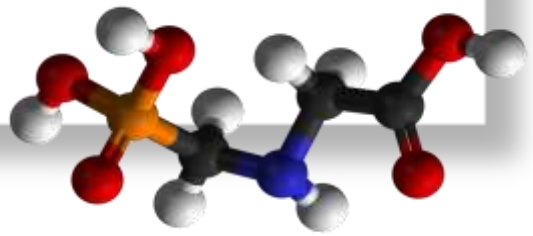
VALIDATION AND DETERMINATION OF GLYPHOSATE AND AMPA IN WATER



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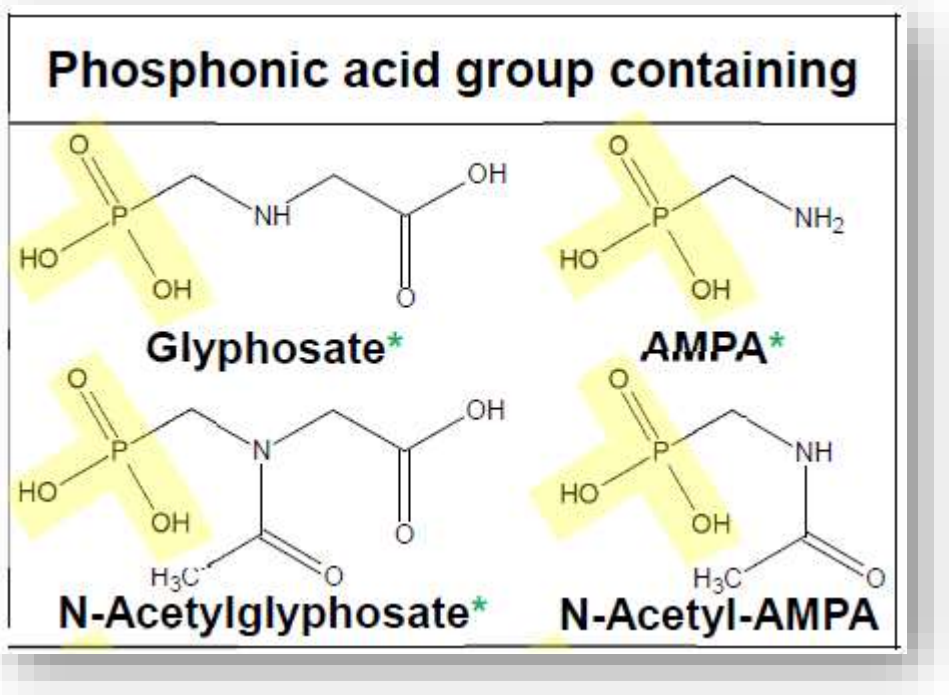
OVERVIEW

- Introduction
- Method validation
- Results of water sample analyses
- Conclusions

INTRODUCTION

- Glyphosate is a broad-spectrum herbicide and widely used in agriculture. The main degradation product is aminomethyl phosphonic acid AMPA which can also be formed from the breakdown of organic phosphonates in detergents.
- Regulation 2008/105/EG defines quality criteria for 20 priority substances and 13 priority hazardous substances.
- A further set of 13 substances including glyphosate and AMPA are candidates for future action.

INTRODUCTION



* Included in the actual Residue Definition or currently discussed to be included

INTRODUCTION

➢The determination of these two herbicides at the sub µg/L level is difficult due to their ionic character, low volatility, low mass and lack of chemical groups that could facilitate their detection.

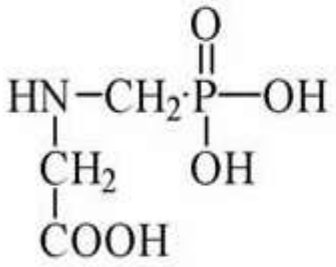
•Methods for the determination:

➢HPLC-FLD, IC-UV, CE-MS.

