Application note | 000772

Food safety

Simultaneous analysis of underivatized anionic and cationic polar pesticides in food

Authors

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Keywords

Acclaim Trinity P1 column, TSQ Altis mass spectrometer, polar pesticides, plant growth regulators, QuPPe, Vanquish Flex UHPLC, mixed mode

Goal

To develop an LC method based on the Thermo Scientific™ Acclaim™ Trinity P1 column in a modified Thermo Scientific™ Vanquish™ UHPLC setup for the simultaneous determination of underivatized anionic and cationic polar pesticides in food

Application benefits

This application can be easily managed in a routine laboratory: single extraction, no derivatization, productivity improvement by a single run for analysis of both anionic and cationic species.

Introduction

One of the primary sources of contamination in vegetables is pesticides. Pesticides can be classified into two groups: those that are extractable from sample matrices using QuEChERS methodology and those that are not. QuEChERS extracts are easily analyzed by LC- or GC-MS/MS workflows (depending on the targeted compounds), while QuPPe[1] extracts require analysis through LC- and IC-MS/MS.

Polar pesticides can be anionic or cationic species. Chlormequat and mepiquat, both plant growth regulators, are cations, while chlorate is a strong oxidant used as a herbicide and biocide. Chlorate has not been used since 2008; however, many plant origin samples still show chlorate residues higher than 0.01 mg/kg.[2] Perchlorate anion also occurs as an environmental contaminant. Industrial processes like rocket fuels and explosives can release perchlorate into the environment; however, water, soil, and fertilizers are potential food contamination sources.

The adverse health effects of these pesticides are well-known. In just under twenty years, the World Health Organization hazard classifications for mepiquat, chlormequat, and chlorate have changed from slightly to moderate hazardous products,[3-4] and analytical analysis demand has increased.

However, simultaneous analysis of both anionic and cationic species remains a challenge. More than one analytical method is often required to perform chlormequat, mepiquat, chlorate, and perchlorate analyses. This could impact productivity in the lab due to column and eluents switching, system stabilization duration, or even a technology change from LC to IC.

To overcome this challenge, a new exclusive HPLC column chemistry has been selected with a new trimodal stationary phase based on Nanopolymer Silica Hybrid (NSH) technology. NSH technology consists of high-purity porous spherical silica particles coated with charged nanopolymer particles. This specific chemistry ensures spatial separation of the anion-exchange and cation-exchange regions and allows both retention mechanisms to function simultaneously and be controlled independently.

This study will demonstrate the benefits of using the Acclaim Trinity P1 column in a complete LC-MS/MS setup in terms of accuracy and reproducibility. The performance of the method will also be assessed using SANTE/12682/2019 guidelines.[5]

Experimental

Reagents and consumables

- Methanol, Optima™ LC/MS Grade, Fisher Chemical™ **(P/N 10031094)**
- Acetonitrile, Optima™ LC/MS Grade, Fisher Chemical™ **(P/N 10489553)**
- Formic acid, Thermo Scientific™ Pierce™ LC-MS grade, 50 mL **(P/N 13454279)**
- Ammonium formate, Optima™ LC/MS grade, Fisher Chemical™ (P/N 11377490)
- Ultra pure water produced by Thermo Scientific™ Barnstead™ Smart2Pure™ Pro water purification **(Model Smart2pure Pro UV/UF 16LPH)**
- Fisherbrand™ 1 mL plastic syringe PP **(P/N 14955-456)**
- Thermo Scientific™ Titan3™ syringe filter, 17 mm PVDF membrane **(P/N 44513-PV)**
- 1.2 mL, 9 mm glass vials **(P/N 1.2-UHRSV)**
- Pre-slit PTFE vial caps (P/N 9-SCK(B)-ST1X)
- Acclaim Trinity P1 column 100 x 2.1 mm, 3 µm **(P/N 071389)**

LC-MS/MS setup

The detailed design used for this study is outlined below and in Figure 1.

- Thermo Scientific™ Vanquish™ Flex UHPLC system, modified and consisting of:
  - Thermo Scientific™ Vanquish™ Dual Pump F **(P/N VF-P32-A)**
  - Thermo Scientific™ Vanquish™ Split Sampler FT **(P/N VF-A10-A)**
  - Thermo Scientific™ Vanquish™ Column Compartment H **(P/N VH-C10-A)**
  - Thermo Scientific™ Dionex™ ICS-6000™ SP Analytical – Gradient with Degas **(P/N 22181-60001)**
  - Thermo Scientific™ Dionex™ GM-4 2 mm gradient mixer (P/N 049136)
- Thermo Scientific™ TSQ Altis™ triple quadrupole mass spectrometer **(TSQ02-10002)** equipped with the Thermo Scientific™ OptaMax™ Duet NG source housing (OPTON-32104)
- Thermo Scientific™ Dionex™ PEEK Viper™ assembly, 0.007 i.d., 9.0 in. (229 mm), CD (P/N 088835) x2
- Thermo Scientific™ Dionex™ PEEK Viper™ assembly, 0.007 i.d., 7.0 in. [178 mm],ED (P/N 088809) x2
- Thermo Scientific™ Dionex™ PEEK Viper™ loop, 25 µL, 0.007 i.d. (1,007 mm) **(P/N 302893)**
- Thermo Scientific™ Dionex™ PEEK Viper™ loop, 2.5 µL, 0.007 i.d. (100 mm) **(P/N 302899)** x2
- Union tee, HPLC, PEEK, 1/16 in. orifice 0.020 in. thru-hole, 10–32 **(P/N P-727)**
- Thermo Scientific™ NanoViper™ 0.075 mm i.d. x 550 mm l, PEEK **(P/N 6041.5760)**
- Viper cap., 0.13 mm i.d. x 150 mm l, PEEK (P/N 6041.5616)

