Technical note | 000771

Food safety

Acclaim Trinity P1 mixed-mode chromatography column coupled with an inert LC system: an innovative approach for underivatized highly polar pesticides separation

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Keywords

Acclaim Trinity P1 column, glyphosate, mepiquat, chlormequat, Vanquish UHPLC, mixed mode, food

Goal

To develop an LC method based on the Thermo Scientific™ Acclaim™ Trinity P1 column for cationic and anionic polar pesticides analysis in a single run

Application benefit

Lab productivity improvement based on a single run for anionic and cationic highly polar pesticides

Introduction

Despite cultural practice modifications and pesticide residue control efforts implemented in many countries, the European Food Safety Authority reports[1] show that pesticide contamination remains present in many plant samples. Among the 25 most frequently encountered pesticides, anionic or cationic polar pesticides are broadly identified. Facing these results, routine analysis demand is increasing; however, analyzing polar pesticides presents a difficult analytical challenge. The high polarity does not allow the direct analysis by common pesticides approach like reversed-phase HPLC.

A new analytical method based on the Acclaim Trinity P1 column was developed to overcome this challenge. By competing with zwitterionic technology, this column chemistry allowed cationic and anionic polar pesticides analysis in a single run.

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)

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™ DP Analytical – Gradient with Degas **(P/N 22181-60007)**
  - 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. (1007 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)**
- Thermo Scientific™ Viper™ cap., 0.13 mm i.d. x 150 mm l, PEEK **(P/N 6041.5616)**
- Viper cap., 0.5 mm i.d. x 350 mm l, WASTE LINE, VH-D1 (P/N 6083.2425)

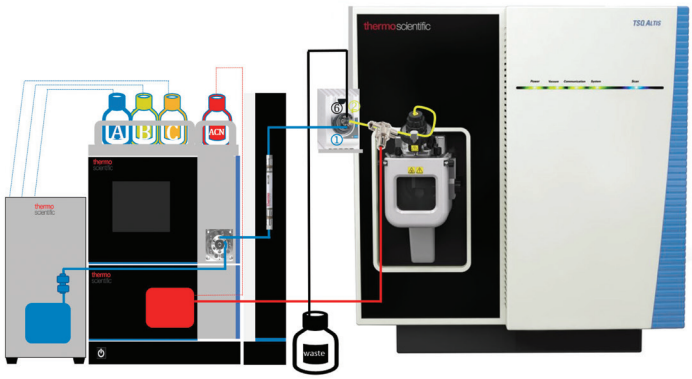

Figure 1. Inert fluidic pathway (blue lines) used to perform glyphosate elution; a second pump is required to add makeup solvent (red lines).

LC conditions

Table 1. LC conditions

Parameter	Setting
LC column	Thermo Scientific™ Acclaim™ Trinity P1 100 x 2.1 mm, 3 µm, **P/N 071389**
Dionex ICS-6000 DP Dual Pump	
Mobile phase A	100 mM Ammonium formate with pH adjusted up to 3 using formic acid
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	15 µL
Sampler wash solution	Water 90 / methanol 10 (vol / vol)

Table 2. Gradient details using the Acclaim Trinity P1 column

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
13	0.4	5	0	95	0
13	0.4	5	95	0	0
16	0.4	5	95	0	0

Table 3. Heartcutting LC conditions

LC column and conditions 2D	
LC column	Thermo Scientific™ Dionex™ IonPac™ AS19-4 µm, 250 x 2 mm, P/N 083223
Mobile phase C	100 mM ammonium acetate, pH = 3 / water (10 / 90 vol/vol)
Elution flow rate	See Table 4
Gradient	See Table 4
Column oven	40 ˚C Still air mode

Table 4. Gradient details using heartcutting

Time (min)	Flow rate (mL/min)	%A	%B	%C	%D
0.000	0.005	0	0	100	0
11.000	0.005	0	0	100	0
11.000	0.35	0	0	100	0
15.000	0.35	0	0	100	0
15.001	0.005	0	0	100	0
16.000	0.005	0	0	100	0

MS conditions

Table 5. MS conditions

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	1
Ion transfer tube temperature	350 °C
Vaporizer temperature	400 °C
Make up	0.4 mL/min acetonitrile
Experiment type	Selected reaction monitoring (SRM)
Dwell time	10 ms
Chromatography peak width	7 s
Collision gas pressure	2.5 mTorr
Q1 resolution	0.7 FHMW
Q3 resolution	1.2 FHMW