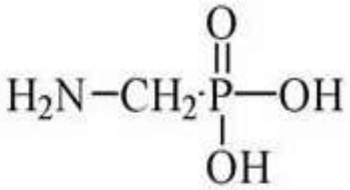
➢MS has been combined with ion chromatography (IC), inductively coupled plasma (ICP), capillary electrophoresis (CE), gas chromatography (GC) and liquid chromatography (LC) for glyphosate and AMPA residue determination.

SAMPLE PREPARATION

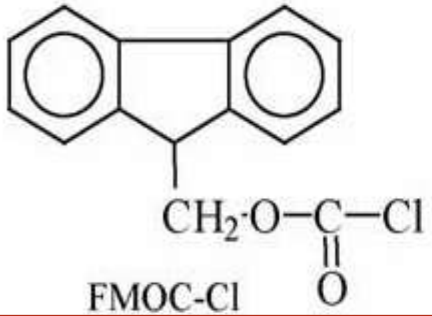
Our method: **(-ESI) LC- MS/MS** after derivatization with **FMOC**



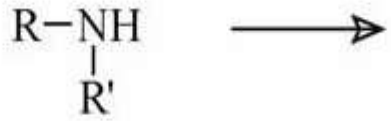
Glyphosate

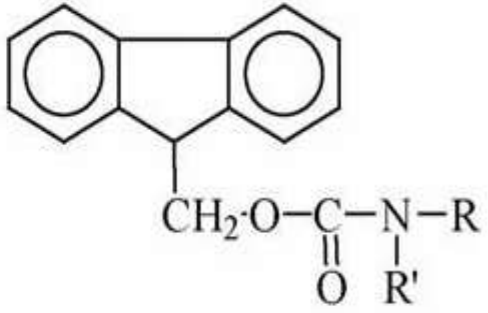


AMPA



FMOC-Cl





SAMPLE PREPARATION

Water sample was acidified to release GLY with 6M HCl to pH 1 and neutralized with 6M KOH.

Sample was derivatized by adding 5% borate buffer (pH9) followed by FMOC, and allowing the reaction to take place overnight.

After night, sample was acidified with formic acid (pH3) and filtered after the addition of water and 0.1M EDTA.

Sample were extract through Bond Elut Plaxa, activated with MeOH and water. The elution was done with MeOH and water. After evaporation, the extract was redisolved in mobile phase and injected into the LC–MS/MS.

