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Note d'application

Determination of Chlorate, Perchlorate, and Bromate in Food Commodities using LC-MS/MS with Atlantis™ Premier BEH™ C_{18} AX Column

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Abstract

This application note describes an efficient method for analysis of chlorate, perchlorate, and bromate in food of plant and animal origin using the Atlantis Premier BEH C_{18} AX Column and ACQUITY™ Premier UPLC™ System coupled to Xevo™ TQ Absolute MS System.

Benefits

- Excellent method performance which meets the European regulatory requirements was achieved for key oxyanions in representative commodities all within a 5-minute run cycle
- Excellent sensitivity at low levels (≤0.1 µg/kg) which allows method flexibility to be realized when sample dilution or reduced sample injection volume is required

Introduction

Chlorate and perchlorate are ubiquitous contaminants that have been detected in food commodities.[1,2] Chlorate is formed as a disinfection byproduct of water used in food production or for cleaning surfaces in contact with food while perchlorate can be found via the use of fertilizers.[3,4] Chlorate and perchlorate pose a high risk to human health especially amongst infants and children by inhibiting iodine uptake while bromate has been classed as a possible human carcinogen.[1,4,5] Dietary intake is reportedly the predominant pathway for human exposure to perchlorate and chlorate.[6] Thus, it is important to routinely monitor these compounds at trace levels in food commodities for compliance with European regulatory requirements.

In Europe, the maximum residue limit (MRL) for chlorate is 0.01 mg/kg in food (Regulation (EC) No. 396/2005).[1] MRLs are set for chlorate in regular food at levels based on occurrence data (Commission Regulation (EU) 2020/749).[1] MRL values for perchlorate are 0.05 mg/kg in fruits and vegetables (except for cucurbitaceae and kale, leafy vegetables and herbs), 0.01 mg/kg for infant formula and 0.02 mg/kg for baby food (Commission Regulation (EU) 2020/685).[1] Chlorate and perchlorate have been previously determined in infant milk using the Anionic Polar Pesticide (APP) Column.[7] However, the established method involved the use of a high ammonium formate buffer concentration which can result in signal suppression.

The combination of the hydrophobic and anion-exchange properties of the Atlantis Premier BEH C_{18} AX Columns provide chromatographic characteristics that facilitate separation and retention of these highly polar and ionic compounds without the need for a high ammonium formate buffer concentration.

In this application note, the performance data from an assessment of the method established for determination of chlorate, perchlorate, and bromate in food matrices (cucumber and infant formula representative of food of plant and animal origin, respectively) using the Atlantis Premier BEH C_{18} AX Column and ACQUITY Premier UPLC System coupled to Xevo TQ Absolute MS System is presented.

Experimental

Samples of cucumber and infant formula were purchased from a local supermarket. Test portions were extracted following QuPPe PO and modified QuPPe AO methods, respectively.[8] Method details are summarized in Figures 1 and 2. MRM parameters are listed in Table 1.

Method performance was assessed using the SANTE guidelines.[9] Matrix-matched standards were prepared over the range of 5–200µg/kg for chlorate, perchlorate, and bromate. Isotopically labelled standards (chlorate-$^{18}O_3$, perchlorate-$^{18}O_4$, and bromate-$^{18}O_3$) were used as internal standards. Internal standards were added just before analysis.

