Trace Analysis of Compounds in Potable Water by On-Line SPE-LC/MS/MS

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Why the Interest in Emerging Contaminants (EC) and Pharmaceuticals and Personal Care Products (PPCPs) in Water?

- > 3,000 drugs and PPCPs
- Interest and fate of drugs, hormones
  - Hormones affecting fertility, changes in gender
- Limited knowledge of treatability for all contaminants
- High public visibility, concern

Requirements for Emerging Contaminant Analysis Methods

- Selectivity/Specificity
- Sensitivity-MRL determination by LCMS/MS
- Target List
- QA/QC
- Ruggedness/matrix effects
- Cost

Innovative LC-MS/MS Approach to EC Analysis

- Cost effective screen for as many analytes as possible
- Reporting levels- 5 to 10 ng/L (ppt)
- Known target analytes and identification from experts
- Accuracy and precision comparable to existing drinking water methods- 70-130% recovery
- Use multiple ion transitions (MRM)-misidentification

Examples of EC Concerns

Pharmaceuticals	
Veterinary and human antibiotics	Trimethoprim, erytromycine, lincomycin, sulfamethaxozole
Analgesics, anti-inflammatory drugs	Codein, ibuprofene, acetaminophen, acetylsalicilyc acid, diclofenac, fenoprofen
Psychiatric drugs	Diazepam
Lipid regulators	Bezafibrate, clofibric acid, fenofibric acid
β-blockers	Metoprolol, propanolol, timolol
X-ray contrasts	Iopromide, iopamidol, diatrizoate
Steroids and hormones	Estradiol, estrone, estriol, diethylstilbestrol
Personal care products	
Fragrances	Nitro, polycyclic and macrocyclic musks,
Sun-screen agents	Benzophenone, methylbenzylidene camphor
Insect repellents	N,N-diethyltoluamide
Antiseptics	Triclosan, Chlorophene
Surfactants and surfactant metabolites	Alkylphenol ethoxylates, 4-nonylphnol, 4-octylphenol, alkylphenol carboxylates
Flame retardants	Polybrominated diphenyl ethers (PBDEs), Tetrabromo bisphenol A, C_{10}-C_{13} chloroalkanes Tris (2-chloroethyl)phosphate
Industrial additives and agents	Chelating agents (EDTA), aromatic sulfonates,
Gasoline additives	Dialkyl ethers, Methyl-*t*-butyl ether (MTBE)

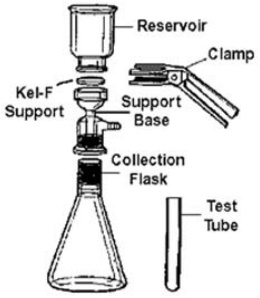
Pesticides/Herbicides
Sugar Substitutes

Sample Preparation Requirements: Basic Steps

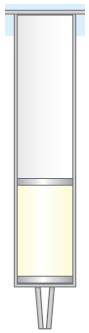
- Collection device
- Collection of water sample 1000 mL
- Shipping/cost
- Analysis

Sample Preparation

Off-line SPE



- 1000 mL transported to the lab
- Trace enrich onto a cartridge or disk
- Elute with few mLs solvent, dry, reconstitute

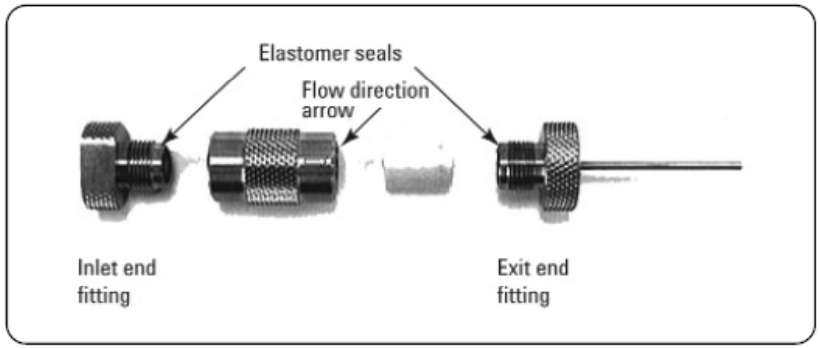


- Amenable LC, % organic solvent
- 1 mL eluent loaded into autosampler vial
- 2-20 uL injected, UHPLC to HPLC UV/MS detection
- End result 98-99% prepared sample is wasted

