AFFINISEP

AFFINIMIP® SPE

Glyphosate

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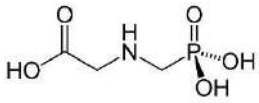
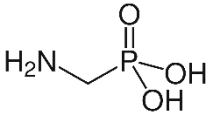
This booklet describes different applications of **AFFINIMIP® SPE Glyphosate** for the analysis of **Glyphosate, AMPA and Glufosinate**. The determination of very low concentrations of these molecules in various waters as well as in very complex matrices is shown.

In addition, as these molecules can be found in surface and underground water, an application with **AFFINIMIP® POCIS Glyphosate** shows the uptake of these molecules as a POCIS passive sampler.

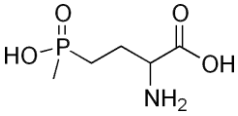
For the analysis of these compounds, several analytical methods have been used such as LC-MS/MS even without derivatization as well as Capillary Electrophoresis –UV.



Glyphosate



AMPA



Glufosinate

Do you know?

Glyphosate and Glufosinate are closely related herbicides referred to as phospho-herbicides. Glyphosate undergoes rapid microbial degradation in plants, soil and water to the metabolite aminomethylphosphonic acid (AMPA). Codex alimentarius has defined a MRL (maximum residue limit) for Glyphosate of 0.05mg/Kg in meat or milk and 30mg/Kg in cereals and for Glufosinate, 2mg/kg of soybean.

- ✓ Efficient for Glyphosate, AMPA, Glufosinate et others metabolites
- ✓ Ready to use kit
- ✓ Tested with LC-MS/MS, Capillary Electrophoresis - UV
- ✓ Do not require derivatization of these molecules
- ✓ Tested on large volume of water, tea, cereals, honey...
- ✓ Simple & Fast process
- ✓ High capacity cartridges

SPE cartridges

+

DETAILED protocole of use

→

A READY TO USE AFFINIMIP® SPE Glyphosate KIT

+

Certificate of analysis

PROTOCOL OF PURIFICATION

Sample preparation

Mix 3g of crushed cereals + 25mL water with 1% formic acid. Sonicate 30 min, centrifuge 10 min. The supernatant is put to pH = 7 with ammonia solution and filtered to form the loading solution.

Purification with a 6mL AFFINIMIP® SPE Glyphosate cartridge

Equilibration
9mL pure Water

Loading
9mL loading solution

Washing of interferences
24mL Water

Elution (E)
8 mL water with HCl 0.1M

Elutions are evaporated under vacuum at 60°C for 2 hours and dissolved in 3 mL of mobile phase containing EDTA-Na2 0,8mM.

HPLC Method with LC – MS/MS

Analysis by HPLC – MS/MS (QTRAP 4000)
Column: Acclaim trinity Q1 100mm x 3mm ID (3µm)
Mobile phase: gradient with Ammonium formiate 50 mM pH = 2.9 (A) – Acetonitrile (B)

Time (min)	% A	% B
0	100	0
3	100	0
3,2	0	100
6,0	0	100
6,2	100	0
10,2	100	0

Flow rate: 0,5mL/min
Injection volume: 20µL.

RESULTS

Recovery of Glyphosate, AMPA and Glufosinate after **AFFINIMIP® SPE Glyphosate** clean-up of Cereals spiked at 92µg/Kg each. No derivatization was performed.

	Glyphosate	AMPA	Glufosinate
Yield (%)	101 %	98 %	93 %
RSD (n=3)	3 %	2 %	3 %

Catalog number:

6mL format with enhanced performances
FS113-15-02B for 25 cartridges
FS113-15-03B for 50 cartridges

12mL format
FS113-02C for 25 cartridges
FS113-03C for 50 cartridges

PROTOCOL OF PURIFICATION

Sample preparation

A mixture of 5mL of apple juice + 15mL water + 200µL Formic acid is put to pH = 7 with ammonia solution to form the loading solution.

Purification with a 6mL AFFINIMIP® SPE Glyphosate cartridge

Equilibration
6mL pure Water

Loading
6mL loading solution

Washing of interferences
12mL Water

Elution (E)
8 mL water with HCl 0.1M

Elutions are evaporated under vacuum at 60°C for 2 hours and dissolved in 1 mL of mobile phase containing EDTA-Na2 0,8mM.

RESULTS

Recovery of Glyphosate, AMPA and Glufosinate after AFFINIMIP® SPE Glyphosate clean-up of Apple juice spiked at 67µg/Kg each. No derivatization was performed.