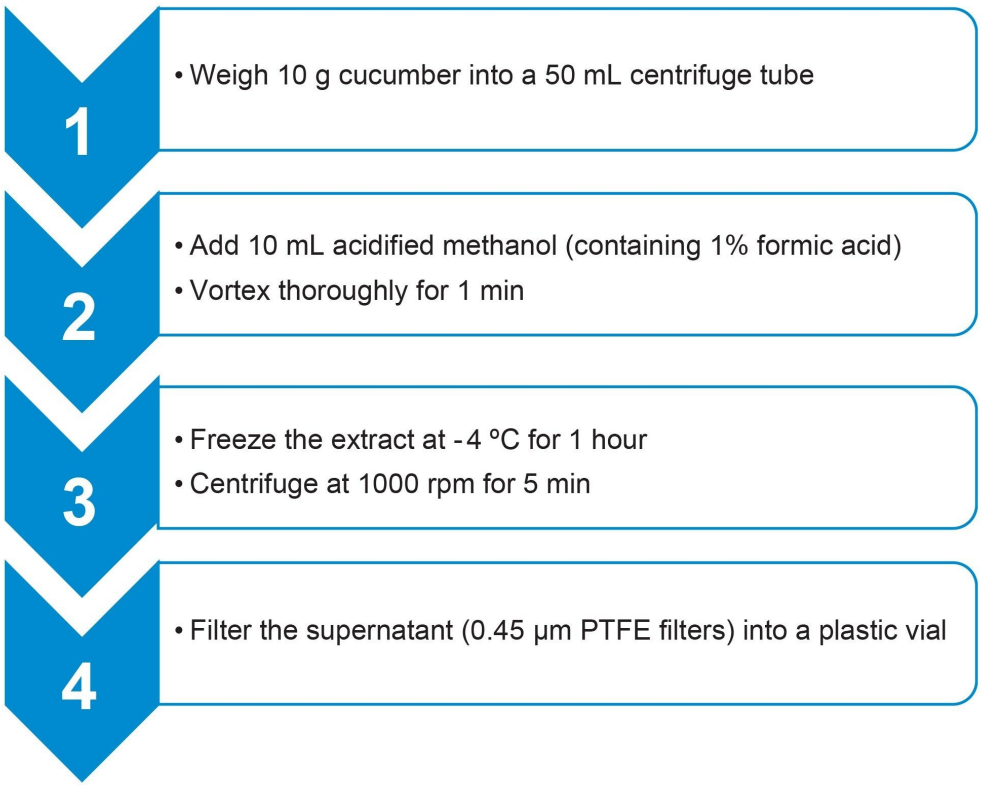

Figure 1. Sample extraction protocol for food of plant origin.

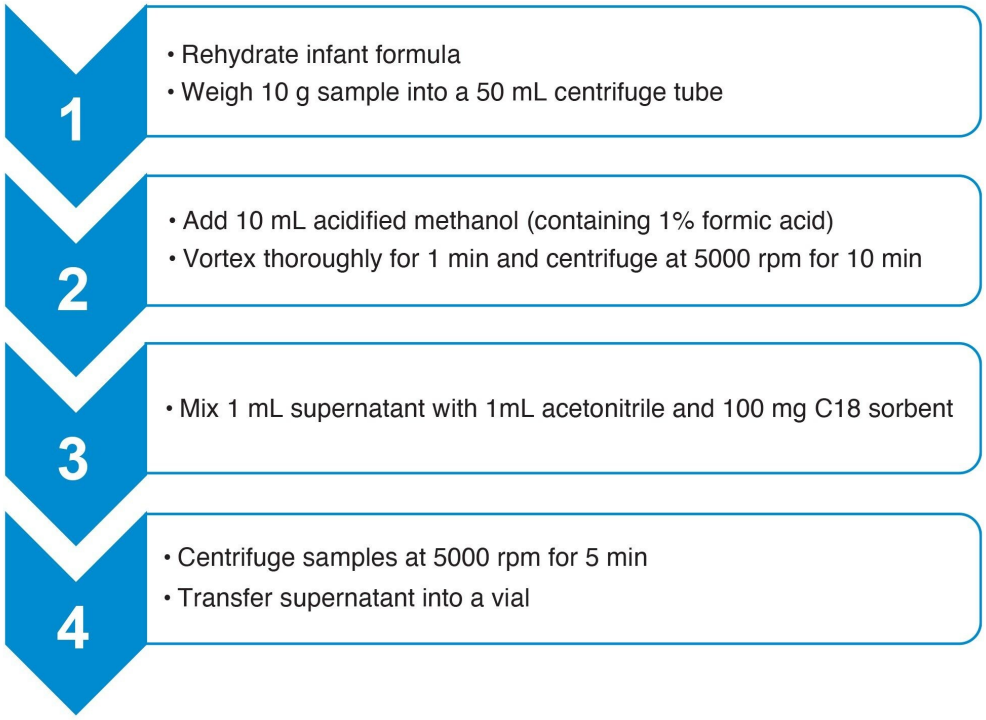

Figure 2. Sample extraction protocol for food of animal origin.