LC-MS/MS conditions

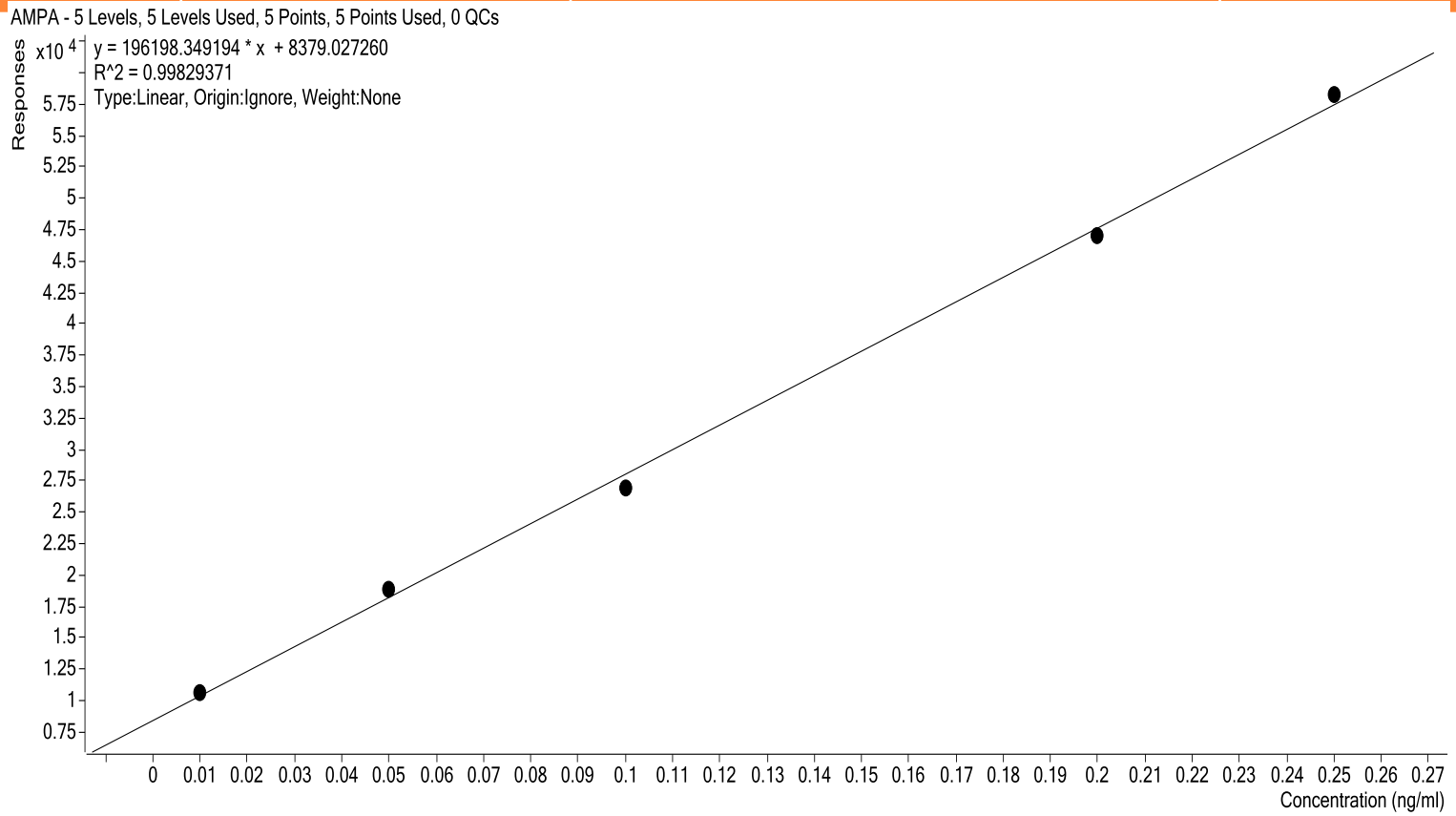
HPLC	Agilent 1200
Column	Zorbax C18, 100 mm x 4.6 mm, 1.8 µm, Agilent
Oven temp	40 °C
Mobile phase	A: MeOH (10 mM NH_4HCOOH, pH 9) B: water (10 mM NH_4HCOOH, pH 9) The elution program was started with 70% B and hold 5 min 70% B. It was linearly decreased to 10% B in 15 min.
Flow rate	0.25 ml/min
Injection	5 µl
MS	Agilent 6410
Ionization	ESI (-)
Nebulizer gas	40 psi
Gas flow	10 L/min at 350 °C
Capilary	4000 V
Fragmentor	100 V

MRM conditions for QQQ

Analyte	R_{time} (min)	Precursor (m/z)	Product (m/z)	Fragmentation (V)	CV (mV)	Polarity
GLY	2.53	390.2 390.2	168 150	100 100	5 15	negative
GLY-D3	2.53	393.2	170.8	100	15	negative
AMPA	14.12	332 332	136 110	100	15 10	negative

Validation parameters - Calibration

Analyt	Range (µg/L)	Equation	R^2
GLY	25-500	Y=0.613464x-0.009063	0.9979
AMPA	**10-250**	**Y=196198.34x+8379.03**	**0.9983**