Table 6. SRM transitions

Compound	Polarity	Precursor *(m/z)*	Product *(m/z)*	Collision energy (V)	RF Lens (V)	Source fragmentation
Glufosinate	Negative	180	63	40	43	0
Glyphosate	Negative	168	150	10	38	0
***N*-acetyl AMPA**	Negative	152	63	20	30	0
Phosphonic acid	Negative	81	79	15	43	0
Chlormequat	Positive	122	58	20	55	0
3-MPPA	Negative	151	63	33	35	0
Mepiquat	Positive	110	98	20	55	0
Fosetyl aluminum	Negative	109	81	12	38	0
Ethephon	Negative	143	107	10	40	40
***N*-acetyl glufosinate**	Negative	222	180	16	46	0
Chlorate	Negative	83	67	20	42	0
Perchlorate	Negative	99	83	20	30	0
AMPA	Negative	110	63	20	38	0

Software

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

Sample preparation

Commercial standard solutions were stored at -20 ˚C. One mix solution at 1 ppm was prepared for standard compounds using ultrapure water. Internal deuterated standard mix (ISTD) solution at 1 ppm was prepared with water and stored at -20 ˚C. Samples matrices were prepared using a slightly modified EURL QuPPE protocol[4] (Figure 2) by first weighing 10 g of fresh sample in a 50 mL polypropylene tube and then adding 10 mL of extraction solution (methanol with 1% formic acid). Sample homogenization was performed with a Fisherbrand™ 150 Homogenizer with a stainless steel probe for 5 minutes. The final volume was adjusted up to 20 mL with acidified methanol. A 50 mL tube was frozen 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. 200 µL of matrix, 100 µL ISTD stock solution 1 ppm, 10 µL standard stock solution 1 ppm, and 690 µL of water were added to the vial.

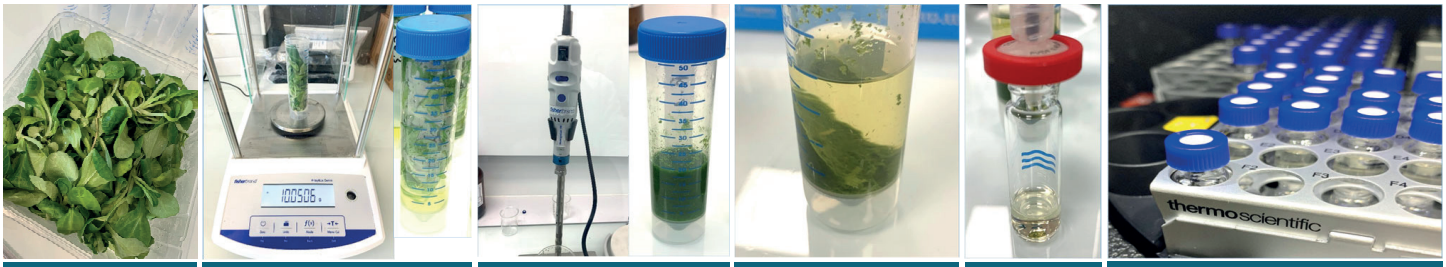

Figure 2. Sample preparation process

Results and discussion

Concept proof

The mixed-mode Acclaim Trinity P1 column allows separation of the most common polar herbicides glyphosate and glufosinate. Figure 3 shows baseline resolution between both herbicides and good peak shape even for AMPA and glyphosate. Retention times are 0.8, 3,3, and 5.7 for AMPA, glufosinate, and glyphosate, respectively.