Sample Preparation

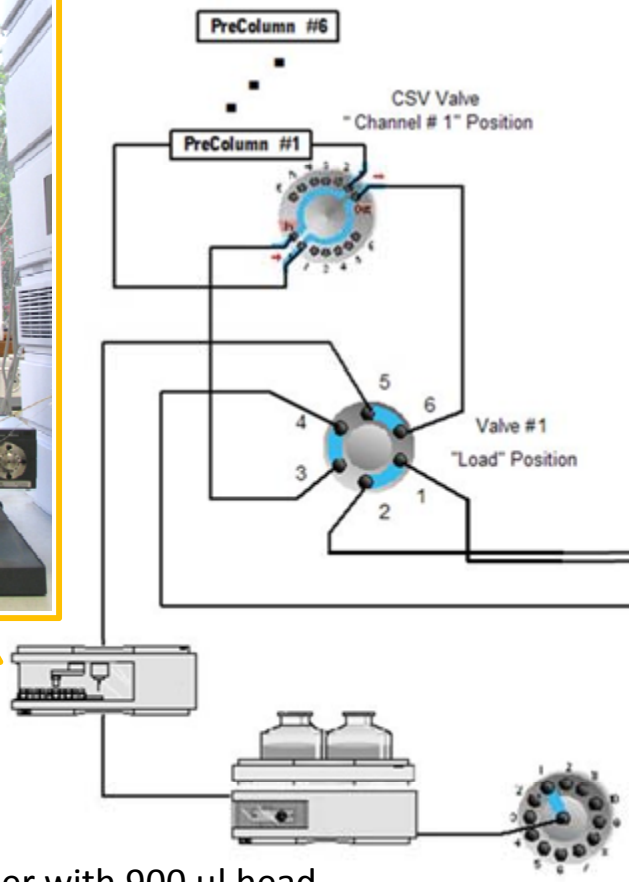
On-line SPE (trace enrichment-SPE)

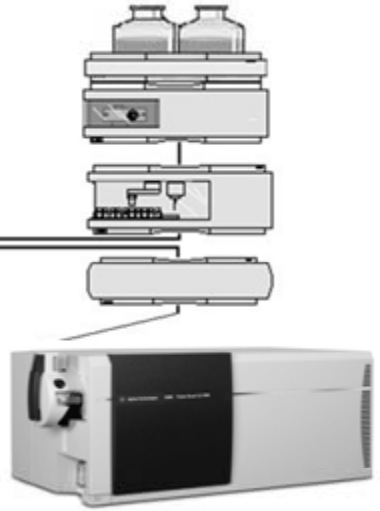
- 100% of the prepared sample is loaded
- Volume can be <5 mL
- Combined with more sensitive detection (MS/MS)



