		25		
	2.20	75		25		
	2.21	5		95		to waste
injection (μL)	2					
detection mode	ESI^-					
MRM conditions	analyte	precursor	product	DP (V)	CE (eV)	CEX (V)
	BrO_3^-	126.9	110.9	−10.0	−32.0	−15.0
	$Br^{18}O_3^-$	132.9	97.0	−10.0	−46.0	−13.0
ISV (V)	−3800					
collision gas (psig)	10					
heater (°C)	550					
EP (V)	−10					

[a] DP, declustering potential; CE, collision energy; CEX, collision cell exit potential; ISV, ion spray voltage; EP, exit potential.

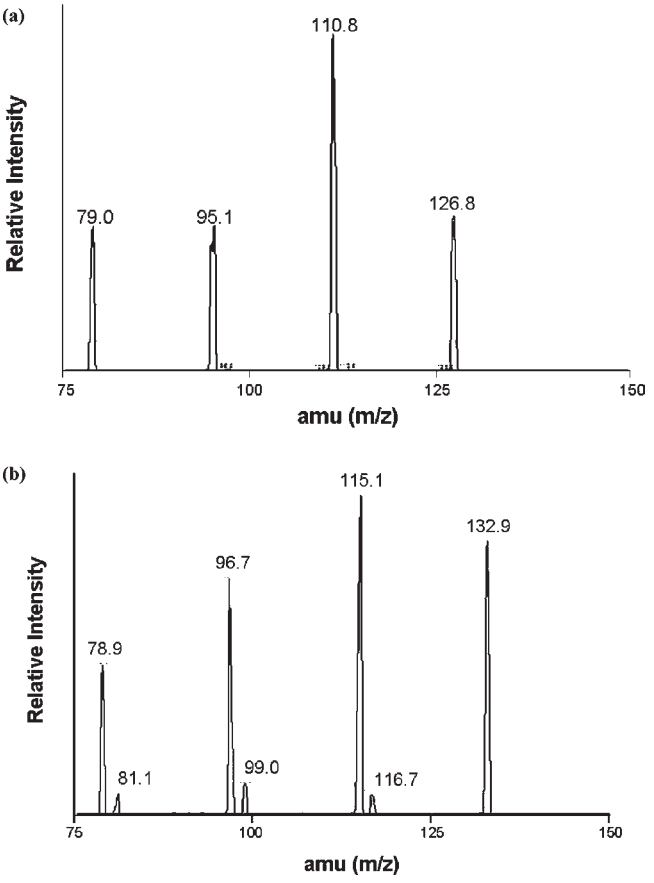

Figure 1. Product scans for bromate and ^{18}O-bromate IS for precursor ions: (a) m/z 126.8 for $^{79}BrO_3^-$ and (b) m/z 132.9 for $^{79}Br^{18}O_3^-$.

The recovery of bromate spiked onto unseasoned potato snacks was examined by extracting the LQC (20 ppb), MQC (200 ppb), and HQC (800 ppb), n = 5 at each level, as described above, except that the IS was not added to the snacks but rather added after the aqueous extract had been isolated. The extracts were then analyzed against the working standards to determine the recovery of the bromate. The accuracy and precision, both intra-assay and interassay, were examined by analyzing replicate (n = 6) QC samples at each level (LQC, MQC, and HQC) on 3