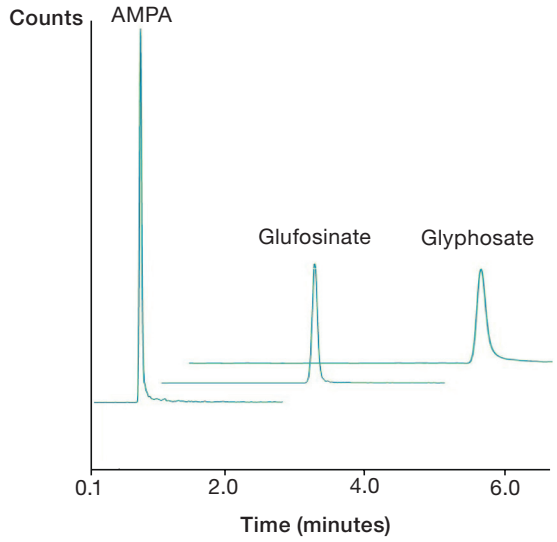

Figure 3. Typical separation was obtained after a 15 µL injection of 100 ppb standard glyphosate, glufosinate, and AMPA on the Acclaim Trinity P1 column.

Impact of system inertness

Phosphated analyte-metal interactions are well documented and reported in literature.[2] These interactions can generate peak shape degradation injection after injection. Figure 4 illustrates glyphosate peak shape differences when elution was performed using a conventional metallic or modified setup system. Leachable metallic contamination impacts column retention, and three distinct pollution effects can be measured. The first is the signal intensity using a conventional metallic LC system: 40% height was lost after one hundred injections. The second was the drastic increase of peak width, up to 84%, and a problematic peak tailing. The latest effect was the retention time shift that can exceed 0.2 minutes.

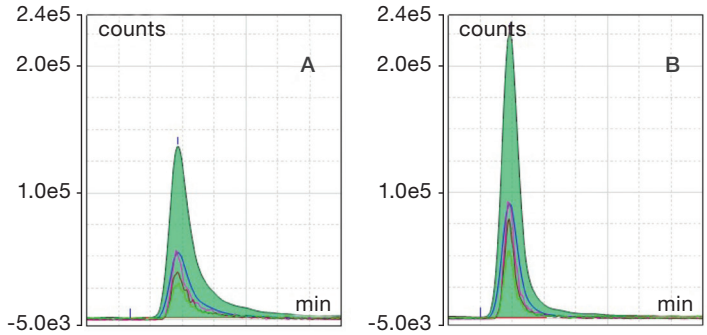

Figure 4. Impact of the LC system material on glyphosate. (A) Elution with a conventional LC system and (B) elution with an inert LC system

Phosphated anions like glyphosate have a poor peak shape using conventional titanium LC systems. Many passivation approaches using strong acids or chelators have been developed to avoid metallic interaction with compounds at the LC system surface. However, these approaches are time-consuming and often rejected by high sample throughput laboratories due to their massive impact on productivity. Ionic chromatography with suppression based on the fully inert fluidic pathway is the dedicated best-in-class technology with a broader range for anionic polar pesticides analyzed. This study replaces the conventional LC pump with an inert pump, the Dionex ICS-6000 DP Pump. Figure 1 illustrates the fluidic pathway of the new LC/MS-MS setup. Using this inert pump, no passivation of the system is required, making it compatible with day-to-day polar pesticides analysis. Specifications of this high-pressure PEEK pump fit perfectly with the backpressure generated by the 3 µm Acclaim Trinity P1 stationary phase at 40 ˚C. A titanium-based Thermo Scientific Vanquish Flex Pump adds a simple makeup solvent to enhance solvent evaporation in the source. This innovative hybrid setup, including a mass spectrometer, is fully controlled by Chromelon CDS 7.3.

Glyphosate peak tailing was evaluated over fifty consecutive injections of a standard solution in water. Peak shape stability assessment is reported in Figure 5. The peak shape measured at five percent of the peak height remained stable, validating the hybrid setup for the following experiments.

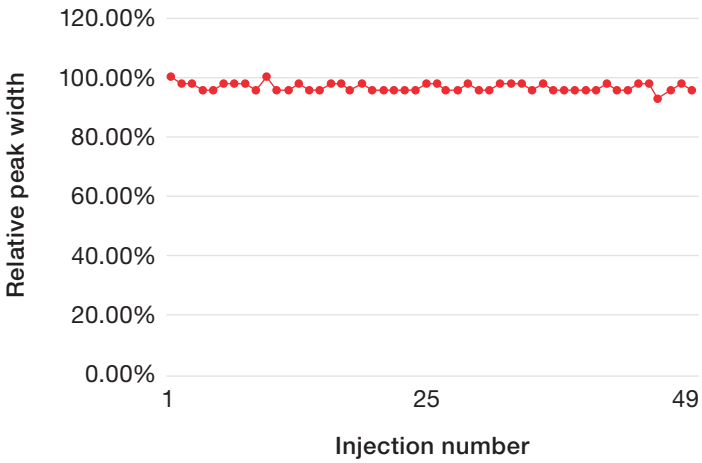

Figure 5. Relative peak width (measured at 5%) for 50 consecutive injections of glyphosate standard solution using the inert pump