Validation parameters – Recovery and repeatability

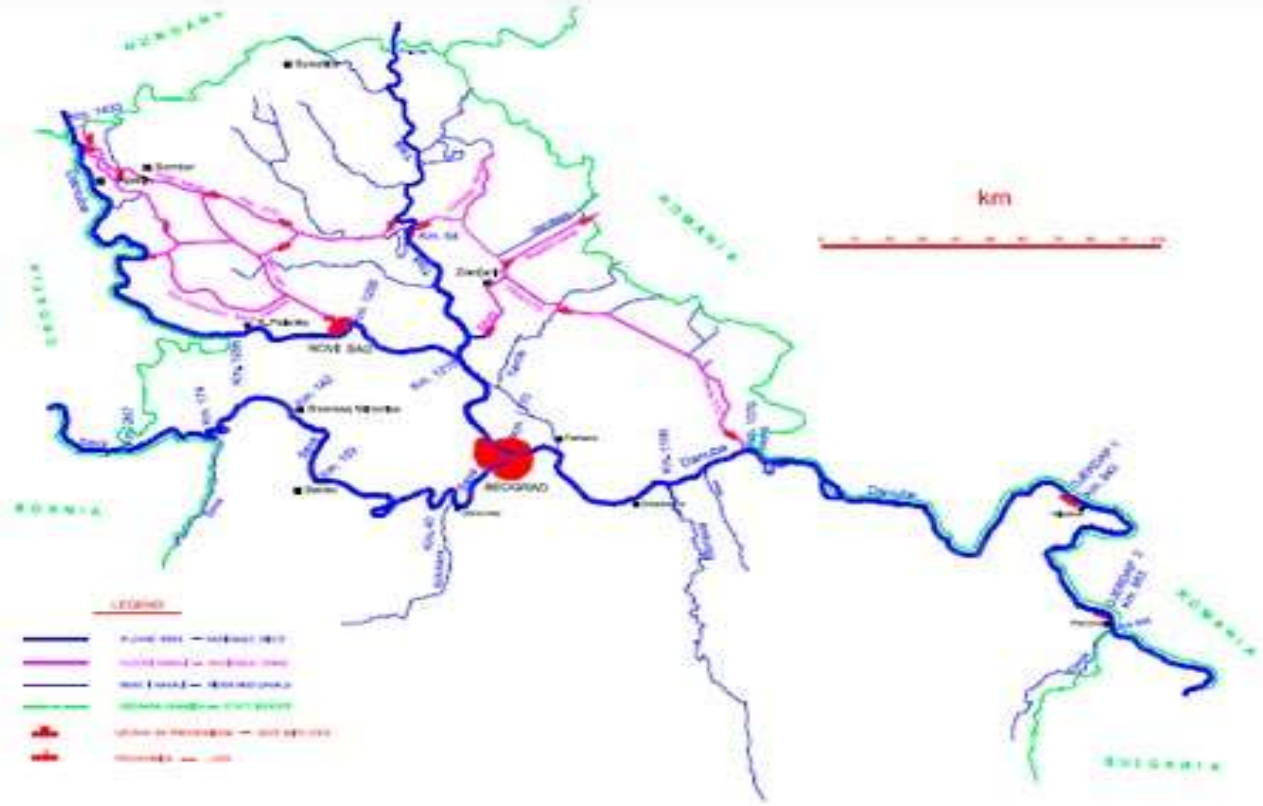
Analite	Level µg/L	Recovery, %	Average recovery, %	% RSDr	SANTE/11813/2017 Rec. 70-120% RSDr <20%
GLY	0.50	99.0	100.7	5.75	
	0.10	98.3			
	0.025	104.9			
AMPA	0.250	94.2	105.6	11.46	
	0.10	104.3			
	0.010	118.3			

LOQ were taken as the lowest fortification level of 0.025 µg/L (GLY) and 0.010 µg/L (AMPA).

LOD were calculated by MassHunter software from the most diluted standard analysed (10 ng/mL) and were estimated to be 5 ng/L for both

