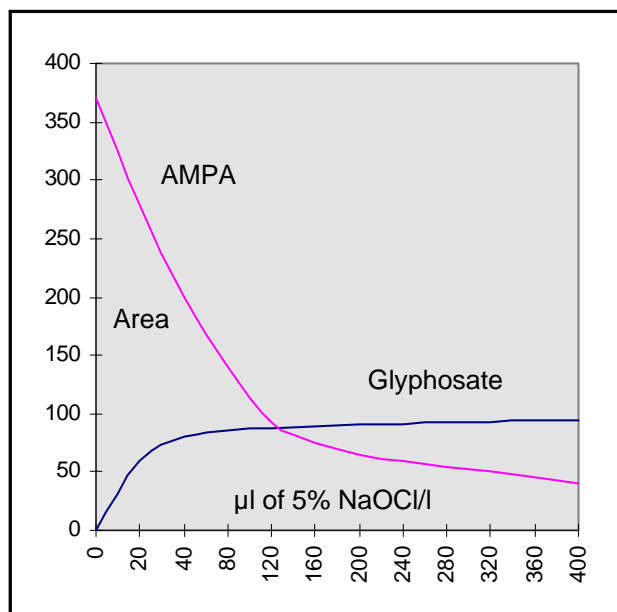


APPLICATION NOTE

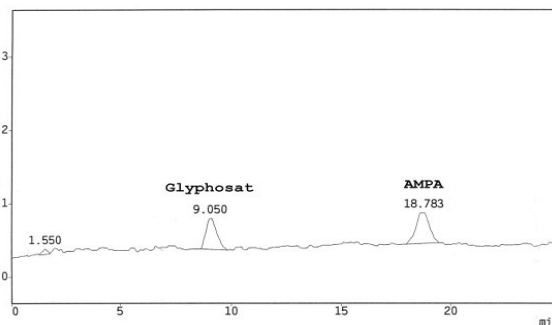
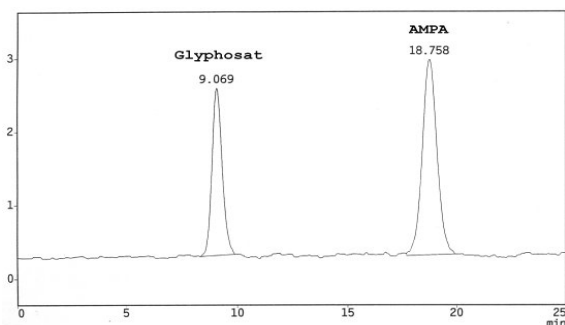
The peak areas of the glyphosate and AMPA derivatives are clearly dependent on the concentration of the oxidising agent. Too much of the oxidant destroys the AMPA, whereas too little oxidises the glyphosate insufficiently. The figure below shows that with a hypochlorite concentration of approx. 100 µl of 5% hypochlorite solution/l diluent, both compounds can be determined with reasonable sensitivity. Depending on which fluorescence detector is used, the absolute detection limit of the derivatives is between 250 and 1500 pg. A time consuming and, therefore, expensive double determination of each sample with and without oxidising agents is not necessary.



Chromatograms

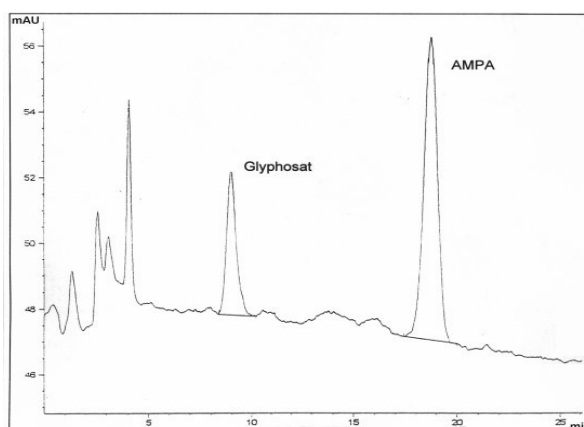
Chromatograms of a Glyphosate/AMPA Standard

Concentration (abs.) per compound: 2.5 ng (left) and 0.25 ng (right)



Chromatogram of surface water spiked with a glyphosate/AMPA standard after cleanup according to the DFG-Method (Deutsche Forschungsgesellschaft)