Extended capabilities of the Acclaim Trinity P1 chemistry

Mixed-mode chromatography provides multiple functionalities on a single chromatographic support. Combining reversed-phase anion-exchange and cation-exchange retention mechanisms, the Acclaim Trinity P1 column chromatography is well-suited to retain hydrophilic ionic and ionizable analytes and does not require addition of ion pairing agents in the mobile phase, significantly improving the MS compatibility. To demonstrate the potential benefits of this mixed-mode column to separate underivatized polar pesticides compounds, a panel of eleven compounds was separated using a ternary gradient based on conventional LC/MS eluents. Separation was driven by the unique stationary phase, mobile phase pH, ionic strength, and organic modifier proportion. Elution required formate buffer, water, and acetonitrile. Figure 6 illustrates the fast separation of eleven compounds in less than 15 minutes. Herbicides and potential metabolites were fully resolved, and chlorate and perchlorate can be analyzed in a short single run. A stationary phase offering ion exchange and reversed-phase capabilities enhanced separation currently obtained with conventional approaches for these underivatized compounds.

As shown in Figure 6, the AMPA retention time was very low next to the void volume. A heartcutting approach was developed to improve retention time stability and avoid loss of response due to the potential matrix effect. AMPA weakly retained was trapped into a loop and released, at the end of the chromatogram, after perchlorate elution on the second column. The second column with another selectivity was implemented in the same column compartment, and in both, two-position, six-port valves were installed to independently analyze the AMPA trapped in the loop. Figure 7 illustrates the fluidic pathway involving two inert pumps. AMPA was eluted isocratically from a strong anion exchange column (Dionex IonPac AS19-4 µm column) using a low pH formate buffer.

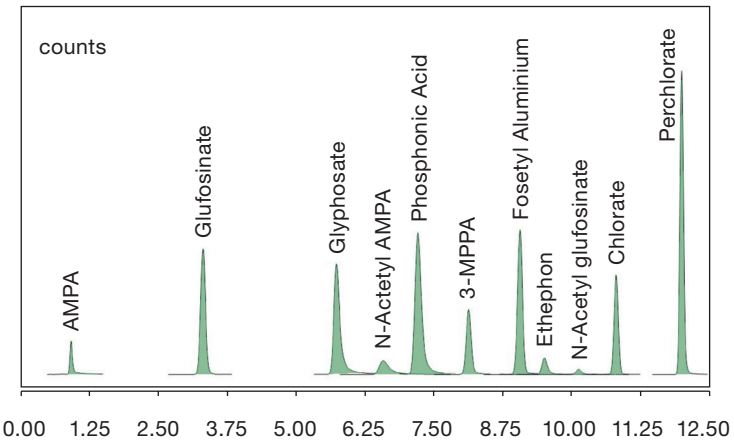

Figure 6. The typical chromatogram obtained after a 15 µL injection of 100 ppb standard solution on the Acclaim Trinity P1 column