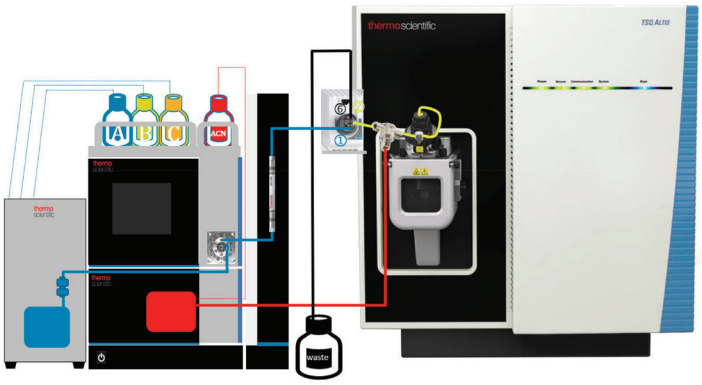


Figure 1. Optimized LC-MS/MS fluidics pathways: elution inert pathway (blue line) and biocompatible makeup pathway (red line)

LC conditions

Table 1. LC conditions

Parameter	Setting
LC column	Acclaim Trinity P1, 100 x 2.1 mm, 3 µm
Mobile phase A	100 mM Ammonium formate, pH = 3
Mobile phase B	Water
Mobile phase C	Acetonitrile
Elution flow rate	0.4 mL/min
Gradient	See Table 2
Column oven	40 ˚C Still air mode
Injection volume	10 µL
Sampler wash solution	Water 90 / methanol 10 (vol / vol)

Table 2. Gradient details

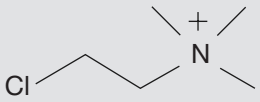
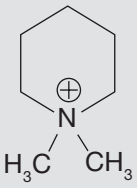
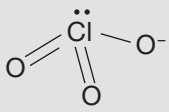
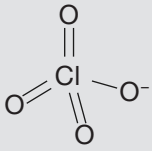
Time (min)	Flow rate (mL/min)	%A	%B	%C	%D
0	0.4	5	95	0	0
2	0.4	5	95	0	0
6.5	0.4	35	65	0	0
7	0.4	60	0	40	0
11	0.4	5	0	95	0
12	0.4	5	0	95	0
12	0.4	5	95	0	0
16	0.4	5	95	0	0

MS conditions

Table 3. MS parameters and settings

Parameter	Setting
Run time	16 min
Ion source	H-ESI
Source positioning	Between M and L
Ionization mode	Positive and negative
Spray voltage	1,000 V in both positive and negative mode
Sheath gas	60
Auxiliary gas	15
Sweep gas	0
Ion transfer tube temperature	325 ˚C
Vaporizer temperature	400 ˚C
Make up	0.4 mL/min acetonitrile
Experiment type	Selected reaction monitoring (SRM)
Dwell time	5 ms
Chromatography peak width	7 s
Collision gas pressure	1.5 mTorr
Q1 resolution	0.7 FHMW
Q3 resolution	1.2 FHMW

Table 4. Chemical details and selected SRM for each pesticide

	Chlormequat	Mepiquat	Chlorate	Perchlorate
Chemical formula				
Retention time (min)	7.28	8.42	10.02	11.45
Ionization mode	Positive	Positive	Negative	Negative
Quan. ion (CE)	122 → 58 (14)	114 → 58 (37)	83 → 67 (20)	99 → 83 (20)
Conf. ion (CE)	124 → 58 (15)	114 → 98 (26)	85 → 69 (20)	101 → 85 (20)
RF (V)	55	55	42	30
Source frag. (V)	0	0	0	0

Software

Thermo Scientific™ Chromeleon™ 7.3 Chromatography Data System was used for data acquisition and analysis.

Sample preparation (modified QuPPE method)

A representative analytical sample of the fresh spinach, 10 g ± 0.1 g, was weighed into a 50 mL centrifuge tube. 10 mL acidified methanol with formic acid (1% vol/vol), internal standards solution were added. The solution was homogenated using a Fisherbrand™ 150 Homogenizer. The final volume was adjusted to 20 mL with acidified methanol. A 50 mL tube was frozen for more than 120 minutes at -20 °C, defrosted, and then centrifuged for 5 minutes at 7,000 rpm. An aliquot (1 mL) of the supernatant was withdrawn using a syringe, filtered through a 0.45 µm syringe filter, and diluted five times with water into the vial.

Results and discussion

Anionic and cationic pesticides separation and detection

Separation of chlormequat, mepiquat, chlorate, and perchlorate was achieved in less than 12 minutes. All retention times were greater than twice the time corresponding to the column void volume, and retention time stability was within the 0.1 min tolerance according to the Document N° SANTE[5] requirements. Each separated compound was detected in Single Reaction Mode (SRM). One quantitation and one confirmation ion were selected for each target compound. Figure 2 shows all selected ions for chlormequat, mepiquat, chlorate, and perchlorate. The peak shape is quite good at 10 ppb and 1 ppb, which are the required MRLs (Figures 3 and 4). After blank subtraction, the signal-to-noise ratio is far above ten at 10 ppb and even at 1 ppb.