- Logistics of getting sample to the lab is simplified

Setup of the Agilent AQUA Online-SPE System





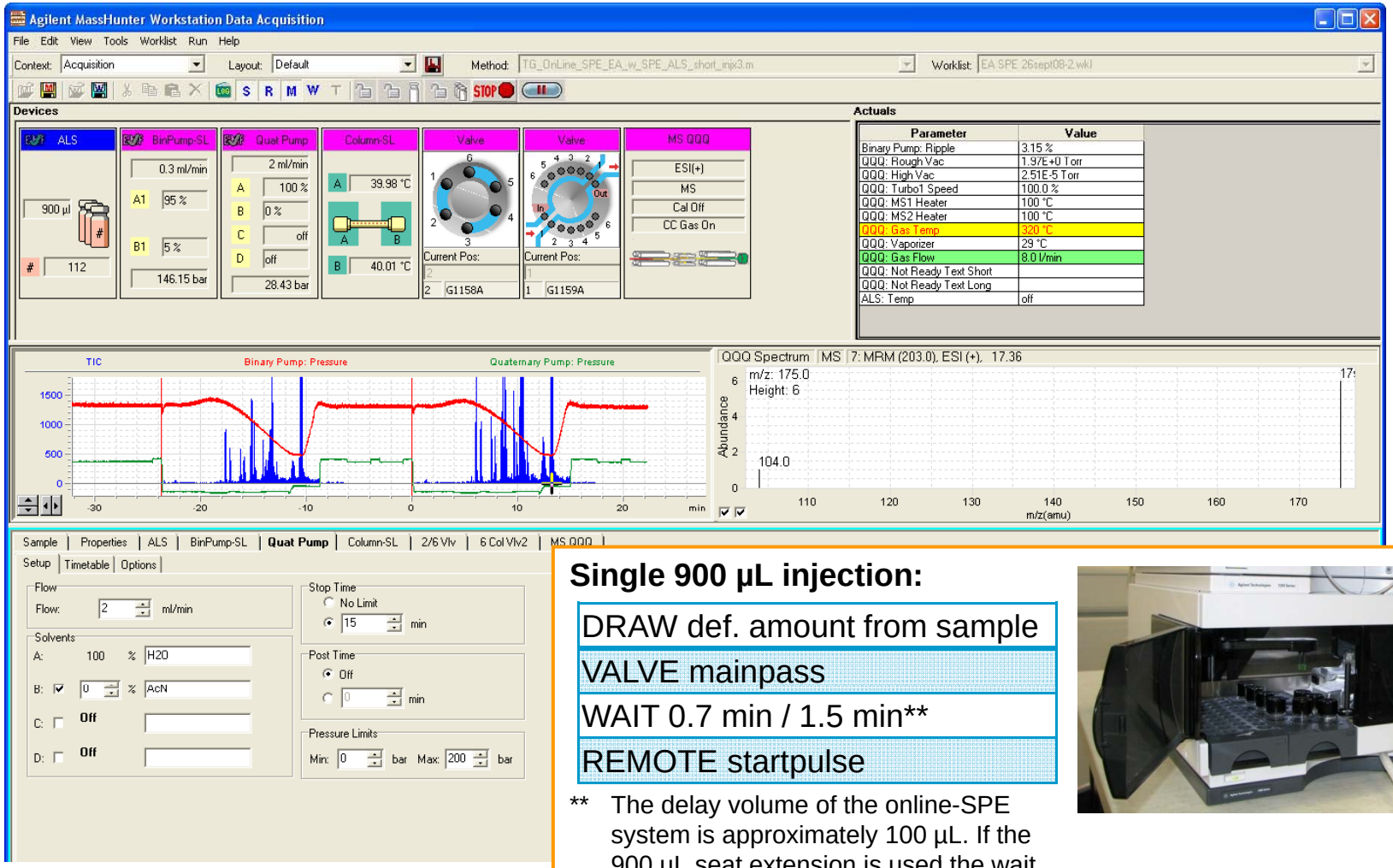
SPE system:

- Quaternary pump
- G1329A autosampler with 900 µl head
- 6 port 2 pos valve
- 12 port 6 pos valve
- 12 port stream selection valve (optional)

RRLC system:

- Binary pump SL
- Well plate sampler SL (not used)
- Column department SL

Method Implementation in Mass Hunter Acquisition



Single 900 µL injection:

DRAW def. amount from sample
VALVE mainpass
WAIT 0.7 min / 1.5 min**
REMOTE startpulse

** The delay volume of the online-SPE system is approximately 100 µL. If the 900 µL seat extension is used the wait time has to be extended to 1.5 min.

On-line SPE method – Autosampler Program(s)

Single 900 µL injection:

DRAW def. amount from sample
VALVE mainpass
WAIT 0.7 min / 1.5 min**
REMOTE startpulse

** The delay volume of the system until the cartridge is approximately 100 µL. If the 900 µL seat extension is used the wait time has to be extended to 1.5 min (flush volume 3 mL).

2-fold 900 µL injection using the multiple draw kit (900 µL seat extension):