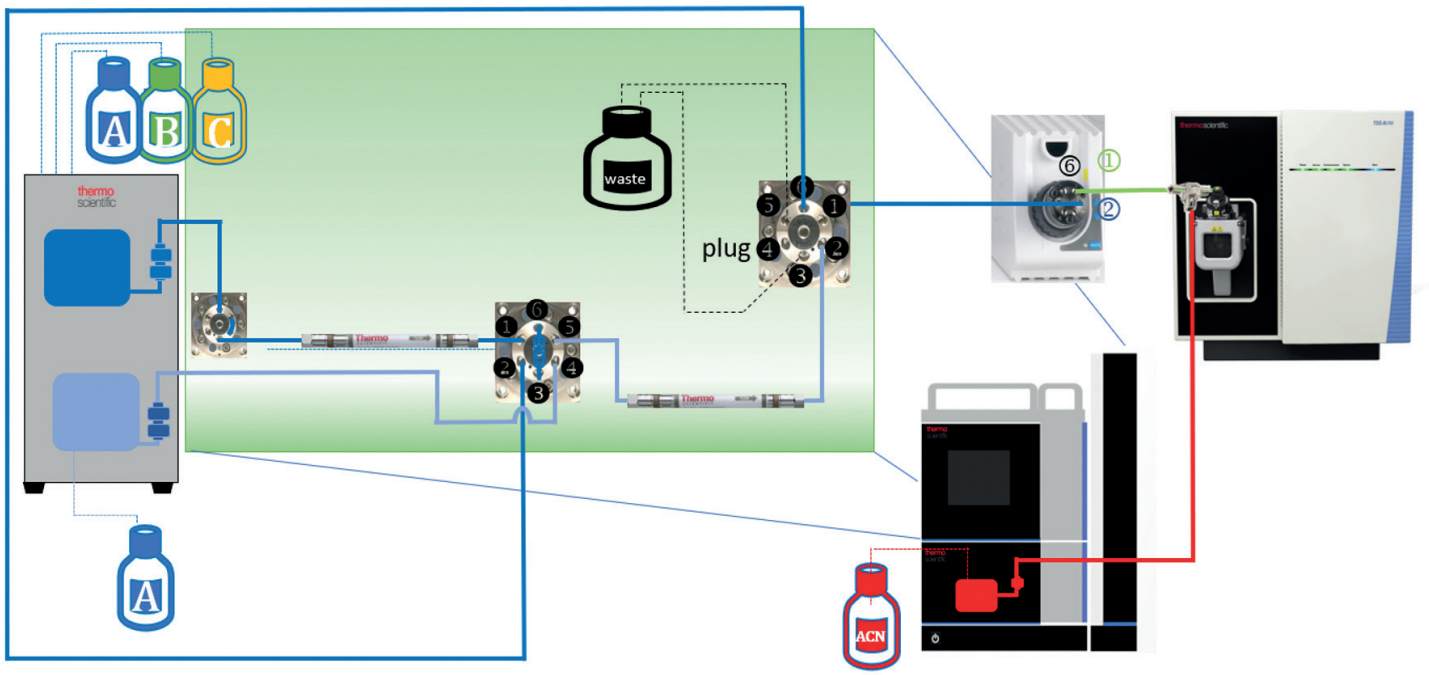

Figure 7. Optimized heartcutting setup with modified fluidics pathways: inert elution pathways (dark and light blue) and biocompatible makeup pathway (red)

Analysis of underivatized glyphosate, metabolites, and analogs was performed without derivatization and suppression, simplifying the routine implementation for nonionic chromatographer experts. Using a heartcutting approach, AMPA was eluted over thirteen minutes after chlorinated peaks: chlorate and perchlorate. AMPA response remains very high despite suppressor-less ionic chromatography (Figure 8).

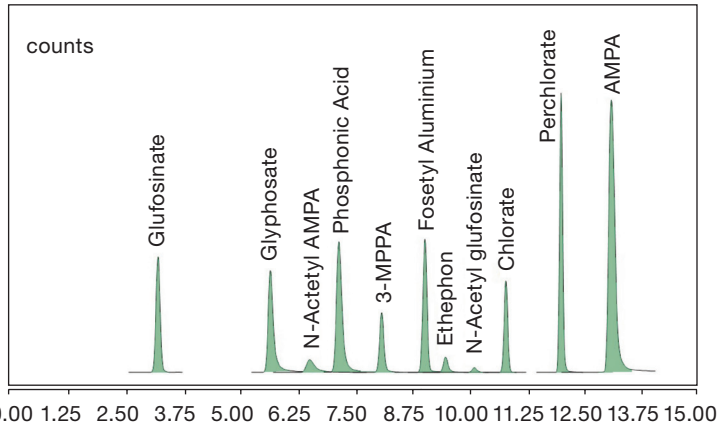

Figure 8. The typical chromatogram was obtained after a 15 µL injection of 100 ppb standard solution injection using an inert heartcutting setup.