Due to the complexity of the separation, three solvents were used to reach the objective: water, ammonium formate buffer, and acetonitrile. Automated blending using the pump proportioning valve coupled with a static mixer facilitated gradient reproducibility and enabled easy system use. Also, a whole inert pathway drastically reduced stationary phase contamination by metal and increased the retention time stability and stabilizing peak shape. Figure 2 represents the simultaneous separation of cationic (chlormequat and mepiquat) and anionic (chlorate and perchlorate) within 16 minutes. The equilibration step expands the run time to 16 minutes, allowing perfect retention time stability. In these conditions, retention times are 7.28 min, 8.42 min, 10.02 min, and 11.45 min, respectively.

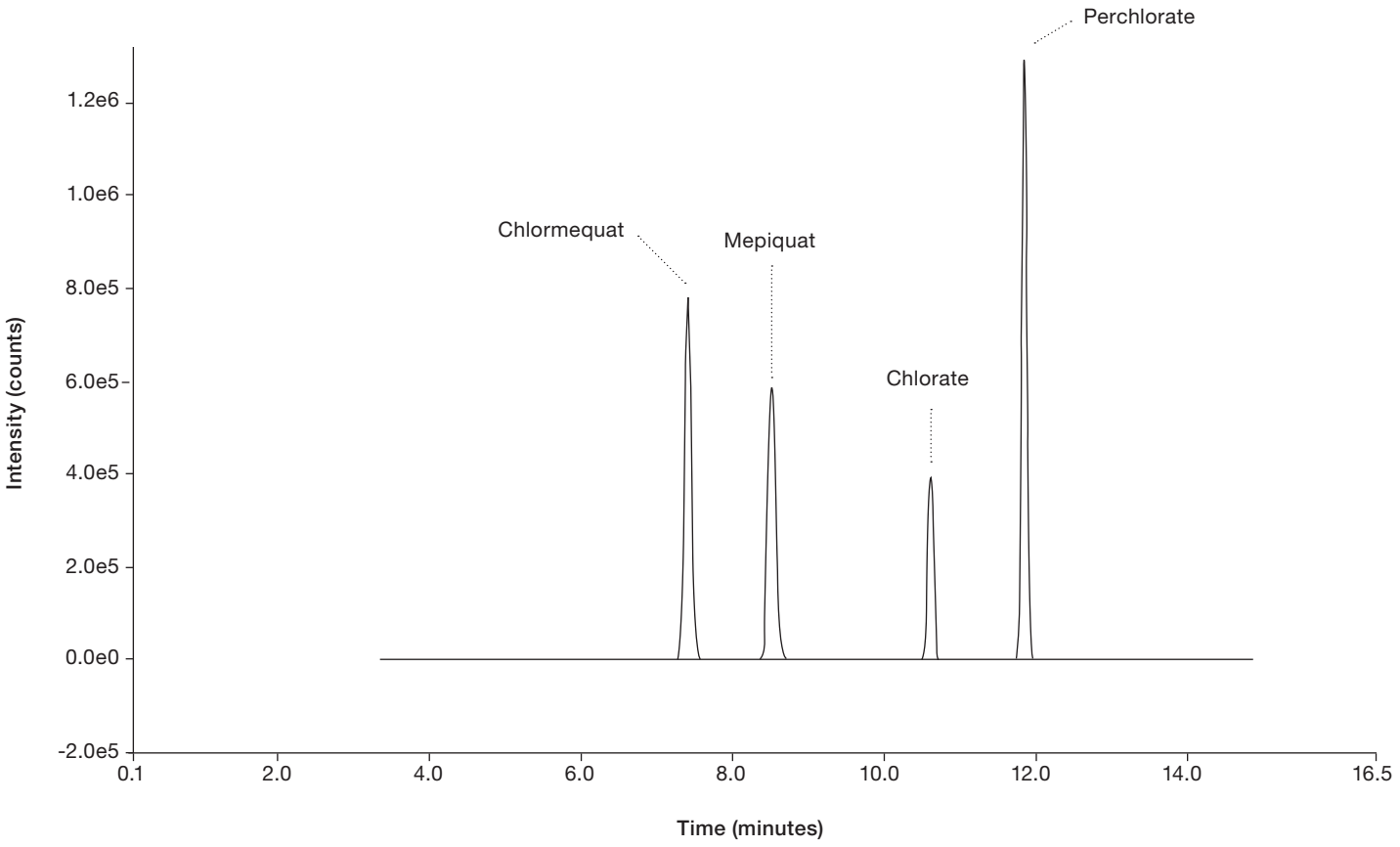


Figure 2. Simultaneous separation of cationic (chlormequat and mepiquat) and anionic (chlorate and perchlorate) within 16 minutes total run time after 10 µL of standard solution. Retention times are 7.28 min, 8.42 min, 10.02 min, and 11.45 min, respectively.