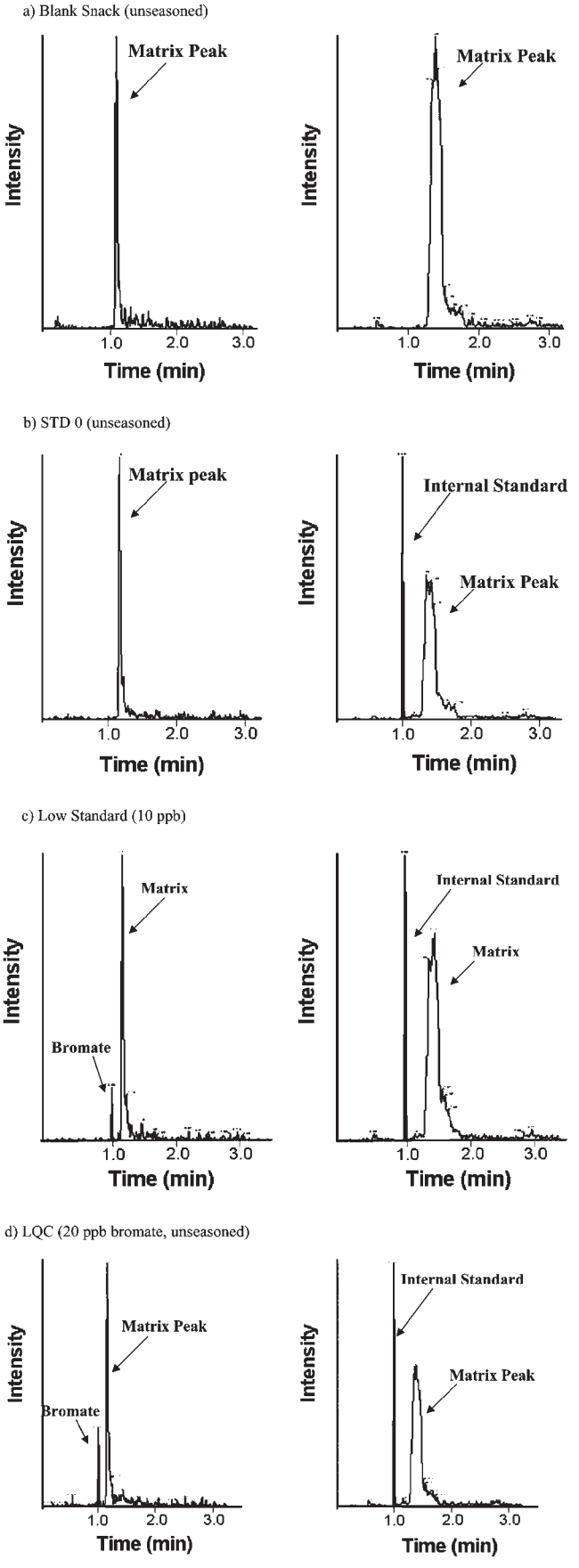

Figure 2. Representative chromatograms for bromate and IS from unseasoned potato snacks: (a) blank snack (unseasoned), (b) STD 0, (c) low standard (10 ppb), and (d) LQC (20 ppb). Identical chromatographic profiles were seen for the other potato snack flavor matrices (data not shown).

Table 2. Performance of LQC Samples in the Bromate Specificity Batch[a]

snack flavors	lot	percent RE for LQC (%)	percent CV for LQC (%)
unseasoned	1	−12.5	10.9
	2	15.0	4.5
barbecue	1	11.0	5.9
	2	−0.5	10.1
	3	11.5	3.8
sour cream and onion	1	10.5	5.9
	2	12.5	8.6
	3	13.5	4.7
cheese	1	−5.0	5.2
	2	16.0	2.8
	3	−5.5	10.7

[a] $n = 3$ per lot.

Table 3. Performance of Quality Controls in Analyte Recovery and Stability Experiments

	percent RE of QC sample sets (%)		
experiment	LQC (20 ppb)	MQC (200 ppb)	HQC (800 ppb)
analyte recovery	−7.0	−4.5	1.6
bench-top stability (24 h, room temperature)	−7.0	−10.5	−6.1
long-term matrix stability (15 days, 4 °C)	−8.5	−7.5	−7.4
autosampler stability (13 days, 11 °C)	3.0	−7.5	−3.9

separate days. A complete plate was run on each day composed of working standards, QC samples, and blank extracts.

The stability of the potato snack extracts was examined at ambient conditions for 24 h and refrigerated for 15 days. For these studies, replicate ($n = 3$) extracts of the LQC, MQC, and HQC were prepared without the IS. The samples were stored under the specified conditions, then prior to analysis, spiked with the IS solution, and analyzed by HPLC−MS/MS. To measure the autosampler stability, a sample batch was prepared fresh and analyzed immediately. The batch was retained in the autosampler at 11 °C for 13 days and then reinjected to determine the storage stability of a prepared batch.