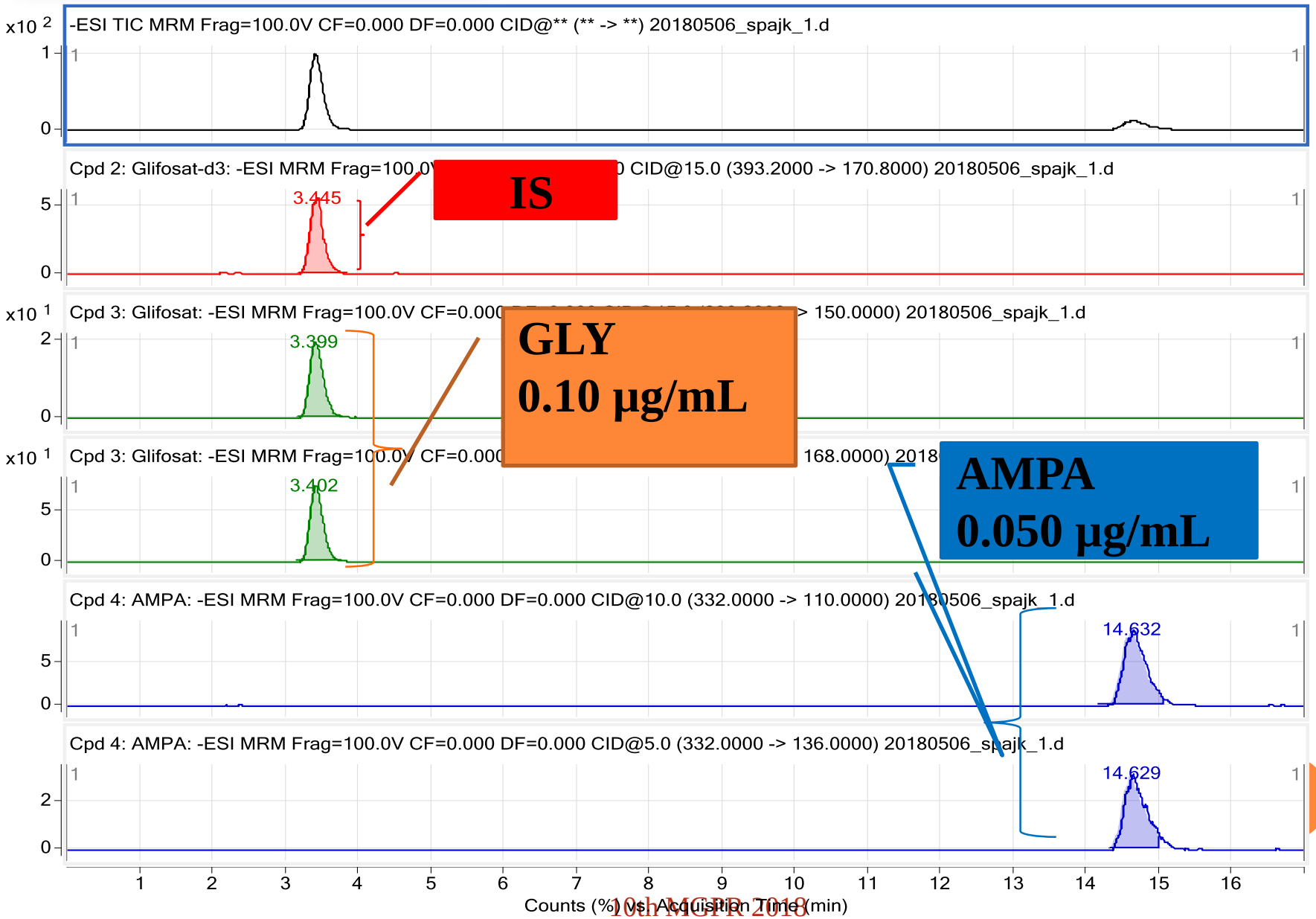
WATER ANALYSES RESULTS

➢Water samples (27) were collected in plastic bottles from six locations of the canal DTD and stored in a freezer at -18 °C until analysis.