Concentration (abs.): 2.5 ng each



HPLC Conditions and Derivatization Parameters

HPLC Conditions	
Operating Mode	binary gradient
Eluent	K200-eluent (potassium dihydrogenphosphate buffer) and RG019-regenerant (potassium hydroxide solution)
Degassing	Helium- or vacuum degassed
HPLC Column	Special "Glyphosate" ion-exchange column
Column Oven	55 °C
Flow Rate	0.4 mL/min
Inject Volume	Up to 100 µL
Post-column Derivatization	
Pinnacle PCX	Dual-pump
Reactor volume	0.5 mL
Reactor temperature	36 °C
Reagent 1	GA116-diluent (potassium dihydrogenphosphate buffer) with ca. 100 µL of 5%, aqueous sodium hypochlorite solution/L.
Reagent 2	Solution of o-phthalaldehyde (OPA) and Thiofluor [®] (2-mercapto-ethanol derivative) in GA104-diluent
Reagent Flow	0.3 mL/min
Detection	
Detection Mode	Fluorescence detection
Excitation Wavelength	330 nm
Emission Wavelength	465 nm
Flowcell	Analytic; pressure stable up to 7 bar

APPLICATION NOTE

Caution! Extreme pH-range! As a consequence of the alkaline regenerant (pH 13), components made from Vespel may not be present in the HPLC system, instead these must be exchanged for components made from pH-inert materials (Tefzel or PEEK). For advice, please contact your LC-representative or consult your technical manual.

An inert version (Titanium, PEEK) of the HPLC system is not necessary, a piston seal wash for the pump heads, however, is recommended.

To avoid corrosion of the system and contamination of the ion-exchange column with metal ions, passivating is recommended; this is especially valid for older systems. For advice, please contact your LC-representative or consult your technical manual.

Gradient Program

Flow rate: 0.4 mL/min; Column temperature: 55°C

Step	Time [min]	Interval [min]	K200 [%]	RG019 [%]	
Equil.			100	0	Equilibration
0	0	0	100	0	Injection up to 50 µl
1	0 - 15	3	100	0	Isocratic
2	15 - 17	2	0	100	Step
3	17 - 25	8	100	0	Re-Equilibration

Contamination of the Column

Only few nanomoles of heavy-metal ions on the "Glyphosate" ion-exchange column cause drastic peak broadening and, consequently, loss of sensitivity. To avoid contamination with heavy-metal ions, passivating of older HPLC systems is recommended (see above). However, should heavy-metal ions contaminate the column; it can be removed effortlessly with the RESTORE® reagent.

APPLICATION NOTE

Chemicals and Columns

Post-column Derivatization Unit

Catalog No.	Description
1153-1052	PINNACLE PCX; dual-pump; 500 µL reactor

Glyphosate Kit

Catalog No.	Description
0352-0010	Application Kit Glyphosate/AMPA, consists of:
1954150	Glyphosate column, cation-exchange, K ⁺ -form, 4 x 150 mm
1953020	Glyphosate guard column, cation-exchange, K ⁺ -form, 3 x 20 mm
O120	o-Phthalaldehyde (OPA), "Chromatographic Grade™", 5 g
GA104	OPA Diluent, "Chromatographic Grade™", 4 x 950 mL
3700-2000	Thiofluor [®] , "Chromatographic Grade™", 10 g
GA116	Hypochlorite diluent, 4 x 950 mL
K200	Glyphosate eluent, 5 mM KH ₂ PO ₄ , pH 2.0, 4 x 950 mL
RG019	Column regenerant, 950 mL
1700-0140	RESTORE [®] for metal ion decontamination of column or guard
1700-0080	Glyphosate/AMPA test mixture, 1.5 mL, 2.5 µg/mL

Analytical Column for the Glyphosate Analysis



- 1) Chromatographically-certified for Glyphosate analysis
- 2) Isocratic, 20-minute method
- 3) High resolution and precision
- 4) Retention times not affected by sample matrix
- 5) Non-metallic column frits

The Pickering glyphosate column is designed and tested only for glyphosate analysis, using the method described above. In comparison to other columns it provides shorter run time (20-minutes), the peaks bases are narrower and reproducibility is guaranteed, run to run and column to column. Each of Pickering's glyphosate columns is individually tested on a standardized HPLC and post-column analysis system prior to shipment.

