Waters™

アプリケーションノート

Determination of Chlormequat Chloride Residues in Cereals by LC-MS/MS

Simon Hird, Henry Foddy, Stuart Adams

Waters Corporation

Abstract

Recent reports from the US show residues of the growth regulator, chlormequat chloride, in a range of cereal products, despite the lack approval for its use on cereals in the US other than for some imports. Waters previously developed a LC-MS/MS method for the determination of highly polar and cationic pesticides and growth regulators in various food commodities. The method is based upon extraction with the established Quick Polar Pesticides method (QuPPe) and separation using the BEH™ Amide column. This application note shows the evaluation of the performance of this method for chlormequat chloride, in a representative cereal sample, using an ACQUITY™ Arc System and Xevo™ TQ-S cronos Tandem Quadrupole Mass Spectrometer. Samples of different cereal flours were spiked at 0.05 mg/kg. The accuracy of the method, using an internal standard, was evaluated and apparent recovery found to be within the range 94 to 102% and repeatability (RSD $_r$) < 4% RSD. Additional verification of performance was obtained from the analysis of QC reference materials, the results from which compared well with the assigned values. This demonstrated that the method could be suitable for checking compliance with any MRLs/tolerances and has the potential for determination at much lower concentrations, for example in the absence of any MRLs/tolerances and for food business operators due diligence testing and brand protection.

Benefits

- Provides a single extraction (QuPPe) and LC-MS/MS method suitable for the determination of residues of the plant growth regulator chlormequat chloride in cereals

- The chromatographic method, using a MS-friendly mobile phase, provides excellent peak shape integrity, maintains selectivity, and offers retention of a highly polar cationic analyte
- The performance of the method, as demonstrated by this evaluation, provides users with evidence of its suitability for both official control and due diligence testing

Introduction

Chlormequat chloride is a quaternary ammonium compound developed as a plant growth regulator.[1] It works by inhibiting the hormone gibberellins, resulting in the development of a more compact plant, producing thicker stalks, which aids in cereal crop harvesting and increases productivity. Chlormequat chloride is not currently approved for use on cereals in the US, but in 2023, the EPA proposed its approval for use on several domestically produced grain crops (wheat, barley, oats, triticale).[2] However, prior to finalizing the proposal, the EPA must establish tolerances for chlormequat in these crops and take measures to address potential health hazards to field workers and wildlife. Chlormequat chloride is, however, approved for such use in Europe and Canada, so maximum residue levels/limits (MRLs) have been set for various cereals. Canada has a variety of MRLs for chlormequat chloride depending on the sample type; 5 mg/kg for wheat, 15 mg/kg for wheat bran and 20 mg/kg for wheat germ, whereas the EU has a single MRL for wheat at 7 mg/kg.[3,4] To facilitate import of chlormequat-treated grains from these countries to the US, the EPA established import tolerances that do not apply to domestically grown grains (*e.g.* 40 mg/kg in oats).[5] In the absence of an established tolerance, action level or approved exemption, the pesticide allowance on, or in, food is defined as "zero tolerance," which is recognized to be any level below the limit of detection using an applicable analytical method, typically 0.01 mg/kg.[6]

A recent study showed that the analysis of samples of cereal-based foods purchased in the US from 2022 and 2023 showed detectable levels of chlormequat chloride in all but two of 25 conventional oat-based products.[7] Concentrations ranged from non-detectable to 0.29 mg/kg, indicating a high prevalence of chlormequat chloride in oats, albeit it at much lower concentrations than the foreign MRLs or import tolerances. Although the amount of chlormequat found in the oat foods was very low, public perception is a serious reality when it comes to pesticides in food and can have a huge impact on damage to brand.

Analytical methods are required to check regulatory compliance for chlormequat chloride residues in cereals and by the food industry for their own brand protection and due diligence activities. This may mean confirming whether a residue exceeds the MRL/tolerance where approved, or an import tolerance or enforcement of some form of zero tolerance policy in cases where no approved use exists. Historically, monitoring of chlormequat

chloride residues in cereals and their related processed products was restricted mainly by the lack of reliable and/or satisfactory analytical methods. Some highly polar, ionic pesticides are not "amenable" to common multi-residue methods and often need alternative conditions for extraction and LC retention/separation. The QuPPe (Quick Polar Pesticides) method allows for the simultaneous extraction of many highly polar, ionic pesticides, including chlormequat chloride, followed by various chromatographic options.[8] Monitoring for many of these pesticides and growth regulators is now mandatory in some national control programs and is of significant interest to the cereal and food industries.[9]

This application note describes the evaluation of method performance for the determination of residues of chlormequat chloride in representative cereal samples. The method uses a "dilute and shoot" approach: generic extraction with no clean-up, LC-MS/MS with the BEH Amide column on an ACQUITY Arc System coupled to the Xevo TQ-S cronos Tandem Quadrupole Mass Spectrometer and waters_connect™ for quantitation software.