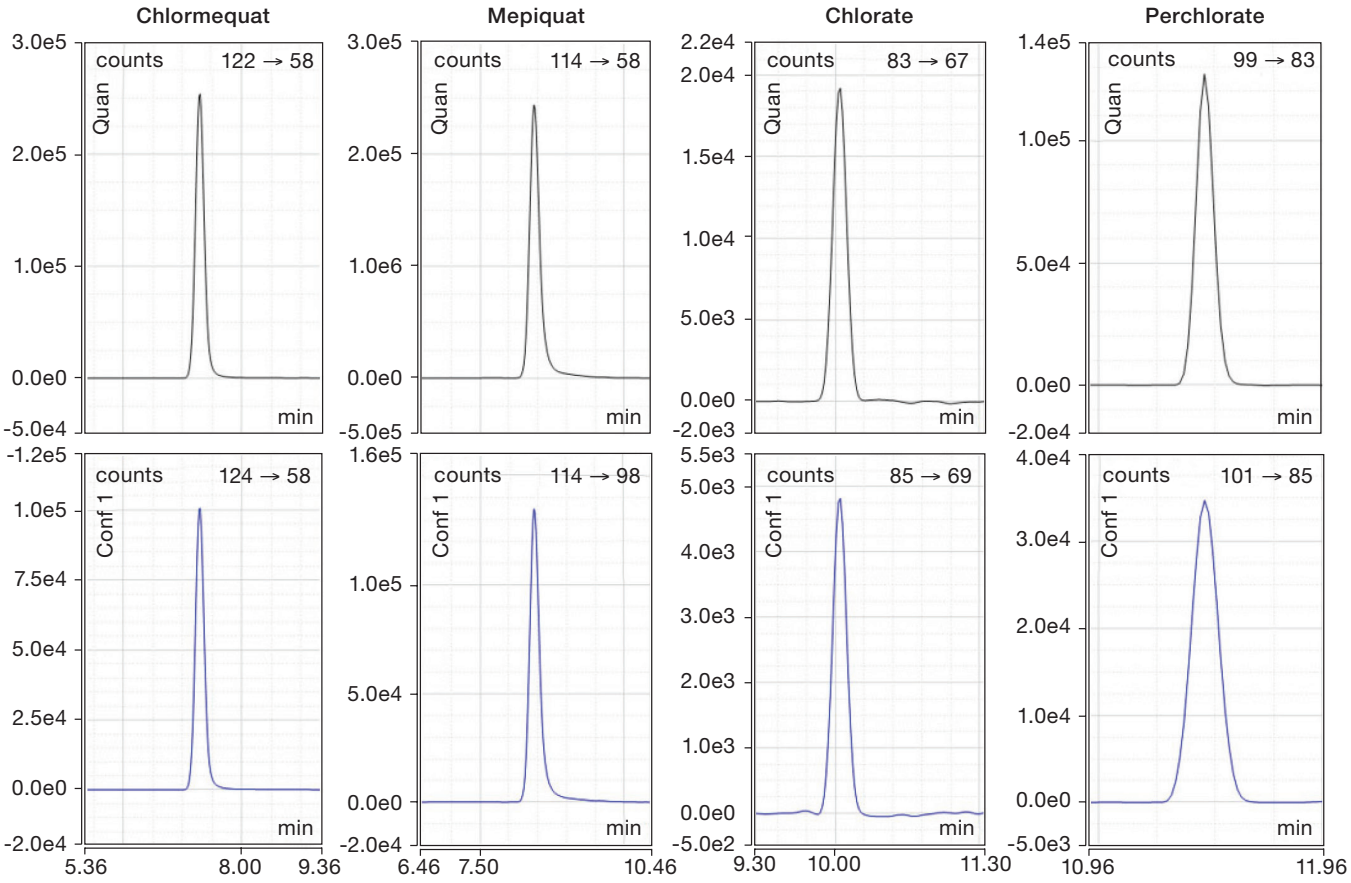


Figure 3. Chromatographic peaks for each analyzed compound were obtained after a 10 µL injection of 10 ppb standard solution (in vial concentration). (Black traces: quantification ion, blue traces: confirmation ions)