Batch Analysis. For each analysis batch, a complete set of working standards (including a blank and STD 0) was run at the start of the analysis (front curve) and at the end of the analysis (back curve). A complete set of QC samples (LQC, MQC, and HQC) was run immediately after the front standard curve, roughly in the middle of the batch, and just before the back standard curve. Study specimens were distributed evenly between the QC samples. The peak area ratio (bromate peak area/IS peak area) was determined for each working standard, QC sample, and study specimen. The peak area ratio of the working standards was plotted versus the bromate concentration in each working standard to generate a regression curve. The concentration of bromate in the standards, QC samples, and study specimens were then determined by interpolation from the $1/x^2$ quadratic regression curve.

RESULTS AND DISCUSSION

Specificity and Sensitivity. The specificity of the HPLC−MS/MS assay was demonstrated by analyzing triplicate samples from various lots of four potato snacks as blanks (no IS), STD 0 (IS spike), and LQC specimens. The test included four potato snack seasonings: unseasoned (two lots), barbecue (three lots), sour cream and onion (three lots), and cheese (three lots). Although there were extraneous matrix peaks seen in each extract that eluted after the analytes retention time, there were no detectable signals at the retention time of the analyte or the IS in any the blank samples, indicating a lack of significant interferences (**Figure 2**). The STD 0 samples for all lots were free of any detectable bromate, and the LQC samples for each lot were all within ±16% relative error (percent RE), with a percent coefficient of variation (percent CV) of less than 11%,

Table 4. Performance of Working Standards in Validation Runs

	standard 1 (10.0 ppb)	standard 2 (15.0 ppb)	standard 3 (50.0 ng/mL)	standard 4 (100 ng/mL)	standard 5 (850 ng/mL)	standard 6 (1000 ng/mL)
run number 1	10.4	16.3	50.5	106	794	978
	13.7[a]	12.8	48.5	98.0	910	1010
intrarun mean	10.4	14.6	49.5	102	852	994
intrarun percent RE	4.0	−2.7	−1.0	2.0	0.2	−0.6
n	1	2	2	2	2	2
run number 2	9.2	14.0	50.1	99.1	826	1020
	11.2	14.8	52.4	989[a]	0.0243[a]	1010
intrarun mean	10.2	14.4	51.3	99.1	826	1020
intrarun percent RE	2.0	−4.0	2.6	−0.9	−2.8	2
n	2	2	2	1	1	2
run number 3	10.1	21.4[a]	43.0	102	911	979
	9.10	17.3	48.8	106	817	991
intrarun mean	9.60	1.73	4.59	10.4	86.4	98.5
intrarun percent RE	−4.0	15.3	−8.2	4.0	1.6	−1.5
n	2	1	2	2	2	2
mean concentration found (ng/mL)	10.0	15.0	48.9	102	852	998
inter-run SD	0.875	1.79	3.20	3.75	55.0	17.8
inter-run percent CV	8.8	11.9	6.5	3.7	6.5	1.8
inter-run percent RE	0.0	0.0	−2.2	2.0	0.2	−0.2
n	5	5	6	5	5	6

[a] Deactivated because it did not meet acceptance criteria.

Table 5. Performance Summary of Precision and Accuracy Batches

samples	batches	percent RE (%)	percent CV (%)
working standards	intrarun (batch 1)	from −2.7 to 4.0%	NA[a]
	inter-run (batches 1, 2 and 3)	from −2.2 to 2.0%	from 1.8 to 11.9%
QC samples	intrarun (batch 1)	from 0.5 to 7.0%	from 3.0 to 12.6%
	inter-run (batches 1, 2 and 3)	from 1.5 to 7.5%	from 5.2 to 13.4%

[a] NA = not applicable.

demonstrating that the various seasonings did not impact quantitation, accuracy, or precision at the LQC level (**Table 2**). Representative chromatograms for a low working standard (10 ppb) and a LQC (20 ppb) are shown in **Figure 2**. The low working standard and LQC each had a signal/noise ratio of greater than 10:1 in all matrices. The limit of detection (LOD; S/N > 3) was consistently found to be between 1 and 2 ppb under the current conditions. The LOD achieved is superior to previously reported methods for the analysis of bread-based materials, which ranged from 5 to 30 ppb. The specificity of the LC−MS/MS approach was excellent for examining bromate levels in snacks because no interfering peaks were noted in any samples examined.