	Glyphosate	AMPA	Glufosinate
Yield (%)	96 %	86 %	92 %

HPLC Method with LC – MS/MS

Analysis by HPLC – MS/MS (QTRAP 4000)
Column: Acclaim trinity Q1 100mm x 3mm ID (3µm)
Mobile phase: gradient with Ammonium formiate 50 mM pH = 2.9 (A) – Acetonitrile (B)

Time (min)	% A	% B
0	100	0
3	100	0
3,2	0	100
6,0	0	100
6,2	100	0
10,2	100	0

Flow rate: 0,5mL/min
Injection volume: 20µL.

Catalog number:

6mL format
FS113-02B for 25 cartridges
FS113-03B for 50 cartridges

12mL format
FS113-02C for 25 cartridges
FS113-03C for 50 cartridges

PROTOCOL OF PURIFICATION

Sample preparation

Mix 3g of dry tea +50mL water with 1% formic acid. Sonicate 30 min, centrifuge 10 min. The supernatant is filtered and put to pH = 7 with ammonia solution to form the loading solution.

Purification with a 6mL AFFINIMIP® SPE Glyphosate cartridge

Equilibration
6mL pure Water

Loading
3mL loading solution

Washing of interferences
12mL Water

Elution (E)
8mL water with HCl 0.1M

Elutions are evaporated under vacuum at 60°C for 2 hours and dissolved in 3 mL of mobile phase containing EDTA-Na2 0,8mM.

HPLC Method with LC – MS/MS

Analysis by HPLC – MS/MS (QTRAP 4000)
Column: Acclaim trinity Q1 100mm x 3mm ID (3µm)
Mobile phase: gradient with Ammonium formiate 50 mM pH = 2.9 (A) – Acetonitrile (B)

Time (min)	% A	% B
0	100	0
3	100	0
3,2	0	100
6,0	0	100
6,2	100	0
10,2	100	0

Flow rate: 0,5mL/min
Injection volume: 20µL.

RESULTS

Recovery of Glyphosate, AMPA and Glufosinate after AFFINIMIP® SPE Glyphosate clean-up of flavoured Black Tea spiked at 1,67mg/kg each. No derivatization was performed.

	Glyphosate	AMPA	Glufosinate
Yield (%)	103 %	81 %	100 %
RSD (n=3)	10 %	12 %	6 %
Matrix effect	-3 %	23 %	6%

Catalog number:

6mL format
FS113-02B for 25 cartridges
FS113-03B for 50 cartridges

12mL format
FS113-02C for 25 cartridges
FS113-03C for 50 cartridges

PROTOCOL OF PURIFICATION

Sample preparation

10 grams of honey are mixed with 30 mL of ultrapure water - 1% formic acid and magnetically stirred during 30 minutes. The mixture is then filtered through 0.45µm filter and neutralized to pH 7 with ammonia solution to form the loading solution.

Purification with a 6mL AFFINIMIP® SPE Glyphosate cartridge

Equilibration
6mL pure Water

Loading
3mL loading solution

Washing of interferences
12mL Water

Elution (E)
8mL water with HCl 0.1M

Elutions are evaporated under vacuum at 60°C for 2 hours and dissolved in 3 mL of mobile phase containing EDTA-Na2 0,8mM.

HPLC Method with LC – MS/MS

Analysis by HPLC – MS/MS (QTRAP 4000)
Column: Acclaim trinity Q1 100mm x 3mm ID (3µm)
Mobile phase: gradient with Ammonium formiate 50 mM pH = 2.9 (A) – Acetonitrile (B)

Time (min)	% A	% B
0	100	0
3	100	0
3,2	0	100
6,0	0	100
6,2	100	0
10,2	100	0

Flow rate: 0,5mL/min
Injection volume: 20µL.

RESULTS

Recovery of Glyphosate, AMPA and Glufosinate after AFFINIMIP® SPE Glyphosate clean-up of Honey spiked at 400 µg/kg each. No derivatization was performed.

	Glyphosate	AMPA	Glufosinate
Yield (%)	104 %	89 %	100 %
RSD (n=3)	4 %	8 %	8 %
Matrix effect	-16 %	-36 %	-19 %

Catalog number:

6mL format
FS113-02B for 25 cartridges
FS113-03B for 50 cartridges

12mL format
FS113-02C for 25 cartridges
FS113-03C for 50 cartridges

PROTOCOL OF PURIFICATION

Sample preparation

3g Dried and crushed *Datisca Cannabina* cannabis like plant mixed with 60 mL water - 1% Formic acid were sonicated for 30 mn and centrifuged at 4000 RPM for 10 mn. After filtration, the solution is neutralized at pH = 7 with ammonia solution. The loading solution is obtained after a 0.45µm filtration.