LC Conditions

LC system:	ACQUITY Premier UPLC System with FTN Sample Manager
Column:	Atlantis Premier BEH C_{18} AX Column, 2.1 x 100 mm, 5 µm (p/n: 186009408)
Vials:	Waters™ TruView™ LCMS Certified Clear

	Glass, 12×32 mm (p/n: 186005669CV)
Mobile phase A:	10 mM ammonium formate + 0.1% formic acid in water
Mobile phase B:	0.1% formic acid in acetonitrile
Flow rate:	0.5 mL/min
Column temperature:	50 °C
Sample temperature:	10 °C
Injection volume:	2.5 µL

Gradient Table

Time (min)	%A	%B	Curve
0	20	80	Initial
1	60	40	6
4	10	90	2
5	20	80	6

MS Conditions

MS system:	Xevo TQ Absolute MS

Ionization mode:	ESI -
Acquisition mode:	MRM
Capillary voltage:	0.5 kV
Desolvation temperature:	600 °C
Desolvation gas flow:	1000 L/hr
Cone gas flow:	150 L/hr
Source temperature:	150 °C
Software:	waters_connect™ for quantitation

Compound	Retention time (min)	MRM transition	Cone voltage (V)	Collision energy (eV)
Chlorate	1.45	**83>67**	55	15
Chlorate		83>51	55	17
Chlorate-$^{18}O_3$		89>71	33	15
Perchlorate	1.97	**99>83**	65	18
Perchlorate		99>67	65	42
Perchlorate-$^{18}O_4$		107>89	55	20
Bromate	1.25	**127>111**	50	18
Bromate		127>95	50	22
Bromate-$^{18}O_3$		133>97	55	24

Table 1. MRM parameters for chlorate, perchlorate, and bromate, optimum dwell time was set automatically using the auto-dwell function (quantitative transitions in bold).

Results and Discussion

The sensitivity of the method was evaluated by assessment of the response of matrix-matched standards prepared in infant formula at 0.1–20 µg/kg. Excellent sensitivity (≤0.1 µg/kg) and selectivity were demonstrated from analysis of matrix-matched standards. The MRL for chlorate and perchlorate is set at 0.01 mg/kg in infant formula. This method allows the quantification of the 3 analytes at ≤0.0001 mg/kg (1/100 MRL). This allows method flexibility to be realized when sample dilution or reduced sample injection volume is required. Representative chromatograms at the lowest level reported, are shown for the 3 analytes in Figure 3.

Linearity was assessed by use of matrix-matched bracketed calibration curves over a range of 5–200 µg/kg for cucumber and infant formula as shown in Figures 4 and 5. The coefficients of determination and residuals were within the SANTE acceptance criteria demonstrating good repeatability. Peak shape and retention times were stable throughout the run in both matrices. This was facilitated by the presence of anion-exchange sites on the column which allows the use of a reduced buffer concentration. Ion ratios and retention times were within the required tolerances as summarized in Table 2.