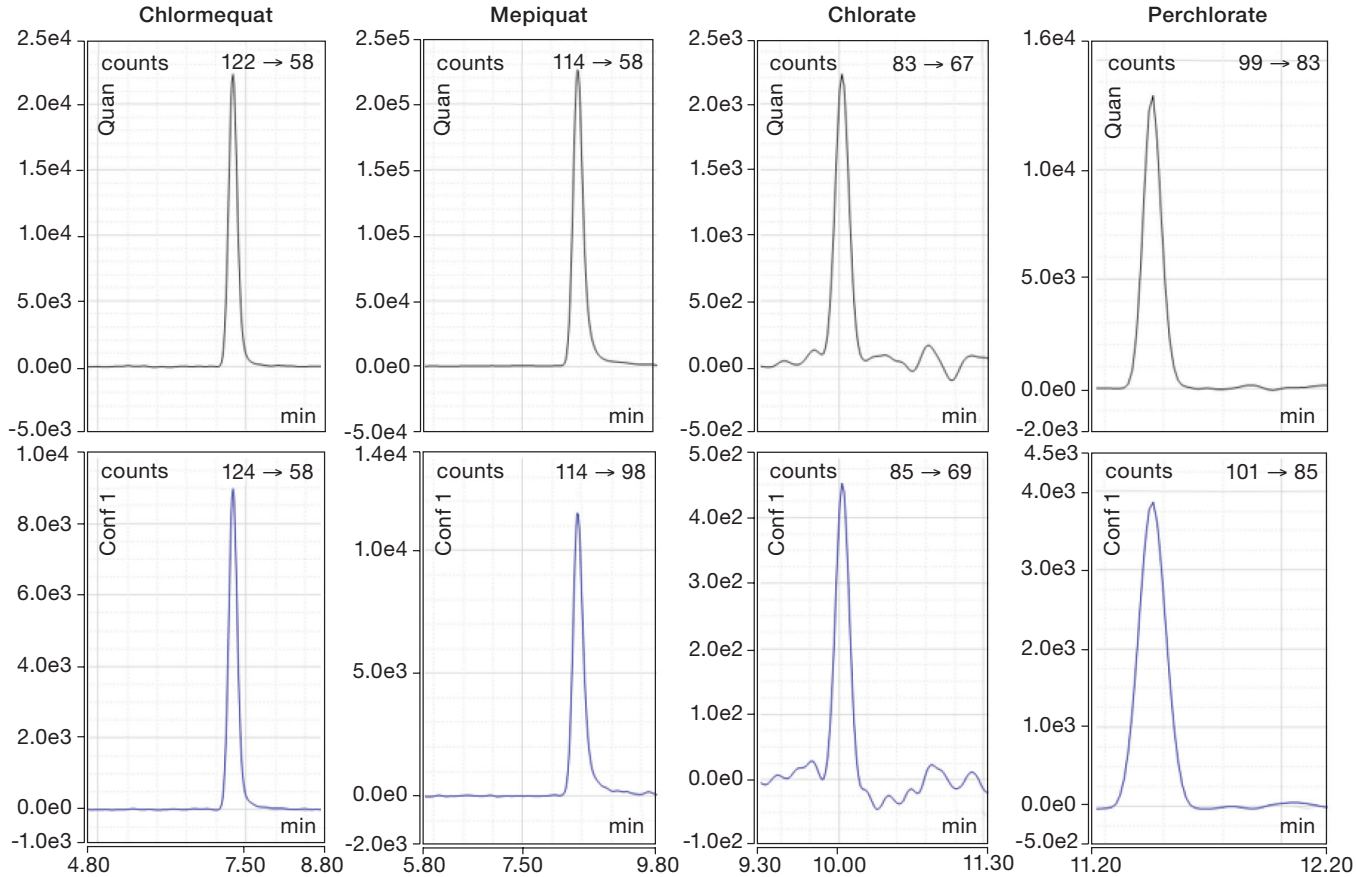


Figure 4. Chromatographic peaks for each analyzed compound were obtained after a 10 µL injection of 1 ppb standard solution (in vial concentration). (Black traces: quantification ion, blue traces: confirmation ions)

Anionic and cationic pesticides quantification

Table 5 shows Maximum Residue Limits (MRLs) expressed in mg/kg for each targeted compound in different matrices.[6] Regarding our sample preparation, the minimum amount to be detected in the vial is 1, 2, or 5 ppb for chlormequat, mepiquat, and chlorate/perchlorate, respectively. The minimum expected value for chlormequat is 0.01 mg/kg and 0.02 mg/kg for mepiquat. The MRL is slightly higher for both chlorate and perchlorate and is fixed at 0.05 mg/kg.

Table 5. MRLs expressed in mg/kg for each targeted compound in different matrices (source European Commission)

	Chlormequat	Mepiquat	Chlorate	Perchlorate
	Referential document			
Matrix	*Reg. (EU) 2020/1565*	*Reg. (EU) 2021/976*	*Reg. (EU) 2020/749*	*Reg. (EU) 2020/685*
Spinach	0.01	0.02	0.70	0.05
Spices	0.05	0.10	0.07	0.05
Teas	0.05	0.10	0.05	0.75
Barley	7.00	4.00	0.05	0.05
Wheat	7.00	3.00	0.05	0.05
Oat	15.00	3.00	0.05	0.05

To determine the correct sample pesticide residue amount in complex matrices, isotopically labelled internal standard (ILIS) solution was added to the 50 mL sample preparation tube. This allowed for correcting nebulization variation, which could be affected by source contamination or ion suppression due to matrix and/or salt. Makeup solvent added to the setup drastically reduced the source fouling sample after sample. Three ILISs were added to the sample at a constant amount of 100 ppb. The peak of each ILIS is represented in Figure 5. Also, SRM parameters and expected intensity for 100 ppb equivalent are mentioned. No isotopically labeled internal standard for chlorate was added to assess chlorate quantification better.

The linearity of the method was evaluated using a linear 1/X weighted model. Data were reprocessed using internal standard mode except for chlorate. Figure 6 shows calibration curves for chlormequat, mepiquat, chlorate, and perchlorate from 0.5 ppb up to 500 ppb. The linearity of response is achieved even between 0.5 and 10 ppb. Accuracies calculated for each calibration level remain within the 20% tolerance (data not shown), and R^2 is above 0.9950.