Purification with a 6mL AFFINIMIP® SPE Glyphosate cartridge

Equilibration
9mL pure Water

Loading
6mL loading solution

Washing of interferences
24mL Water

Elution (E)
8 mL water with HCl 0.1M

Elutions are evaporated under vacuum at 60°C for 2 hours and dissolved in 3 mL of mobile phase containing EDTA-Na2 0,8mM.

Analytical conditions according to p 14

RESULTS

Recovery of Glyphosate, AMPA and Glufosinate after AFFINIMIP® SPE Glyphosate clean-up of dryed plant spiked at 333µg/Kg each. No derivatization was performed.

	Glyphosate	AMPA	Glufosinate
Yield (%)	70 %	72 %	81%
RSD (n=3)	5 %	7 %	6 %

Catalog number:

AFFINIMIP® SPE Glyphosate
6mL format with enhanced performances
FS113-15-02B for 25 cartridges
FS113-15-03B for 50 cartridges

12mL format
FS113-02C for 25 cartridges
FS113-03C for 50 cartridges

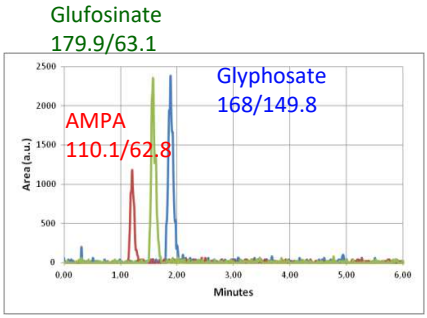
PROTOCOL OF PURIFICATION

Purification with a 6mL AFFINIMIP® SPE Glyphosate cartridge

Equilibration
6mL pure Water
Loading
100mL spiked water
Washing of interferences
6mL Water
Elution (E)
8mL water with HCl 0.1M

Elutions are evaporated under vacuum at 60°C for 2 hours and dissolved in 3 mL of mobile phase containing EDTA-Na_2 0,8mM.

RESULTS



Chromatograms obtained after a clean-up with AFFINIMIP®SPE Glyphosate for 100mL water spiked at 3µg/L of each molecule

Recovery of Glyphosate, AMPA and Glufosinate after AFFINIMIP® SPE Glyphosate clean-up of water spiked at 3µg/L each. No derivatization was performed.

	Glyphosate	AMPA	Glufosinate
Yield (%)	91 %	70 %	80 %
RSD (n=3)	2 %	1 %	6 %

HPLC Method with LC – MS/MS
Analysis by HPLC – MS/MS (QTRAP 4000)
Column: Acclaim trinity Q1 100mm x 3mm ID (3µm)
Mobile phase: gradient with Ammonium formiate 50 mM pH = 2.9 (A) – Acetonitrile (B)

Time (min)	% A	% B
0	100	0
3	100	0
3,2	0	100
6,0	0	100
6,2	100	0
10,2	100	0

Flow rate: 0,5mL/min
Injection volume: 20µL.

Catalog number:
6mL format with enhanced performances
FS113-15-02B for 25 cartridges
FS113-15-03B for 50 cartridges
12mL format
FS113-02C for 25 cartridges
FS113-03C for 50 cartridges

Efficient clean-up and enrichment

PROTOCOL OF PURIFICATION

Sample preparation

Purification with a 6mL AFFINIMIP® SPE Glyphosate cartridge

Equilibration
6mL pure Water

Loading
3 to 500 mL

Washing of interferences
3mL Water

Elution (E)
4-8 mL water with HCl 0.1M

RESULTS

Recovery yields of glyphosate and AMPA after **AFFINIMIP® SPE Glyphosate** clean-up of mineral water spiked at 25µg/mL.
Loading volume 3mL
Analysis done by CE without derivatization

Analytes	Recoveries %
Glyphosate	85
AMPA	87

CE analysis (no derivatization)
Column: fused-silica capillary of 60.2 cm (effective length, 50 cm) x 50 µm ID at 25°C
Mobile phase: 7.5 mM phthalic acid - 51.3 mM histidine running buffer (pH 6.5, ionic strength of 21.8 mM, buffer capacity 25 mM L^{-1} pH^{-1}) containing 1 mM CTAB
Voltage : +25kV
Detection UV-DAD (240nm)

Publication:
Preliminary recovery study of a commercial molecularly imprinted polymer for the extraction of glyphosate and AMPA in different environmental waters using MS, B. Claude, C. Berho, S. Bayoudh, L. Amalric, E. Coisy, R. Nehmé, P. Morin, *Environ Sci Pollut Res*, 24: 12293 (2017).