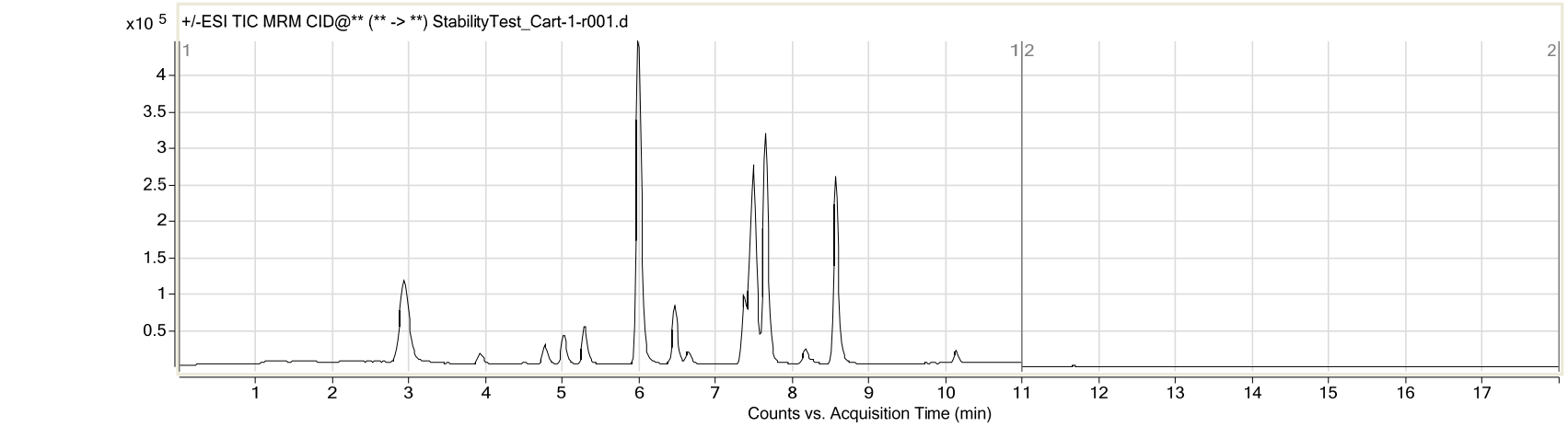
DRAW def. amount from sample
EJECT def. amount into seat
DRAW def. amount from sample
VALVE mainpass
WAIT 1.5 min
REMOTE startpulse

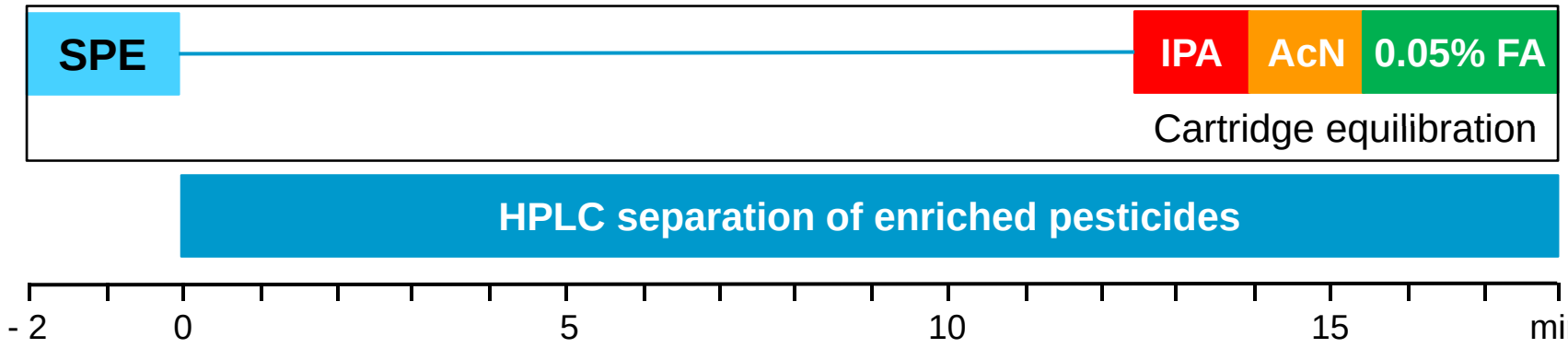
Online-SPE Method – Loading Pump

Mobile phase:	A: 0.05% formic acid B: acetonitrile C: isopropanol			
SPE cartridge**:	PLRP-s available 2.1 x 12.5mm or 4.6 x 12.5mm			
Temperature:	ambient			
Flow (load):	2 mL/min			
Loading and equilibration:	0.00 min	0 % B	0 % C	2.0 ml/min
	0.10 min	0 % B	0 % C	0.1 ml/min
	12.50 min	0 % B	0 % C	0.1 ml/min
	12.60 min	0 % B	100 % C	2.0 ml/min
	12.70 min	0 % B	100 % C	2.0 ml/min
	14.00 min	0 % B	100 % C	2.0 ml/min
	14.10 min	100 % B	0 % C	2.0 ml/min
	15.50 min	100 % B	0 % C	2.0 ml/min
	15.60 min	0 % B	0 % C	2.0 ml/min

** *SPE cartridges fit in the Agilent Guard Column Hardware Kit (P.N. 820888-901)*
All connections between the analytical system and the SPE hardware have been made by using 0.18 mm ID PEEK tubing (P.N. 5042-6462) and finger-tight PEEK fittings (P.N. 5063-6591).