Glyphosate and other metabolites and analogs showed the same SRM transitions. Figure 9 demonstrates good separation for fosetyl aluminum, phosphonic acid, *N*-acetyl AMPA, and 3-MPPA. Appropriate time acquisition windows and stable retention times were required to avoid false positives or over-quantification of these specific compounds potentially impacted by similar isobaric fragments generated. The compatibility of this innovative methodology was assessed with a challenging food matrix: wheat. Modified QuPPe extract was spiked with different levels of glyphosate from 0.2 to 2 mg/kg, corresponding to 10 to 100 ppb in a vial. These values remained lower than the expected MRL in the wheat matrix[3]: 10 mg/kg. Results are shown in Figure 10. The lack of glyphosate was checked in the unspiked wheat matrix (chromatogram A). In B to D chromatograms, increasing amounts of glyphosate are represented. At all spiked levels, confirmation was visible, and ion ratios were maintained even in the matrix. Potential interference was well separated from the glyphosate peak facilitating automatic identification and quantification.

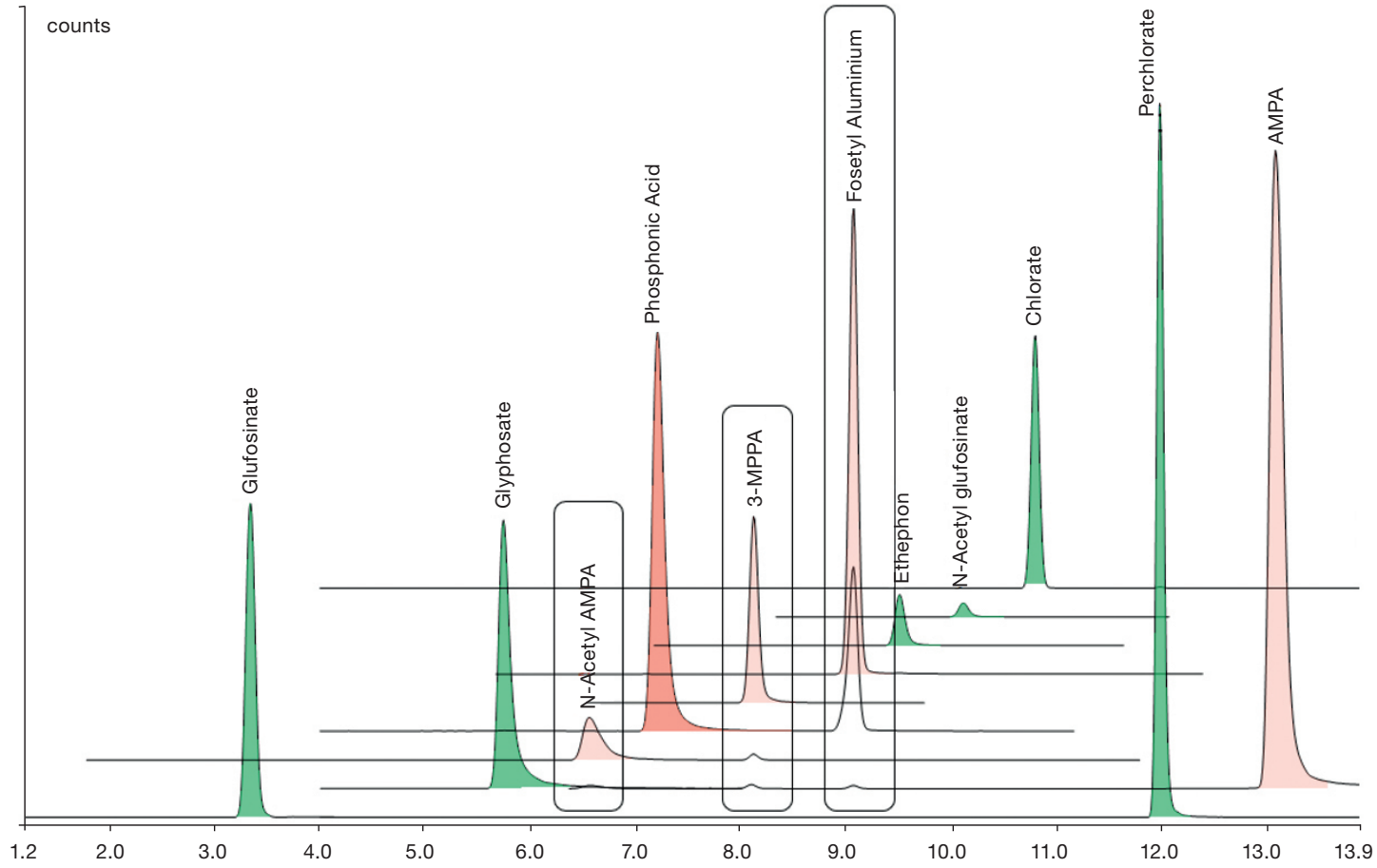

Figure 9. Boxes illustrate potential interferences with isobaric transitions. The typical chromatogram was obtained after a 15 µL injection of 100 ppb standard solution.