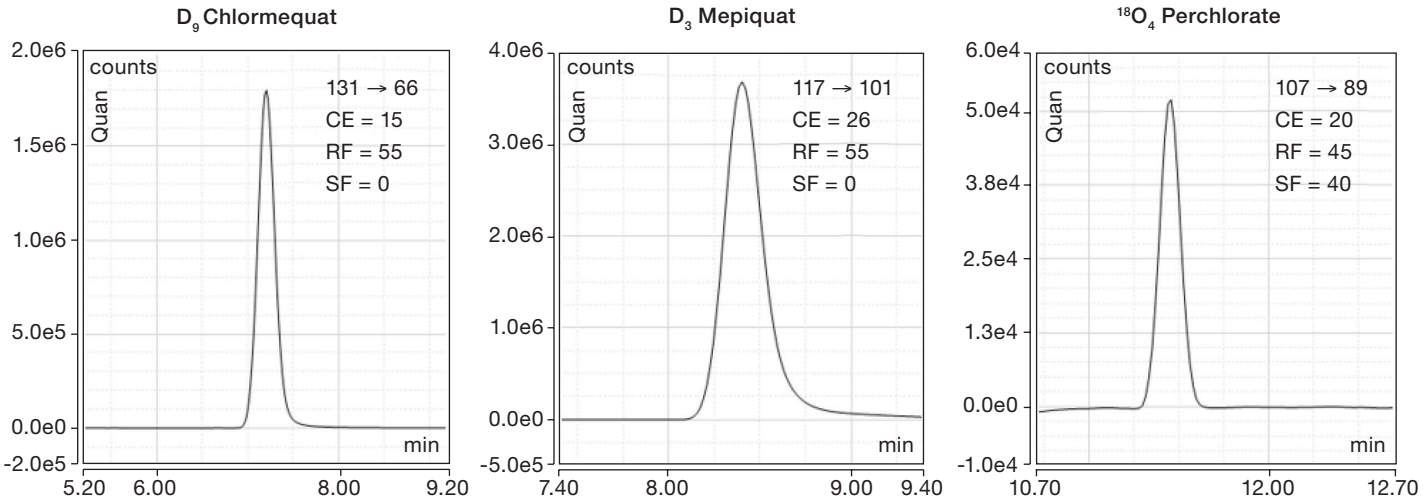


Figure 5. Chromatographic peaks and MS detection parameters for each internal standard

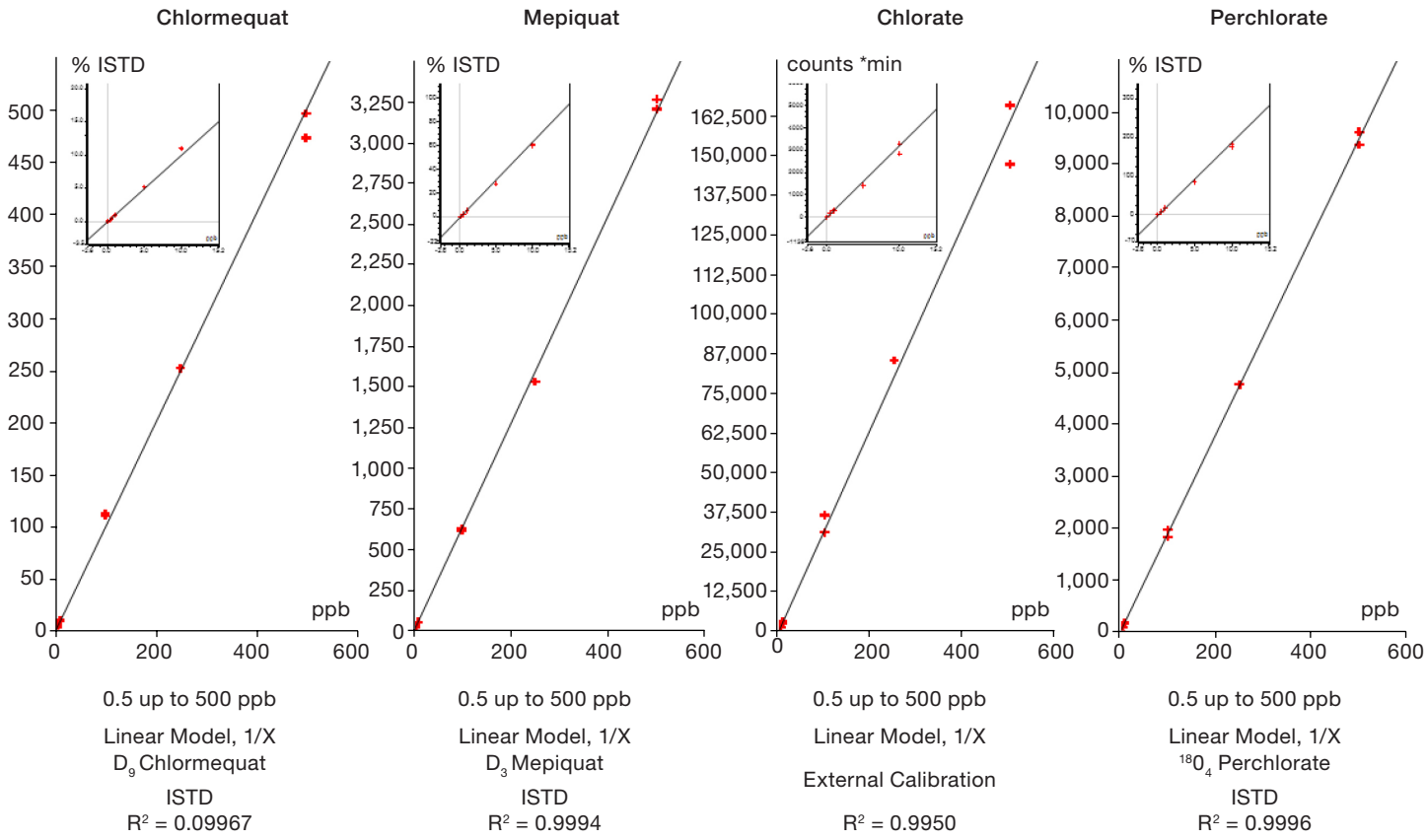


Figure 6. Calibration curves for chlormequat, mepiquat, chlorate, and perchlorate, 0.5 ppb to 500 ppb in vial concentration. Insets: zoom on 0.5 to 10 ppb range.

Compiled data from QC at 10 ppb analyzed over three consecutive days are shown in Table 6. The system stability assessment seems very desirable, with a relative standard deviation below 2% for values corrected with ILISs: chlormequat, mepiquat, and perchlorate. Chlorate data are more dispersed but remain below 7%. These collected data are fully compliant with SANTE guidelines, and the conformity of each QC is validated.