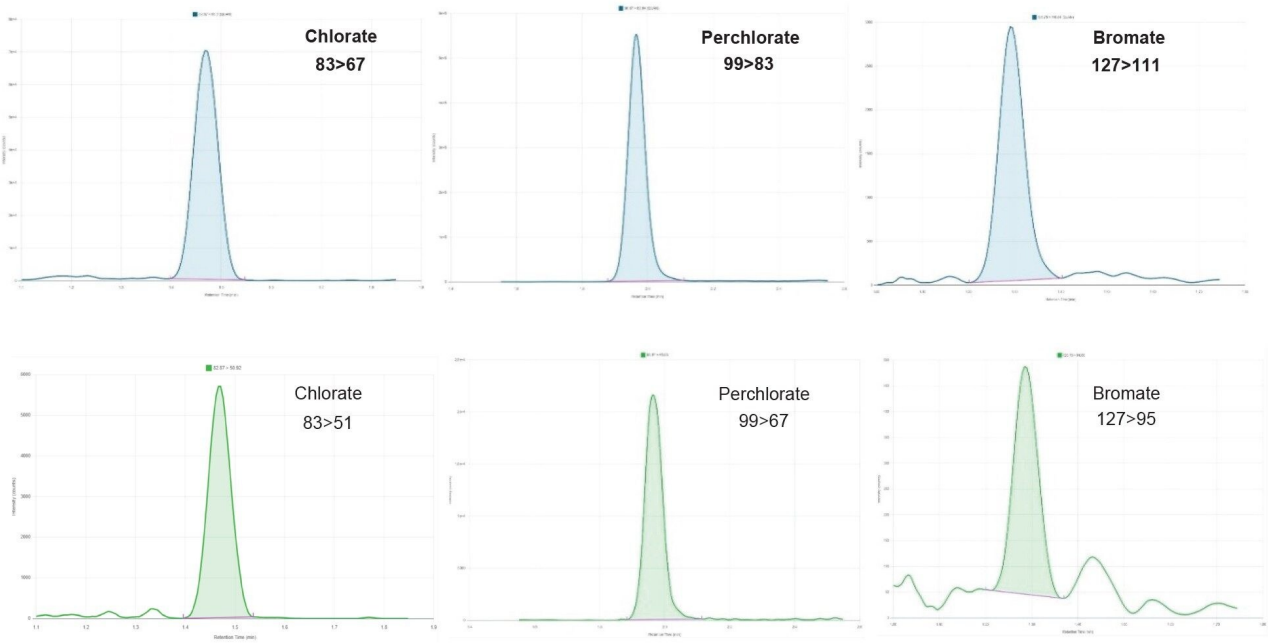

Figure 3. Chromatograms of chlorate, perchlorate, and bromate from the analysis of matrix-matched standards (infant formula) at 0.1µg/kg.

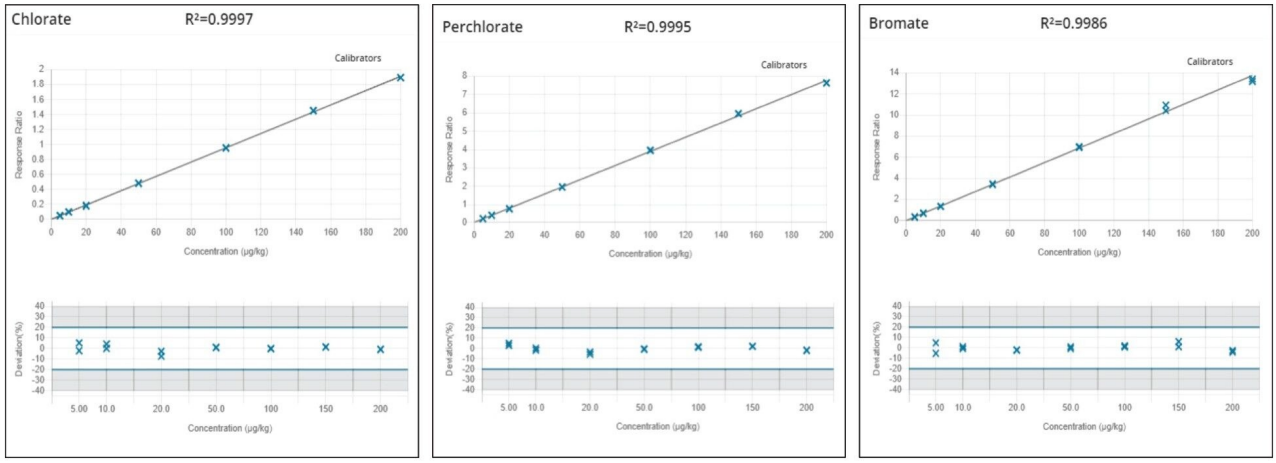

Figure 4. Calibration and residual plots for chlorate, perchlorate, and bromate in cucumber (5–200 µg/kg).

