Automated determination of glyphosate, AMPA and glufosinate in food by online ligand exchange SPE-LC-MS/MS

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Introduction

In recent times the application of glyphosate is heavily discussed. Because of its widespread application the broad-spectrum herbicide glyphosate can be detected in many different food matrices. An automated method is needed which is also applicable to complex organic food matrices and therefore provides low limits of quantification for glyphosate, its metabolite AMPA and the structurally related compound glufosinate.

Worldwide discussions

- Worldwide discussions about possible cancerogenic and/or mutagenic effects
- March 2015: International Agency for Research on Cancer (IARC): probably carcinogenic to humans (Group 2A)
- 2016: European Food Safety Authority (EFSA) and German Federal Institute for risk assessment (BfR): no carcinogenic risk to humans if it is used in the proper manner for the intended purpose; 2016: opinion supported by the WHO/FAO Joint Meeting on Pesticide Residues (JMPR)
  - November 2017: extension of the licence of glyphosate in the EU for 5 more years
- Many (not always factual) reports in press
- Effective actions driven by NGOs (e. g. analysis of glyphosate in urine)
- Claims for MRL of 0,01 mg/kg in food

Analytical challenges

- log KOW = -3.3

→ Glyphosate approx. 2000-fold better soluble in water than in octanol; HPLC is preferred

- Chromatography on C18-columns is difficult because of high polarity

→ FMOC-acylation: Fluorenyl group increases hydrophobicity → better retention on C18 columns

- Matrix effects
- Applicability for complex matrices (e. g. cocoa, tea)
- Automatization for high-throughput feasible

Workflow

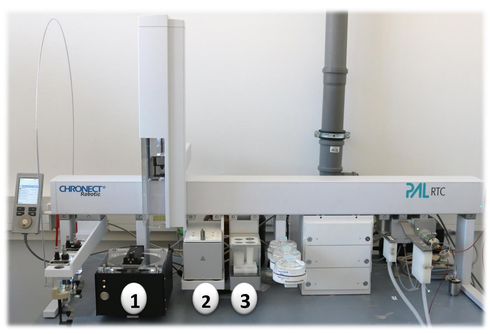
Manual extraction	**MeOH/water 1/1, addition of labeled internal standards**
	shaking for 1 min, centrifugation
Automated derivatization	**Addition of buffer solution and FMOC, 20 min reaction time at 50 °C, addition of formic acid, centrifugation**
Injection	**400 µL**
SPE	**CHRONECT µSPE with CHROSPHE glyphosate cartridges**
	(ligand exchange, zircon oxide based)
HPLC	**Column: YMC Triart C18 (water/acetonitrile, pH 9)**
MS/MS	**Quantification by isotope dilution**

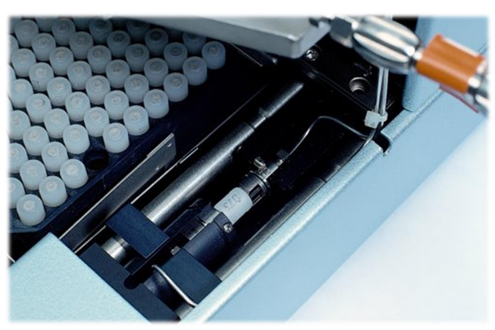
Processes are organized simultaneously by the CHRONOS software to enhance sample throughput.

Approximately 60 samples can be analyzed within 24 hours.

Equipment

- **RTC PAL with centrifuge (1), agitator (2) and vortexer (3)**
- **CHRONECT µSPE system**
- **SPE: CHROSPE glyphosate cartridges (ligand exchange, zircon oxide based)**
- **Agilent 1290 UHPLC system**
- **SCIEX 5500 TripleQuad**