Online-SPE – Timetable for Loading and Analysis





Equilibration of SPE cartridges, SPE and separation of pesticides is overlapped and results in a total runtime for the method of 20 minutes.

Example 1:High Sensitivity Analysis of Pesticides

- Dqdo}hg#khuelflgh#frp srxqgv

Atrazine
Carbendazim
Carbetamid
Chloridazon
Chlorotoluron
Chloroxuron
Desethylatrazine
Desisopropylatrazine
Desmetryn
Diflubenzuron
Diuron
Fenuron
Irgarol 1051
Isoproturon
Linuron
Metamitron
Methabenzthiazuron
Metoxuron
Metsulfuron methyl
Monolinuron
Monuron
Neburon
Prometryn
Propazin
Simazine
Terbutryn
Terbutylazine
Trietazin

Example 1:High Sensitivity Analysis of Pesticides

- V|vwhp #frqiljxudwlrq

AQUA Online-SPE system consisting of

- Quarternary pump
- G1329A autosampler with 900 µl head
- 6 port 2 pos valve
- 12 port 6 pos valve

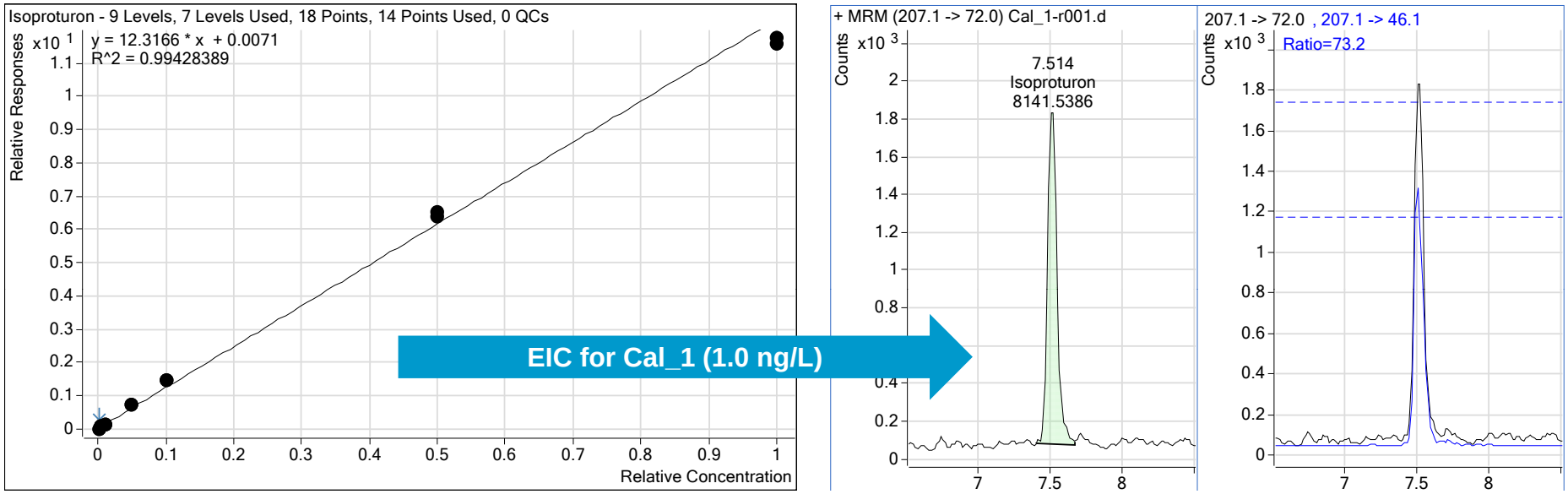
UHPLC system consisting of

- 1290 Infinity binary pump
- 1290 Infinity autosampler (not used)
- 1290 Infinity thermostatted column compartment