The spike at the required lowest authorized value 0.01 mg/kg or 1 ppb in the vial was performed using the spinach matrix. Figure 7 illustrates the area increase of each peak corresponding to targeted compounds while the ILISs areas remain stable. The reference trace (red dotted line) shows that the "virgin" spinach matrix already contains a significant amount of chlorate and perchlorate, probably due to the process of disinfection with chlorinated water used for the spinach leaves. Calculated amounts are graphically reported in Figure 8. The average (n=6) for all tested compounds stays at 1.2 and 0.8 ppb. Consolidated data for chlormequat, mepiquat, and perchlorate are close to the mean value, while chlorate values are more dispersed and sometimes above the ±20% limit.

Table 6. Quality control (QC) conformity assessment at 10 ppb

		Chlormequat			Mepiquat			Chlorate			Perchlorate		
Day	Sample	Conc. (ppb)	Expected value (ppb)	Conformity	Conc. (ppb)	Expected value (ppb)	Conformity	Conc. (ppb)	Expected value (ppb)	Conformity	Conc. (ppb)	Expected value (ppb)	Conformity
1	First QC	10.815	10.000	Yes	9.567	10.000	Yes	11.500	10.000	Yes	10.363	10.000	Yes
	Latest QC	10.932	10.000	Yes	9.669	10.000	Yes	10.608	10.000	Yes	10.576	10.000	Yes
2	First QC	10.730	10.000	Yes	9.530	10.000	Yes	9.525	10.000	Yes	10.634	10.000	Yes
	Latest QC	10.733	10.000	Yes	9.548	10.000	Yes	10.894	10.000	Yes	10.676	10.000	Yes
3	First QC	10.701	10.000	Yes	9.694	10.000	Yes	10.925	10.000	Yes	10.171	10.000	Yes
	Latest QC	10.438	10.000	Yes	9.610	10.000	Yes	10.998	10.000	Yes	10.332	10.000	Yes
	RSD% =	**1.53%**			**0.70%**			**6.17%**			**1.91%**		

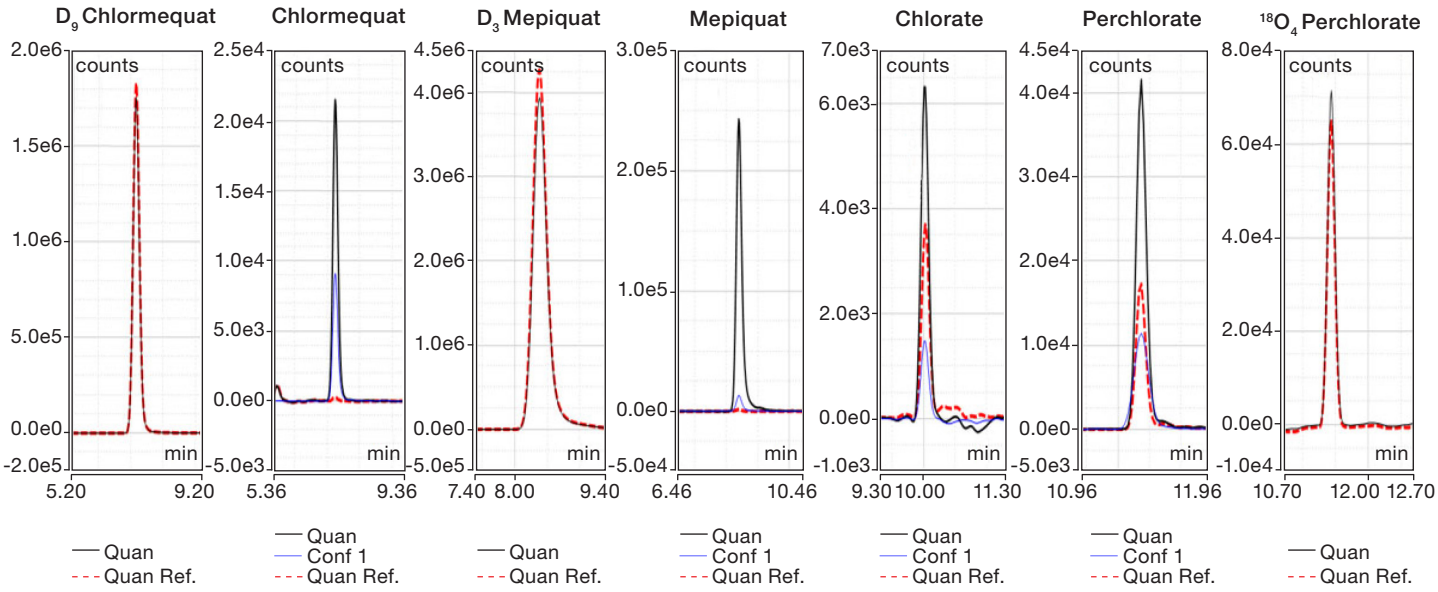


Figure 7. Extracted ion chromatograms (XICs) obtained after injection of 10 µL of spinach matrix spiked with 1 ppb of each targeted compound (except for ILISs, amount is fixed at 100 ppb). The red dotted line represents the unspiked spinach matrix.