Bromate Recovery in Snack Extracts. The recovery of bromate from unseasoned snacks was examined by preparing replicate ($n = 5$) samples for each QC level (LQC, MQC, and HQC) using the sample preparation procedure described above, except that the IS solution was not added until the aqueous extract was isolated from the snacks. Therefore, the IS cannot correct for any sample loss during extraction. The QC samples were then analyzed versus the working standards (**Table 3**). The intrabatch percent RE results indicate that the bromate extraction efficiency across all concentration levels was ≥93%. The recovery data demonstrates the effectiveness of the simple sample preparation approach for extracting bromate from the snack matrix.

Accuracy and Precision. Three complete 96-well batches (working standards, QC samples, and study specimens) were analyzed to assess the single day (intra-assay) and multiple day (interassay) performance of the method. The results for the working standard for each batch as well as the overall summary statistics (intra-assay and interassay) for the working standards

Table 6. Performance of QC Samples in Validation Runs

	LQC (20.0 ng/mL)	MQC (200 ng/mL)	HQC (800 ng/mL)
run number 1	20.8	211	883
	23.1	203	894
	17.0	216	825
	18.1	206	783
	21.7	201	895
intrarun mean	20.1	207	856
intrarun SD	2.53	6.11	50.0
intrarun percent CV	12.6	3.0	5.8
intrarun percent RE	0.5	3.5	7
n	5	5	5
run number 2	20.6	197	797
	23.6	185	848
	23.5	190	850
	21.2	187	790
	19.7	209	861
intrarun mean	21.7	194	829
intrarun SD	1.75	9.74	33.1
intrarun percent CV	8.1	5.0	4.0
intrarun percent RE	8.5	−3.0	3.6
n	5	5	5
run number 3	28.9[a]	215	762
	19.5	193	736
	19.2	203	795
	23.6	219	799
	22.7	203	859
intrarun mean	22.8	207	790
intrarun SD	3.93	10.4	46.3
intrarun percent CV	17.2	5.0	5.9
intrarun percent RE	14.0	3.5	−1.3
n	5	5	5
mean concentration found (ng/mL)	21.5	203	825
inter-run SD	2.89	10.6	49.2
inter-run percent CV	13.4	5.2	6.0
inter-run percent RE	7.5	1.5	3.1
n	15	15	15

[a] Theoretical value > 20%.

are shown in **Tables 4** and **5**, respectively. The intra-assay RE values for all working standards were within ±4.0% for the three batches (**Table 4**). The percent RE and percent CV values for the interassay working standards ranged from −2.2 to 2.0% and from 1.8 to 11.9%, respectively. The percent RE and percent CV measurements for the QC samples from each batch and the overall summary statistics (intra-assay and interassay) are shown in **Tables 5** and **6**, respectively. The intra-assay percent RE measurements for all QC levels were within ±14% for the three batches, and the interassay percent RE and percent CV values spanned from 1.5 to 7.5% and from 5.2 to 13.4%, respectively. The results for the QC samples indicate that the method is both accurate and precise for the analysis of bromate from potato snacks.

Extract Stability and Autosampler. The stability of bromate in the prepared extract was examined under both ambient (24 h) and refrigerated (15 days) storage by extracting the LQC, MQC, and HQC samples without the addition of the IS and then adding the IS immediately prior to analysis. The percent RE measured for the three QC levels in the ambient temperature and long-term stability tests ranged from −10.5 to −6.1% and from −8.5 to −7.4%, respectively (**Table 3**). These percent RE values are comparable to the QC performances in the three accuracy and precision batches. The data attest to the robustness of the assay and, particularly, the high stability of bromate in potato snack extracts at ambient and refrigerated storage conditions. The stability of a prepared batch was also examined to ensure a wide window opportunity for sample analysis by analyzing one of the batches immediately and then after 13 days of storage in an autosampler set at 11 °C. All QC samples had percent RE and percent CV within ±15% and ≤15%, respectively, demonstrating at least 13 days of stability in the autosampler.

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