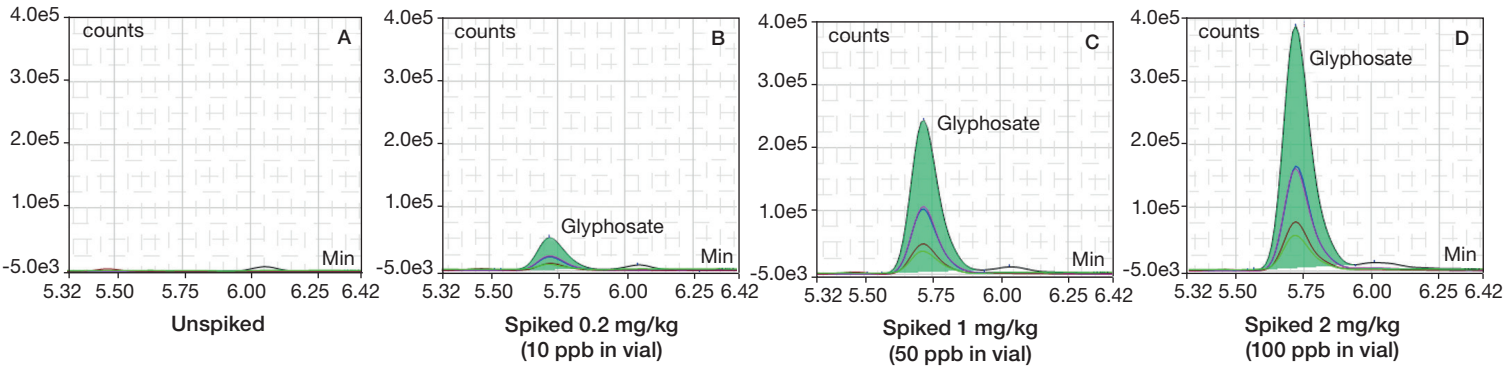

Figure 10. Chromatograms were obtained after injection of 15 µL of wheat modified QuPPe extracts native or spiked at three increasing levels from 0.2 mg/kg to 2 mg/kg.

Table 7. Retention time statistics for eleven anionic polar pesticides were analyzed with the Acclaim Trinity P1 column

	Glufosinate	Glyphosate	N-Acetyl AMPA	Phosphonic Acid	3-MPPA	Fosetyl Aluminium	Ethephon	N-Acetyl glufosinate	Chlorate	Perchlorate	AMPA
Maximum	3.344	5.743	6.586	7.206	8.126	9.066	9.501	10.125	10.790	11.977	13.106
Average	3.312	5.718	6.558	7.187	8.105	9.044	9.484	10.091	10.770	11.967	13.075
Minimum	3.273	5.687	6.525	7.157	8.071	9.011	9.445	10.034	10.708	11.957	13.045
Standard deviation	0.019	0.018	0.018	0.015	0.012	0.012	0.013	0.025	0.021	0.004	0.019
Relative standard deviation	**0.58%**	**0.31%**	**0.31%**	**0.20%**	**0.15%**	**0.14%**	**0.14%**	**0.24%**	**0.20%**	**0.03%**	**0.14%**