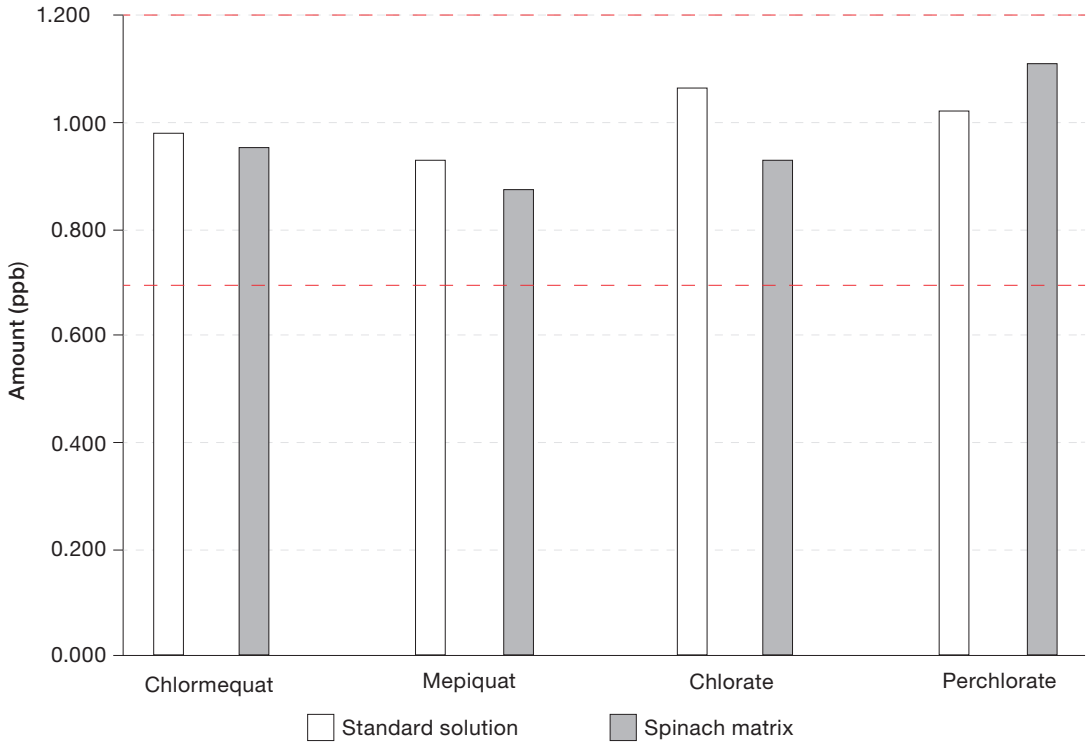


Figure 8. Average values obtained after injection of standard solution (white bar) or spinach matrix spiked for each compound of interest at the 0.01 mg/kg or 1 ppb level in the vial (gray bar)

The inter-day recovery rate is aligned with SANTE/12682/2019 guidelines for chlormequat, mepiquat, and perchlorate. All values are corrected using ILISs between 60 and 140% (Table 7). As previously seen, chlorate values are slightly over the authorized range ±20%.

These inter-day experiments also demonstrate that a labeled chlorate ILIS must correct values despite the perfect system stability.

Table 7. The inter-day recovery rate for spinach matrix spiked with 10 ppb of each following compounds: chlormequat, mepiquat, chlorate, and perchlorate

			Chlormequat			Mepiquat		
Sample	Day	Repetition	Conc. (ppb)	Spike level concentration (ppb)	Recovery rate (%)	Conc. (ppb)	Spike level concentration (ppb)	Recovery rate (%)
Spinach d5 spiked 10 ppb	1	1	10.926	10.000	**109%**	8.883	10.000	**89%**
Spinach d5 spiked 10 ppb	1	2	10.494	10.000	**105%**	8.470	10.000	**85%**
Spinach d5 spiked 10 ppb	2	1	10.940	10.000	**109%**	8.773	10.000	**88%**
Spinach d5 spiked 10 ppb	2	2	10.631	10.000	**106%**	8.536	10.000	**85%**
Spinach d5 spiked 10 ppb	3	1	10.812	10.000	**108%**	10.858	10.000	**109%**
Spinach d5 spiked 10 ppb	3	2	10.381	10.000	**104%**	10.427	10.000	**104%**

			Chlorate			Perchlorate		
Sample	Day	Repetition	Conc. (ppb)	Spike level concentration (ppb)	Recovery rate (%)	Conc. (ppb)	Spike level concentration (ppb)	Recovery rate (%)
Spinach d5 spiked 10 ppb	1	1	12.488	10.000	**125%**	10.110	10.000	**101%**
Spinach d5 spiked 10 ppb	1	2	11.226	10.000	**112%**	10.791	10.000	**108%**
Spinach d5 spiked 10 ppb	2	1	8.345	10.000	**83%**	11.109	10.000	**111%**
Spinach d5 spiked 10 ppb	2	2	8.447	10.000	**84%**	11.083	10.000	**111%**
Spinach d5 spiked 10 ppb	3	1	9.442	10.000	**94%**	11.433	10.000	**114%**
Spinach d5 spiked 10 ppb	3	2	7.344	10.000	**73%**	11.044	10.000	**110%**

Conclusion

This work describes an LC-MS/MS workflow based on a modified Vanquish Flex system, together with an Acclaim Trinity P1 column, coupled with TSQ Altis mass spectrometer. This methodology shows the simultaneous determination of underivatized anionic and cationic polar pesticides in food. This method is suitable for checking regulated MRLs and is fully validated and compliant with SANTE/12682/2019 guidelines for chlormequat, mepiquat, and perchlorate. To meet the chlorate validation, ILIS use is required. Implementation in the laboratory is easy, and the analytical process purposed allows the production of faster results compared to multi-application combination.

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