The required guard column is also packed with an ion-exchange resin and tested. Even with the most carefully crafted sample preparation methods, it is possible to contaminate the analytical column with metal ions. With the 1953020 guard in place, normally the guard column, only, will become contaminated. The application of Pickering's RESTORE[®] removes metal ions in a few minutes.

Catalog No.	Description
1954150	Glyphosate column, cation-exchange, K ⁺ -form, 4 x 150 mm
1953020	Glyphosate guard column, cation-exchange, K ⁺ -form, 3 x 20 mm

o-Phthalaldehyde Reagent

Primary amines such as AMPA or glycine (oxidation product of glyphosate) form highly fluorescent compounds when reacted with o-Phthalaldehyde (OPA) and a mercaptan under basic conditions. At a pH >9 and ambient temperature, reaction is generally complete within 1 - 30 seconds. The products of this reaction, 1-alkyl-2-alkylthio-substituted isoindoles, exhibit optimal excitation at 330 nm and maximal emission at 465 nm.

For an oxygen-sensitive reagent like OPA to remain stable for days instead of hours, it is important to start with the purest and most stable ingredients available, and to store and use the reagent under anaerobic conditions.

Using the chemicals described below, a long-lasting (up to ten days) OPA reagent for post-column derivatization of primary amines can be prepared. Each chemical is accompanied by clear instructions for formulating the reagent in your laboratory within minutes.

APPLICATION NOTE

Catalog No.	Description
O120	o-Phthalaldehyde (OPA), "Chromatographic Grade™", 5 g

o-Phthalaldehyde Diluent

For the glyphosate analysis Pickering offers a sodium borate buffer with pH 10.4 capable to buffer acidic eluents (pH 2).

Catalog No.	Description
OD104	OPA diluent for amino acid analysis, "Chromatographic Grade™", 4 x 950 mL

Thiofluor®

Pickering's Thiofluor®, a solid, nearly odorless nucleophile, is a superior substitute for 2-Mercaptoethanol in the preparation of OPA reagents. It forms a more stable and longer-lasting fluorophore with OPA than does 2-Mercaptoethanol, while possessing the same fluorescence properties.

Unlike the volatile 2-Mercaptoethanol, Thiofluor® will not migrate through the gas manifold and regulator of the OPA reagent pressurization system.

Catalog No.	Description
3700-2000	Thiofluor®, "Chromatographic Grade™", 10 g

Hypochlorite Diluent

- 1) Forms with sodium hypochlorite the Glyphosate oxidizing reagent
- 2) Guaranteed free of fluorescing impurities
- 3) Consistent quality, bottle to bottle, lot to lot

This diluent is used to prepare the oxidizing reagent required to convert glyphosate to a primary amine, prior to reacting it with OPA. The reagent is prepared by adding a solution of sodium hypochlorite to the diluent.

The prepared reagent should remain usable for several days, but daily calibration is recommended. Although the hypochlorite concentration will slowly decrease with time, this can be monitored as a change in the relative peak areas of glyphosate and AMPA.

APPLICATION NOTE

CAUTION: Do not use calcium hypochlorite in the oxidizing reagent, even though it is in the USEPA Method 547 recipe. It can be justified stoichiometrically, but not chemically, since it will react with the phosphate buffer to form a precipitate which will block the reactor or the flowcell.

Catalog No.	Description
GA116	Hypochlorite diluent, 4 x 950 mL

Potassium Phosphate Buffer

- 1) Eluent buffer for isocratic elution of glyphosate and AMPA
- 2) Guaranteed free of fluorescing impurities
- 3) Filtered, ready to use
- 4) Consistent quality, bottle to bottle, lot to lot

For the isocratic elution of glyphosate on an ion-exchange column according to Pickering's method K200 buffer is recommended. K200 is also used to equilibrate the glyphosate column after regeneration. One 950 mL bottle of K200 is sufficient for over 100 analyses.

Catalog No.	Description
K200	Glyphosate eluent, KH_2PO_4 buffer, pH 2.0, 4 x 950 mL

Column Regenerant for the Glyphosate Analysis

- 1) Strips strongly-bound sample residues from glyphosate column
- 2) Guaranteed free of fluorescing impurities
- 3) Filtered, ready to use
- 4) Consistent quality, bottle to bottle, lot to lot

Pickering always recommends regeneration of an ion-exchange column, and has included this step as part of Method. Considering the wide range of sample matrices which are likely to contact the cation-exchange column in glyphosate analyses, it will be prudent to ensure that any compounds which bind more strongly to the resin will be eluted before the next run.

