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Analysis of Glyphosate Herbicides Using the Hitachi LaChrom Elite® Liquid Chromatography System and Sensivate Elite™ Post Column Reactor

Hitachi High Technologies America, Inc.

Control of broad-leaf weeds in cash crops has been a goal of agricultural interests since the inception of farming. Glyphosate herbicide was discovered in the 1970's by Monsanto for the eradication of competitive weeds in farm areas. This compound inhibits ESPS synthase enzymes in plants, blocking the production of essential aromatic amino acids by photosynthesis. Genetically modified cotton and soybeans have been developed that allow for broad application of these herbicides on these crops. Due to the wide use of glyphosate and its high water solubility, the EPA monitors its levels in ground and drinking water, and observing the levels of glyphosate in water run-off and water supplies is a major concern of environmental agencies in both agricultural and urban areas.

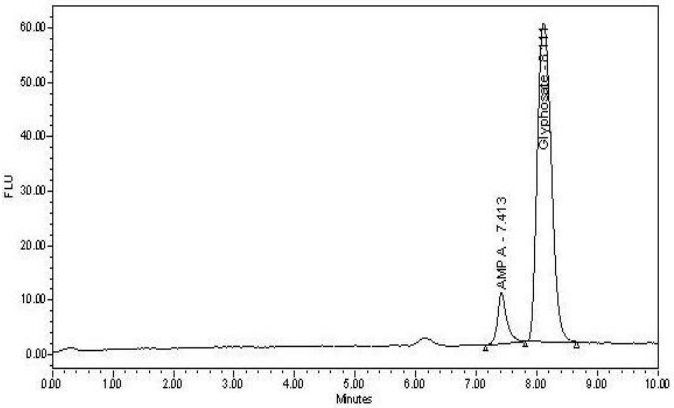
EPA method 547[1] describes the analysis of glyphosate (GLY) and its metabolite, (amino methyl) phosphonic acid (AMPA), via chemical derivatization followed by low level analysis with fluorescence detection. Derivatizing these compounds through exposure to an oxidizing reagent followed by reaction with o-phthaldehyde and thiofluor greatly enhances the sensitivity of this analysis as previously reported in another method for glyphosate analysis[2]. Using a SAX silica ion-exchange column the LaChrom Elite liquid chromatography system coupled with the Sensivate Elite post-column reactor achieves reproducible results. The post-column derivatization uses standard reagents described in EPA method 547.

Experimental Conditions

Module	Conditions and Other
Pump (L-2100)	5mM sodium phosphate monobasic pH 1.9
Autosampler (L-2200)	1 µL inj volume, of 10µg/mL GLY/AMPA mix, 10°C
Column Oven (L-2300)	40°C
FLU Detector (L-2485)	Excitation: 330 nm; Emission: 440 nm
Column	Supelcosil™ Silica SAX-1 250mm X 4.6mm 5µm
Standards	Chem Service GLY/AMPA F1104RPS

* Supelcosil is a trademark of Sigma-Aldrich Co. LaChrom Elite is a registered trademark of Hitachi High Technologies Corporation. All other trademarks are property of their respective owners.

Results



	Peak Name	RT	Area (µV*sec)	Height	K Prime	USP Tailing	USP Resolution	USP Plate Count	Rel. Resol.
1	AMPA	7.41	101083	9406	1.97	1.3		11440	
2	Glyphosate	8.11	938132	58548	2.24	1.2	2.00	5920	2.1

Glyphosate (n=5)

Conc. (ug/mL)	Average FLU	Standard Dev	%RSD
10	742623	6367	0.9
8.5	700206	13872	2.0
7	668249	7686	1.2
6	635872	12398	1.9
4.5	581214	8007	1.4

Discussion

Hitachi's LaChrom Elite Liquid Chromatography System, equipped with a Supelcosil SAX-1 250X4.6mm 5µm particle size ion exchange column and Sensivate Elite post-column reactor, produces reproducible results. The Sensivate Elite automates the derivatization of glyphosate to a fluorescent compound. Together, the LaChrom Elite and Sensivate Elite systems increase the overall throughput of samples, while decreasing the error that might occur with non-automated post-column reaction systems. System suitability and validation of this method have been completed with in-house buffers and reagents.

References:
1- USEPA Official Methods 5 and 547 and 40 CFR 136, United States Environmental Protection Agency, 1990.
2 – Pickering Laboratories, Inc. Application Manual on Glyphosate Analysis, ver. 2.0, July 2002.

Hitachi High Technologies America, Inc.
Life Sciences Division
5100 Franklin Drive
Pleasanton, CA 94588
Toll Free: (800) 548-9001
E-mail: sales-LS@hitachi-hta.com
Web site: www.hitachi-hta.com/ultra