Catalog number:
3mL format
FS113-02.IP for 25 cartridges
FS113-03.IP for 50 cartridges
6mL format
FS113-02B for 25 cartridges
FS113-03B for 50 cartridges

Performance not affected by physico chemical properties of Water Loading with up to 1L

Physico chemical properties of tested waters

Salt concentrations (mg/L) and pH of analyzed solutions

	Ca	Na	Mg	K	HCO3	Cl	NO3	SO4	Fe	pH
Groundwater	15,7	11,3	4,9	1,3	76	9,7	<0,5	1,2	7,5	7,1
Groundwater	22,3	105,7	17	4,7	136	159	8,9	15,8	0,17	6,4
Groundwater	104,1	13,9	6,9	1,8	203	28,1	113,7	33		7,1
Geothermal water	799	5163,5	189,5	71,9		9759,7		702,2	3,2	
Mineral water	80	6,5	24	1	360	3,8	3,7	12,6		7,2

AFFINIMIP® SPE Glyphosate performance for tested waters

Above five waters spiked at various concentrations with AMPA and Glyphosate

Sample	Concentration range	Average Recoveries %
Glyphosate	100 to 750ng/L	>70%
AMPA	100 to 750ng/L	>75%

Method UPLC – MS/MS

Column: UPLC HSS T3 (2.1mm x 100mm, 1,8µm)

Mobile phase: A: Water/Ammonium Acetate 5mM

B: Acetonitrile

Time (min)	A %	B %
0	90	10
2	90	10
7	50	50
7.5	0	100
11	0	100

Flow rate: 0.2mL/min

MS detection: m/z 321 (ESI^-)

Injection volume: 20µL.

Same protocol than previous page

Publication:

B. Claude, C. Berho, S. Bayoudh, L. Amalric, E. Coisy, R. Nehmé, P. Morin, *Environ Sci Pollut Res*, 24: 12293 (2017).

Catalog number:

3mL format

FS113-02.IP for 25 cartridges

FS113-03.IP for 50 cartridges

6mL format

FS113-02B for 25 cartridges

FS113-03B for 50 cartridges

MONITORING OF GLYPHOSATE - AMPA WITH A PASSIVE SAMPLER AFFINIMIP® POCIS Glyphosate

AFFINIMIP® POCIS Glyphosate

Passive Sampling with POCIS

Polar Organic Chemical Integrative Sampler (POCIS) is a passive sampler designed to provide the time weighted average (TWA) concentration of chemicals during a sampling period of several weeks.

AFFINIMIP®POCIS Glyphosate enables the sampling of Glyphosate and AMPA in water (Groundwater, geothermal, mineral…).

Then the powder is collected in an empty SPE column for the extraction of Glyphosate and AMPA

PROTOCOL OF EXTRACTION

Extraction of collected Glyphosate and AMPA from AFFINIMIP® POCIS Glyphosate with a SPE

Extraction of the analytes (E)

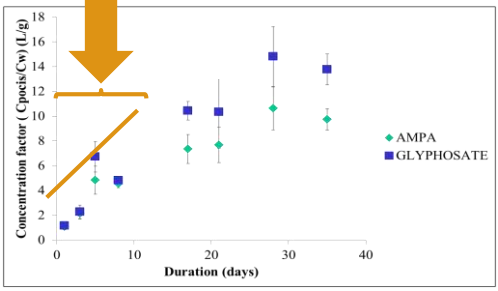
HCl solution (100mM)

The extraction solution is then evaporated and reconstituted with water prior analysis

RESULTS

Laboratory sampling rates estimation for AMPA and glyphosate using the AFFINIMIP® POCIS Glyphosate

Sampling rates: 130mL/day/200mg AFFINIMIP® POCIS Glyphosate in agreement with other pesticides in classical POCIS.



Mineral water (pH = 7) fortified at 500ng/L of AMPA and glyphosate. Concentrations kept constant during whole experiment.

Pesticides concentration in the tank, temperature, TOC and conductivity monitored during the experimental period to verify the stability of physico-chemical conditions in water.