RG019 regenerant is a ready-to-use KOH solution prepared under amine-free conditions. By following the Pickering procedure and regenerating after each run, the user can be confident that baseline anomalies or interfering peaks are related only to the current sample, and are not remnants of prior samples.

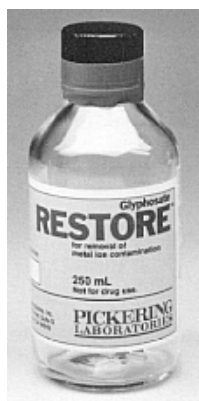
The small volumes of regenerant required for each analysis ensure that one 950 mL bottle of RG019 will last for a long time.

Catalog No.	Description
RG019	Column regenerant, 950 mL

Glyphosate RESTORE®

- 1) Removes metal ion contamination from ion-exchange guard or column
- 2) Treatment restores performance within 1 hour
- 3) Filtered, ready to use

Glyphosate herbicide analysis by post-column HPLC according to USEPA Method 547 employs a cation-exchange column. Many polyvalent metal ions which may be present in the sample, especially iron, will accumulate in the guard or analytical column. As little as 100 nmole of ferric ion, for example, will cause serious degradation of the column performance.



Although larger amounts can actually cause the glyphosate peak to vanish completely, the iron should be removed at the first signs of peak symmetry degradation. Under these circumstances all of the iron will still be in the guard column.

Note: The use of a guard column containing the same cation resin as the column is strongly recommended, as the guard will normally trap all of the metal ions, and will be the only column requiring the use of RESTORE®. Tests in our laboratory have demonstrated that the guard can accumulate sufficient iron to completely adsorb glyphosate without bleeding Fe-(III) into the analytical column.

The traditional metal removal process using EDTA requires a long treatment time, an even longer re-equilibration period, and is still not 100 % effective.

Glyphosate RESTORE® rapidly removes iron, nickel, aluminum, and chromium from the column and guard. The column and guard are ready to use after only a very brief re-equilibration with the K200 (potassium phosphate) eluent.

Glyphosate RESTORE® is strongly acidic and can displace tenaciously-bound Fe-(III) from the ion-exchange medium. In addition, it preserves the balance of K⁺ and H⁺ in the resin, thus avoiding a lengthy re-equilibration.

Four Easy Steps to use RESTORE®

- 1) Remove the analytical column.
- 2) Reverse the guard column and pump RESTORE® through the guard at 0.4 mL/min. for a minimum of 15 minutes, sending the effluent to waste.
- 3) Pump Pickering K200 eluent through the guard column long enough to displace RESTORE®.
- 4) Reconnect the columns in the normal directions and restart the HPLC and post-column systems.

APPLICATION NOTE

If treatment of the guard does not improve chromatographic performance using a glyphosate test mixture, the analytical column might also be contaminated. Remove the guard and reverse the analytical column. Pump RESTORE[®] through the column for a minimum of 45 minutes, directing the effluent to waste.

Catalog No.	Description
1700-0140	RESTORE [®] for metal ion decontamination of column or guard

Glyphosate Test Mixture

This test mixture is a 1.5 mL water solution of glyphosate and AMPA (aminomethyl-phosphonic acid) at a concentration of 2.5 µg/mL. It is a qualitative standard intended to be used to establish an elution profile and for troubleshooting. For precise quantitation, we recommend purchasing these compounds in pure form from a firm specializing in analytical standards for pesticide analysis.

Catalog No.	Description
1700-0080	Glyphosate/AMPA test mixture, 1.5 mL, 2.5 µg/mL

Literature

- 1) M. E. Oppenhuize, J. E. Cowell., *J. Assoc. Off. Anal. Chem.* **1991**, 74, 317 – 323
(**water samples**)
- 2) DFG-Methode 405 (**water-, soil- and food samples**)
- 3) R. Reupert, S. Fuchs, *GIT Laborfachzeitschrift* **1997**, 5, 468 – 474 (**water samples**)
- 4) DIN-Method 38407-22