G6460AA Triple Quadrupole Mass Spectrometer

Example 1:High Sensitivity Analysis of Pesticides

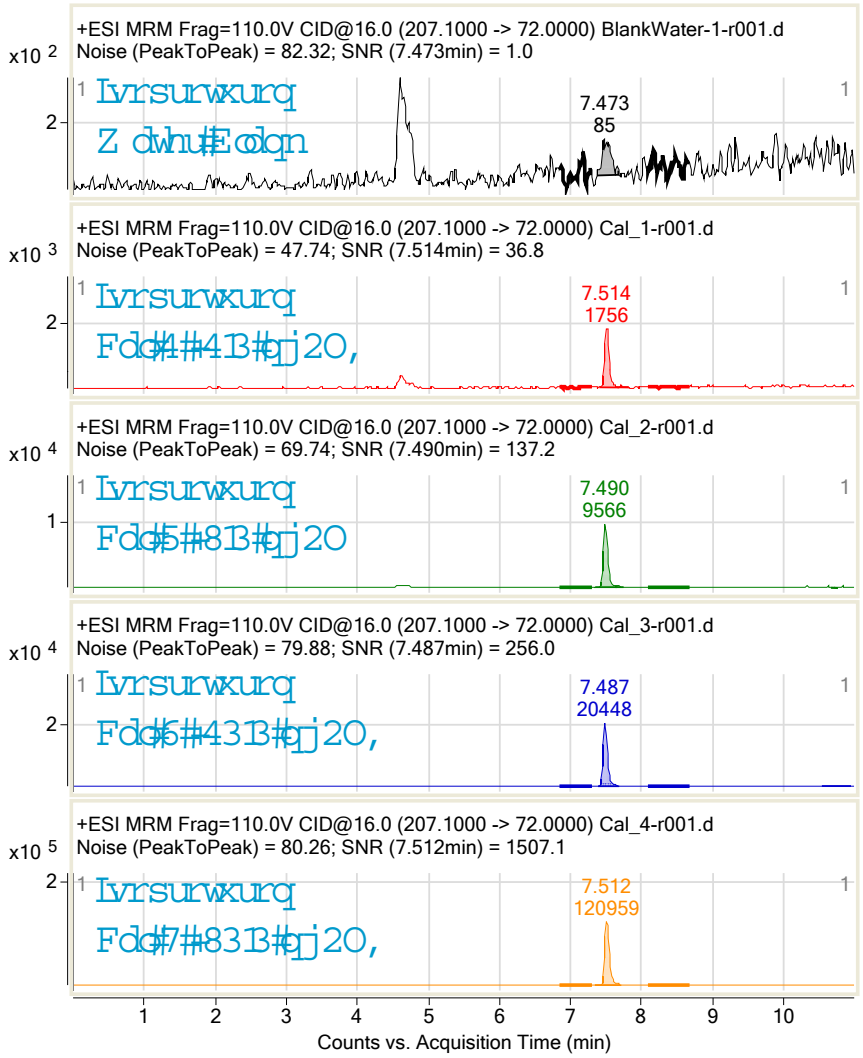
- Ivrsurwxurq

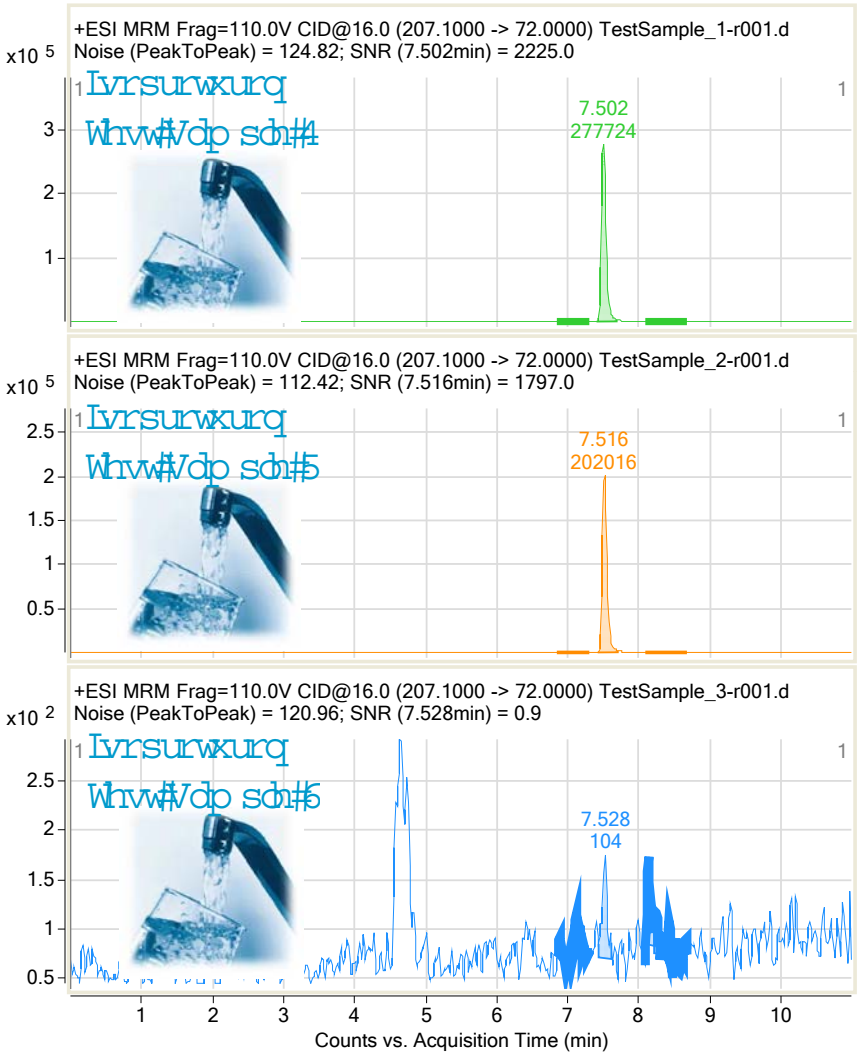


- Quantifier: 207.1 → 72.0
- Qualifier: 207.1 → 46.1