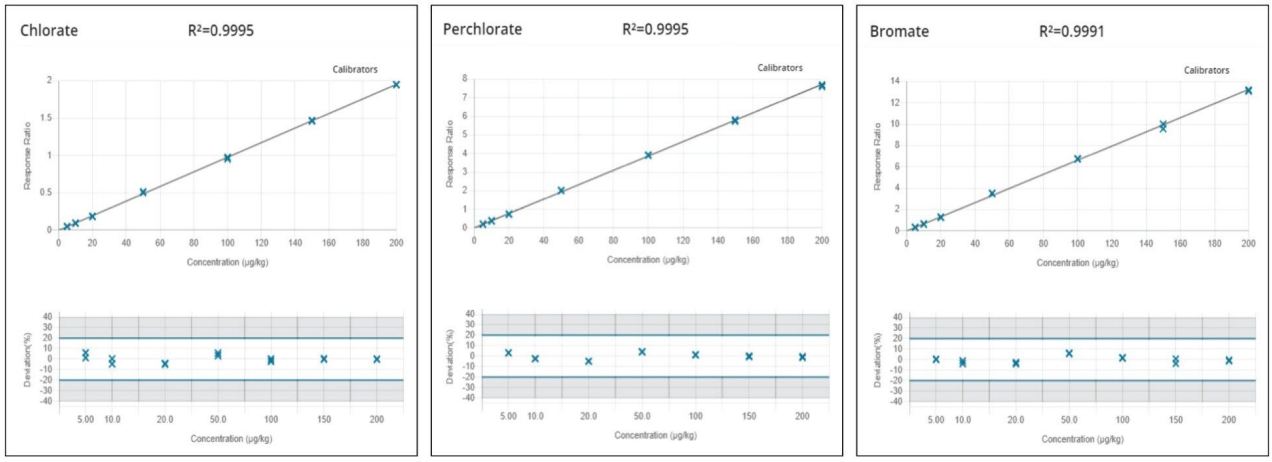

Figure 5. Calibration and residual plots for chlorate, perchlorate, and bromate in infant formula (5–200 µg/kg).

Parameter	SANTE criteria	Chlorate	Perchlorate	Bromate
	Cucumber			
Retention time (min)	±0.1 min	<0.1	<0.1	<0.1
Ion ratio (%)	±30 %	<14	<25	<6
Residuals (%)	±20 %	<7	<6	<6
	Infant formula			
Retention time (min)	±0.1 min	<0.1	<0.1	<0.1
Ion ratio (%)	±30 %	<13	<25	<5
Residuals (%)	±20 %	<6	<5	<6

Table 2. Summary of method parameters for chlorate, perchlorate, and bromate in cucumber and infant formula.

Conclusion

Excellent sensitivity (≤0.1 µg/kg) has been achieved with the Xevo TQ Absolute MS System. This allows method flexibility to be realized when sample dilution or reduced sample injection volume is required. Excellent selectivity and retention have been achieved for the 3 analytes with the Atlantis Premier BEH C_{18} AX Column facilitated by the presence of anion exchange sites on the column. This column allows the use of a reduced buffer concentration making the method more reliable.

Method performance was successfully evaluated using matrix-matched standards prepared in cucumber and infant formula using the EURL Quick Polar Pesticides (QuPPe) PO and modified QuPPe AO methods, respectively. Response for the 3 compounds was linear over a range of 5–200 µg/kg with coefficients of determination, retention times, ion ratios and residuals all within the SANTE acceptance criteria.

References

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