Publications:

Laboratory calibration of a POCIS-like sampler based on molecularly imprinted polymers for glyphosate and AMPA sampling in water, C. Berho, B. Claude, E. Coisy, A. Togola, S. Bayoudh, P. Morin, L. Amalric, *Anal Bioanal Chem* 409: 2029 (2017)

Catalog number:
POCIS-GLY.90.55.A.1 for 1 AFFINIMIP® POCIS Glyphosate
POCIS-GLY.90.55.A.10 for 10 AFFINIMIP® POCIS Glyphosate

Transitions for glyphosate - AMPA and Glufosinate used in water

Analyte	Q1	Q3	Time (ms)	DP (V)	EP (V)	CE (V)	CXP (V)
Glyphosate 1	168	149.8	100	-50	-10	-16	-9
Glyphosate 2	168	62.9	100	-50	-10	-32	-7
AMPA 1	110.1	62.8	100	-50	-10	-24	-9
AMPA 2	110.1	78.8	100	-50	-10	-34	-11
Glufosinate 1	179.9	63.1	100	-50	-10	-58	-9
Glufosinate 2	179.9	135.8	100	-50	-10	-24	-9

Table transitions for glyphosate - AMPA and Glufosinate used for complex matrices

Analyte	Q1	Q3	Time (ms)	DP (V)	EP (V)	CE (V)	CXP (V)
Glyphosate 1	168	62.9	100	-50	-10	-32	-7
Glyphosate 2	168	78.9	100	-50	-10	-50	-3
AMPA 1	110.1	62.8	100	-50	-10	-24	-9
AMPA 2	110.1	78.8	100	-50	-10	-34	-11
Glufosinate 1	179.9	63.1	100	-50	-10	-58	-9
Glufosinate 2	179.9	95.0	100	-50	-10	-24	-5

HPLC Method with LC – MS/MS

Analysis by HPLC – MS/MS (QTRAP 4000)
Column: Acclaim trinity Q1 100mm x 3mm ID (3µm)
Flow rate: 0,5mL/min
Injection volume: 20µL.

Mobile phase: gradient with Ammonium formiate 50 mM pH = 2.9 (A) – Acetonitrile (B)

Time (min)	% A	% B
0	100	0
3	100	0
3,2	0	100
6,0	0	100
6,2	100	0
10,2	100	0

AFFINIMIP® SPE– Product list

Designation	Definition	Reference	Nber of units
AFFINIMIP® SPE Glyphosate	3mL Selective SPE cartridges for glyphosate, AMPA & Glufosinate	FS113-02.IP	25
		FS113-03.IP	50
	6mL Selective SPE cartridges for glyphosate, AMPA & Glufosinate	FS113-02B	25
		FS113-03B	50
	6mL Selective SPE cartridges for glyphosate, AMPA & Glufosinate – Enhanced performances	FS113-15-02B	25
		FS113-15-03B	50
	12mL Selective SPE cartridges for glyphosate, AMPA & Glufosinate	FS113-02C	25
		FS113-03C	50
AFFINIMIP® POCIS Glyphosate	POCIS for the uptake of glyphosate, AMPA & Glufosinate	POCIS-GLY.90.55.A.1	1
		POCIS-GLY.90.55.A.10	10
		POCIS-GLY.90.55.A.50	50

SPE ACCESSORIES – Product list

SPE Accessories	Designation	Definition	Reference
Manifold	**SPE Vaccum Manifold**	12-port model	ACC-MAN1
SPE Adapter & Reservoir kit	**SPE Adapter & Reservoir kit**	Kit of 12 reservoirs 60ml and adapters for use with 1,3 & 6 mL cartridges	ACC-AR1
Mini-Vap	**Mini Evaporator/Concentrator**	6 port Mini-Vap Evaporator/Concentrator for use with 1 to 250mL containers	ACC-VAP1
Mini PUMP	**Mini vacuum pump**	Laboport diaphragm vacuum mini pump, 5.5L/min	ACC-PUMP
Vacuum pump trap	**SPE Vacuum pump trap kit**	1L trap kit	ACC-TRAP

About AFFINISEP

AFFINISEP is **a worldwide expert in sampling and sample preparation methods for** analytical applications in various fields such as water, biological fluids, food and feed analysis. AFFINISEP is leader in **design and development of intelligent polymers including** Molecularly Imprinted Polymers (MIP).

Products	Applications	Matrices	Technologies
• SPE, SLE • POCIS, SPATT	• Sample preparation • Passive sampling	• Water • Biological fluids • Food and feed • Soil	• Molecularly imprinted polymers (MIP) • Other modified polymers • Modified silica

We develop and manufacture a most comprehensive portfolio of solid phase extraction products, SLE, Filtration, 96 well plates and POCIS in a various sectors: food and feed safety and quality, pharmaceutical R&D and quality control, clinical diagnosis, environment and doping.

Furthermore, by exploiting our library of innovative polymers and our know-how in chromatography and solid phase extraction, we have a strong capacity to adapt these polymers to meet any specific requirements and to solve unsatisfied purification and extraction needs. Numerous documents related to our products (Application notebooks, publication references, posters, catalog for different applications…) can be found on our website www.affinisep.com.

ORDERING INFORMATION

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76650 Petit-Couronne, France

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