Experimental

The experimental details used here were the same as those published in an earlier application note, with some minor modifications.[10]

LC Conditions

LC system:	ACQUITY Arc System with FTN-R Sample Manager
Vials:	Polypropylene 12 x 32 mm Snap Neck Vials, with Cap and Preslit PTFE/Silicone Septum, 700 µL (p/n: 186005222)
Column:	ACQUITY UPLC BEH Amide Column (1.7 µm, 2.1 x 50 mm) (p/n: 186004800)
Column temp.:	50 °C
Sample temp.:	10 °C

Injection volume:	1 µL
Mobile phase A:	20 mM ammonium formate (pH 2.95) in water
Mobile phase B:	Acetonitrile

Gradient Table

Time (min)	Flow rate (mL/min)	%A	%B	Curve
0.00	0.5	3	97	Initial
0.25	0.5	3	97	6
2.00	0.5	30	70	6
2.50	0.5	60	40	3
3.00	0.5	60	40	6
3.05	0.5	3	97	6
5.00	0.5	3	97	6

MS Conditions

MS system:	Xevo TQ-S cronos
Ionization mode:	Electrospray (positive ion mode)
Capillary voltage:	0.2 kV
Source temperature:	150 °C
Desolvation temperature:	600 °C
Desolvation gas flow:	1000 L/hr
Cone gas flow:	150 L/hr
Cone voltage:	30 V

MRM Table

Compound	MRM	CE (eV)
Chlormequat Cl	**122>58**	20
	122>63	15
Chlormequat Cl-d4	126>58	20

Data Management

MS acquisition software: waters_connect for quantitation

Quantitation software: waters_connect for quantitation

The method performance was assessed in wheat, buckwheat, and rye flour, spiked at 0.005 and 0.05 mg/kg, in accordance with the SANTE guidelines.[11] Additional evaluation was made by analysis of FAPAS QC materials (T09127QC and T09146QC).

Results and Discussion

Retaining highly polar analytes so that the minimum acceptable retention time for the analyte(s) under examination is at least twice the retention time corresponding to the void volume of the column, can be challenging.[11] The method provides excellent chromatographic retention of this highly polar cationic compound, at 1.35 minutes, with a short 5-minute run time. The void volume time (T0) was 0.34 minutes. Peak shape and retention time were shown to be stable.

Figure 1 shows a typical chromatogram for chlormequat chloride from the analysis of a matrix-matched standard at 0.005 mg/kg, in buckwheat flour, which shows that the method is capable of detection of this analyte in cereal extracts at low concentrations and that final extracts could be diluted further prior to LC-MS/MS.

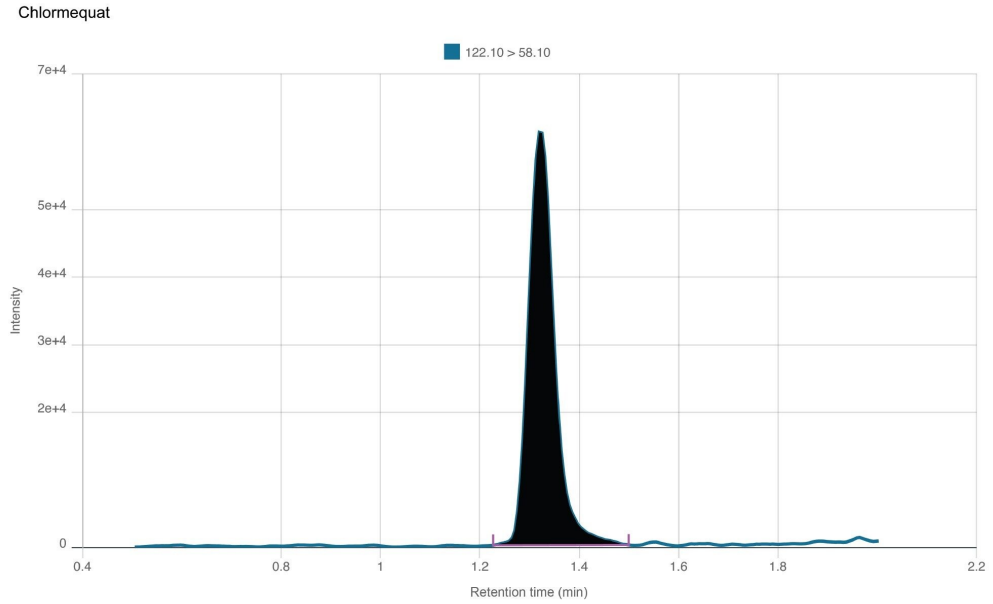

Figure 1. Chromatogram showing chlormequat chloride from analysis of buckwheat flour extract spiked at 0.005 mg/kg.