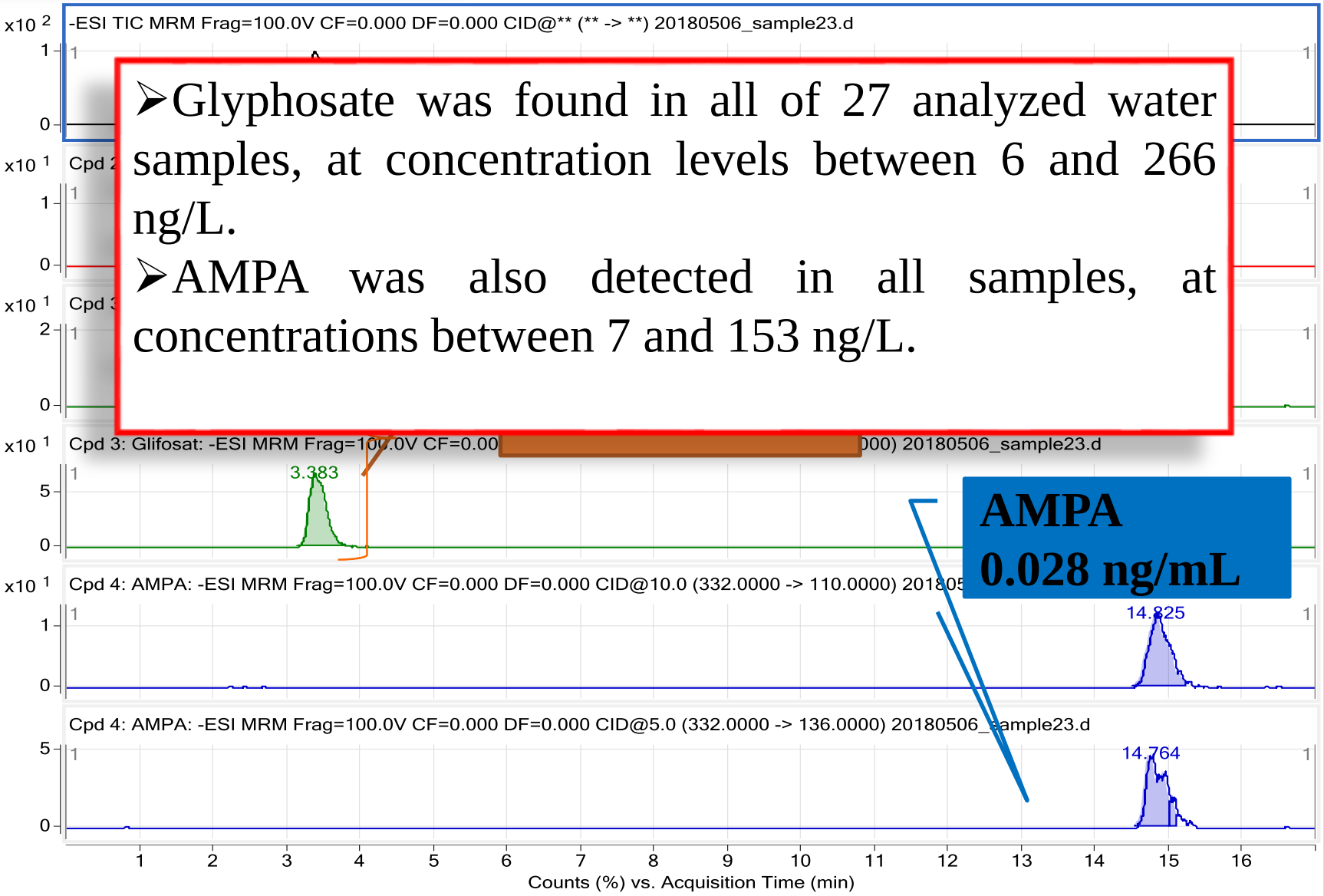
RESULTS

LC-MS/MS hromatogram of standards



RESULTS

LC-MS/MS hromatogram of sample



- Glyphosate was found in all of 27 analyzed water samples, at concentration levels between 6 and 266 ng/L.
- AMPA was also detected in all samples, at concentrations between 7 and 153 ng/L.

AMPA
0.028 ng/mL

CONCLUSION

- The performance characteristics of validated parameters were acceptable according to SANTE/11813/2017 guidance document.
- The concentrations found in our study agree with those reported in other European countries.

THANK YOU FOR YOUR ATTENTION

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