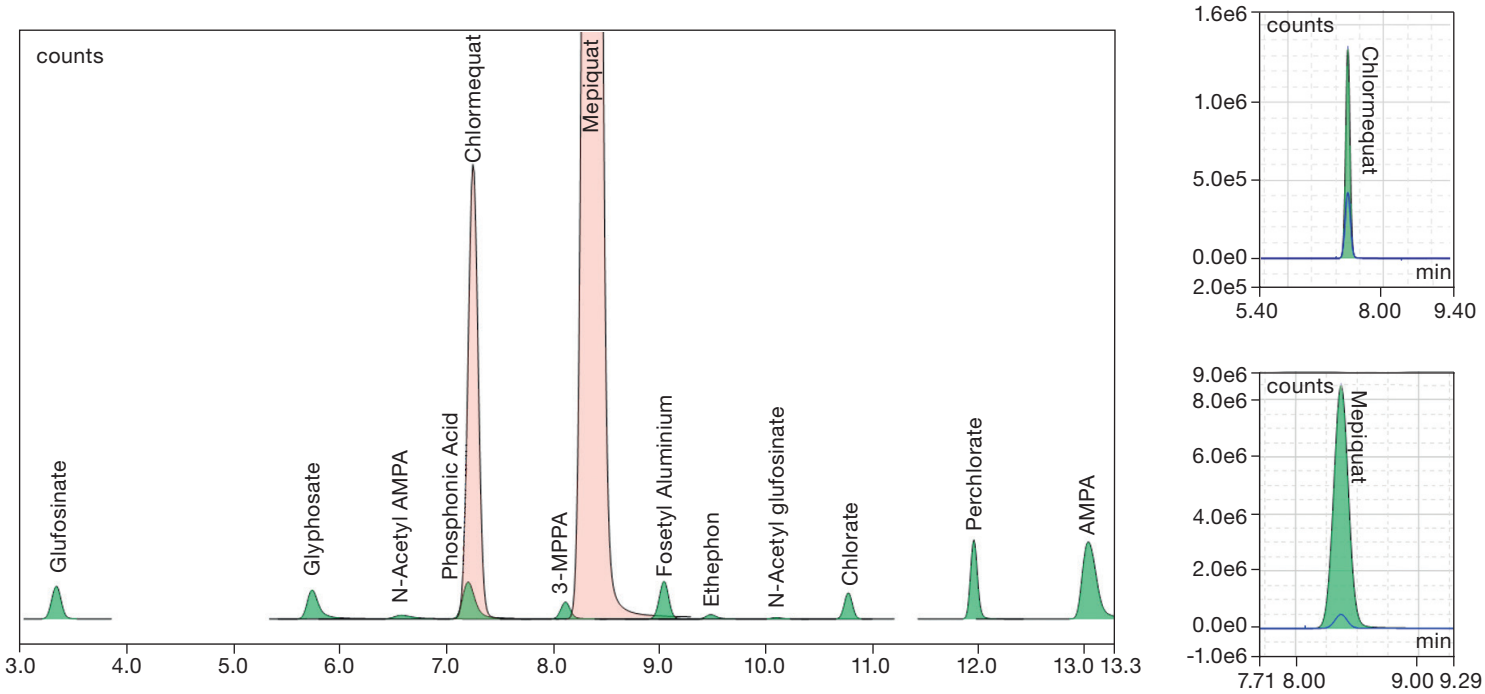

Figure 11. The chromatogram obtained after a 15 µL injection of a 100 ppb standard solution illustrated simultaneous analysis of anionic (green) and cationic (pink) polar pesticides in a single run. The boxes show cationic components extraction with quantification (black trace with area green light highlighted) and confirmation (blue trace) transitions.

Retention time expressed in minutes is reported in Table 7. Sixty-eight consecutive injections, including QC, samples, and calibration standards, were performed to report minimum and maximum and calculate the average and relative standard deviation for each compound. We contributed to stabilize chromatography of phosphated pesticides by granting LC inertness improvement.

The unique chemistry of the Acclaim Trinity P1 column allows development flexibility. The multi-mode surface chemistry is ideal for the simultaneous separation of cationic pesticides like chlormequat and mepiquat and the other eleven anionic polar pesticides (Figure 11). In many laboratories, performing cationic polar pesticides analysis requires two or more distinct LC or IC columns and eluent conditions. Strong cation exchangers located on the outer nanopolymers beads offer productivity improvement and reliable separation between cationic species without run time compromise for these labs.

Conclusion

With the Acclaim Trinity P1 column, the challenge of analyzing anionic and cationic polar pesticides in a single run was overcome. A modified LC setup that improves system inertness enhances chromatographic robustness and performance, allowing this methodology's routine use. Separation without any derivatization or any ion suppressors simplifies the overall analytical workflow, making this solution relatively straightforward to implement.

References

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