Blank samples were prepared and analyzed. The samples of wheat and rye flour selected both contained incurred residues which made assessment of recovery at 0.005 mg/kg impractical. No signal was detected in the blank extracts from buckwheat flour that could lead to compromised quantification. The two transitions gave peaks with ion ratios and retention times within the recommended SANTE tolerances, when compared with the standards.[11] Calibration graphs, using a stable isotope analogue, chlormequat-d4 chloride, as an internal standard, were prepared in solvent and buckwheat flour extracts, over the range 0.0025 to 0.50 mg/kg. No significant matrix effects were observed. Linear fit with 1/x weighing was applied and the correlation of determination (R_2) value from the calibration graphs were $\geqslant$0.999, with individual residuals all <10%, demonstrating reliable quantification. The calibration graph in buckwheat flour extract is given in Figure 2.

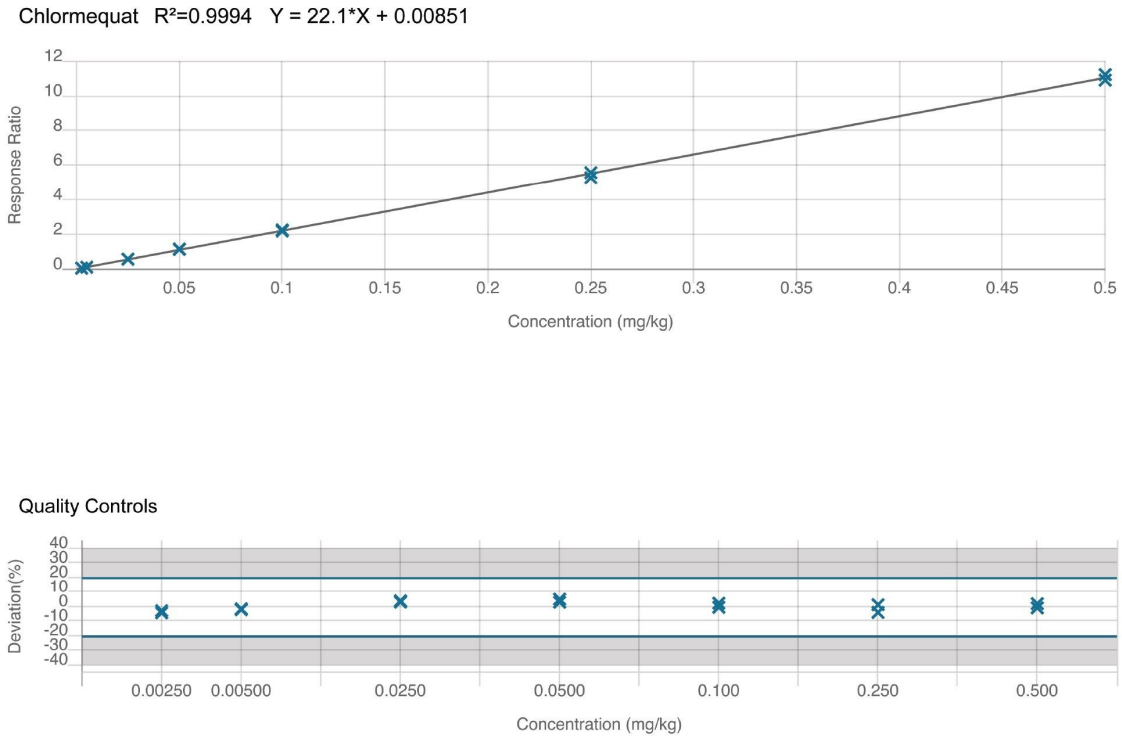

Figure 2. Calibration graph for chlormequat chloride over the range 0.0025–0.50 mg/kg in buckwheat flour extract.

The apparent recovery, after adjustment using the internal standard, was evaluated using the data from the analysis of the samples spiked at 0.05 mg/kg (*n*=5). The mean measured recoveries were within the range 94 to 102%, with repeatability (RSD_r) < 4% RSD, well within the criteria set out in the SANTE guidelines.[11] See Table 1 for more details. The accuracy of the method was further verified at higher concentrations by the analysis of two cereal QC reference materials (T09127QC and T09146QC). The mean values obtained from the analysis of the material (*n*=5) compared very well to the assigned values. See Table 2 for more details.