Chromatograms of complex matrices at LOQ

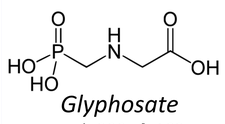
Spiking level: 3 µg/kg

Wheat

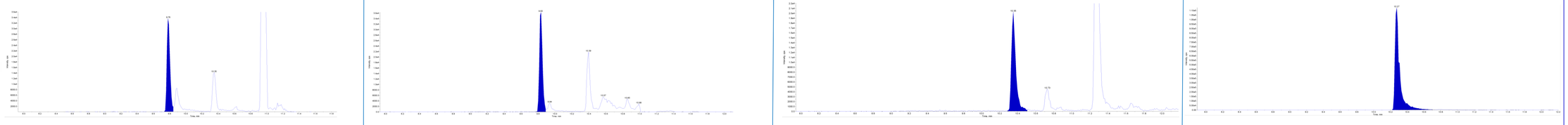
Tea

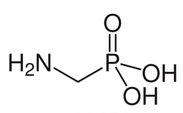
Sun flower seeds

Cocoa paste

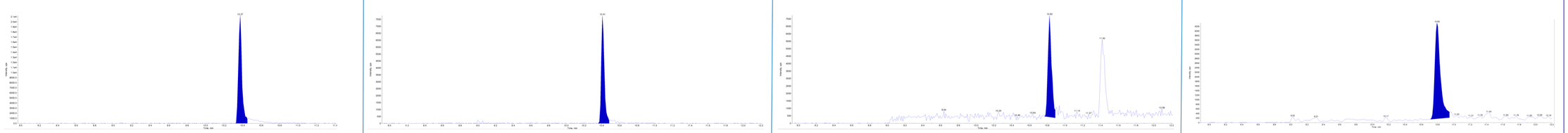


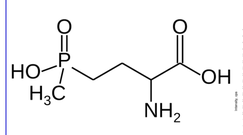
Glyphosate
m/z 390 → 168



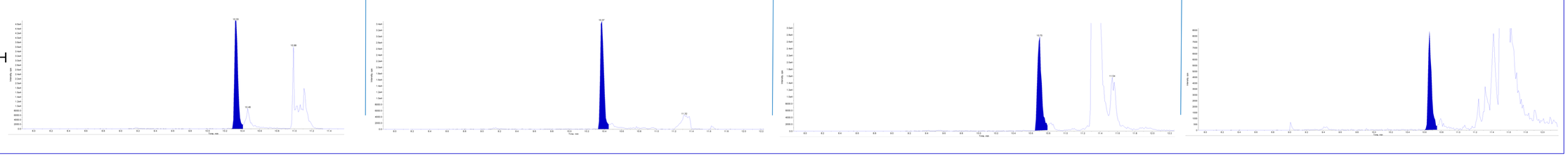


AMPA
m/z 332 → 110





Glufosinate
m/z 402 → 180



Basic Validation

	Glyphosate					AMPA					Glufosinate				
	Wheat	Tea	Sunflower seeds	Cocoa	Criterion according to SANTE/ 11813/2017	Wheat	Tea	Sunflower seeds	Cocoa	Criterion according to SANTE/ 11813/2017	Wheat	Tea	Sunflower seeds	Cocoa	Criterion according to SANTE/ 11813/2017
Signal to noise-ratio at LOQ	≥10:1	≥10:1	≥10:1	≥10:1	≥10:1	≥10:1	≥10:1	≥10:1	≥10:1	≥10:1	≥10:1	≥10:1	≥10:1	≥10:1	≥10:1
Repeatability at LOQ	4,7%	7,0%	9,1%	13%	≤20%	10%	17%	8,9%	18%	≤20%	3,6%	4,8%	5,8%	13%	≤20%
Trueness at LOQ	96%	96%	103%	%	70-120%	95%	86%	75%	%	70-120%	98%	96%	83%	83%	70-120%
Average repeatability at 10-100 µg/kg	5,2%	2,2%	5,1%	5,4%	≤20%	8,0%	3,1%	8,2%	2,2%	≤20%	3,0%	5,5%	4,7%	1,7%	≤20%
Average trueness at 10-100 µg/kg	104%	104%	101%	102%	70-120%	108%	98%	81%	94%	70-120%	101%	97%	97%	94%	70-120%

Validation data also meet the criteria for linearity, retention time and ion ratio according to SANTE/11813/2017.

Conclusions and outlook

The presented method allows a time-efficient sensitive detection and quantification of glyphosate, AMPA and glufosinate in complex food matrices. The manual effort is minimized by applying online SPE. The ligand exchange SPE material reduces matrix effects to a minimum. Signal to noise-ratios at 3 µg/kg are satisfactory, even for complex matrices.
The glufosinate metabolites MPP (3-METHYLPHOSPHINICO-PROPIONIC ACID) and NAG (N-ACETYL-GLUFOSINATE) are currently being added to the method by us.

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