	Mean (mg/kg)	Apparent recovery (%)	RSD (%)
Wheat	0.051	102	4.5
Rye	0.041	94	3.4
Buckwheat	0.049	99	4.7

Table 1. A summary of the apparent recovery and repeatability from analysis of the spiked samples.

		Assigned value (mg/kg)	Mean (mg/kg)	Bias (%)	RSD (%)
T09127QC	Wheat flour	0.21	0.22	3.8	5.6
T09146QC	Oat flour	0.47	0.49	3.8	0.6

Table 2. A summary of the results from the analysis of the QC reference materials.

Conclusion

The results of the evaluation exercise have shown the method to be a sensitive and reliable means for the determination of residues of chlormequat chloride in cereals, down to concentrations well below typical MRLs/tolerances and could be used for zero tolerance scenarios. It was successfully assessed according to the SANTE guidelines and by analysis of independent reference materials. The procedure can also be applied to other commodities after suitable validation. This cost-effective method can be easily implemented in routine testing laboratories, and this evaluation shows the method may be suitable for checking compliance with MRLs/tolerances and has the potential for determination at much lower concentrations, for example in cases where no MRLs exist for food business operators due diligence testing and brand protection.

References

1. Rademacher, W. Chemical Regulators of Gibberellin Status and their Application in Plant Production. *Ann. Plant Rev*. (2016) 49:359–404.

2. EPA Proposes to Register New Uses of Pesticide Chlormequat Chloride < https://www.epa.gov/pesticides/epa-proposes-register-new-uses-pesticide-chlormequat-chloride> . 26 April 2023.

3. https://pest-control.canada.ca/pesticide-registry/en/mrl-search.html <https://pest-control.canada.ca/pesticide-registry/en/mrl-search.html> .

4. Commission Regulation (EU) 2019/552 of 4 April 2019 amending Annexes II and III to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for azoxystrobin, bicyclopyrone, chlormequat, cyprodinil, difenoconazole, fenpropimorph, fenpyroximate, fluopyram, fosetyl, isoprothiolane, isopyrazam, oxamyl, prothioconazole, spinetoram, trifloxystrobin, and triflumezopyrim in or on certain products.

5. https://www.govinfo.gov/content/pkg/FR-2020-05-26/pdf/2020-10331.pdf < https://www.govinfo.gov/content/pkg/FR-2020-05-26/pdf/2020-10331.pdf> .

6. https://www.ecfr.gov/current/title-40/chapter-I/subchapter-E/part-180/subpart-A/section-180.5 < https://www.ecfr.gov/current/title-40/chapter-I/subchapter-E/part-180/subpart-A/section-180.5> .

7. Temkin A. *et al.* A pilot Study of Chlormequat in Food and Urine from Adults in the United States from 2017 to 2023. *J Expo. Sci. Environ. Epidemiol*. (2024). https://doi.org/10.1038/s41370-024-00643-4 < https://doi.org/10.1038/s41370-024-00643-4> .

8. Anastassiades, M *et al*. Quick Method for the Analysis of Numerous Highly Polar Pesticides in Food Involving Extraction with Acidified Methanol and LC-MS/MS Measurement. 2021. *Food of Plant Origin* (QuPPe-PO-Method v12)

9. Commission Implementing Regulation (EU) 2023/731 of 3 April 2023 concerning a coordinated multiannual control programme of the Union for 2024, 2025 and 2026 to ensure compliance with maximum residue levels of pesticides and to assess the consumer exposure to pesticide residues in and on food of plant and animal origin and repealing Implementing Regulation (EU) 2022/741.

10. Determination of Cationic Polar Pesticides and Plant Growth Regulators Using UPLC-MS/MS with the ACQUITY UPLC BEH Amide Column. Waters Application Note 720007201. 2021.

11. Document No. SANTE/11312/2021 V2. Guidance Document on Analytical Quality, Control, and Method Validation Procedures for Pesticides Residues Analysis in Food and Feed. 2024.

Featured Products

ACQUITY Arc LC System <https://www.waters.com/nextgen/global/products/chromatography/chromatography-systems/acquity-arc-system.html>

Xevo TQ-S cronos <https://www.waters.com/waters/nav.htm?cid=135027354>

waters_connect Software Solutions <https://www.waters.com/waters/nav.htm?cid=135040165>

720008361, May 2024