- Peak width: 16.1 s
- Data points: 18
- Linear range: < 1 ng/L to 1 µg/L

Example 1: High Sensitivity Analysis of Pesticides

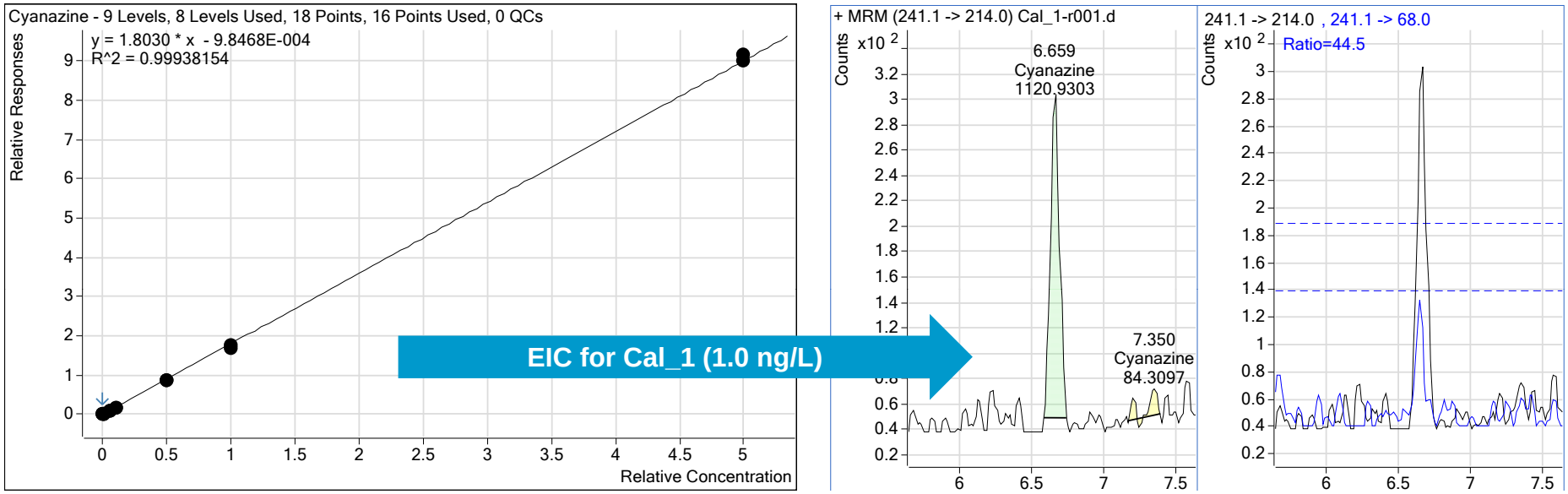
- Ivrsurwxurq +V2Q#Fdf1#shdnCwrCshdn,





Example 1: High Sensitivity Analysis of Pesticides

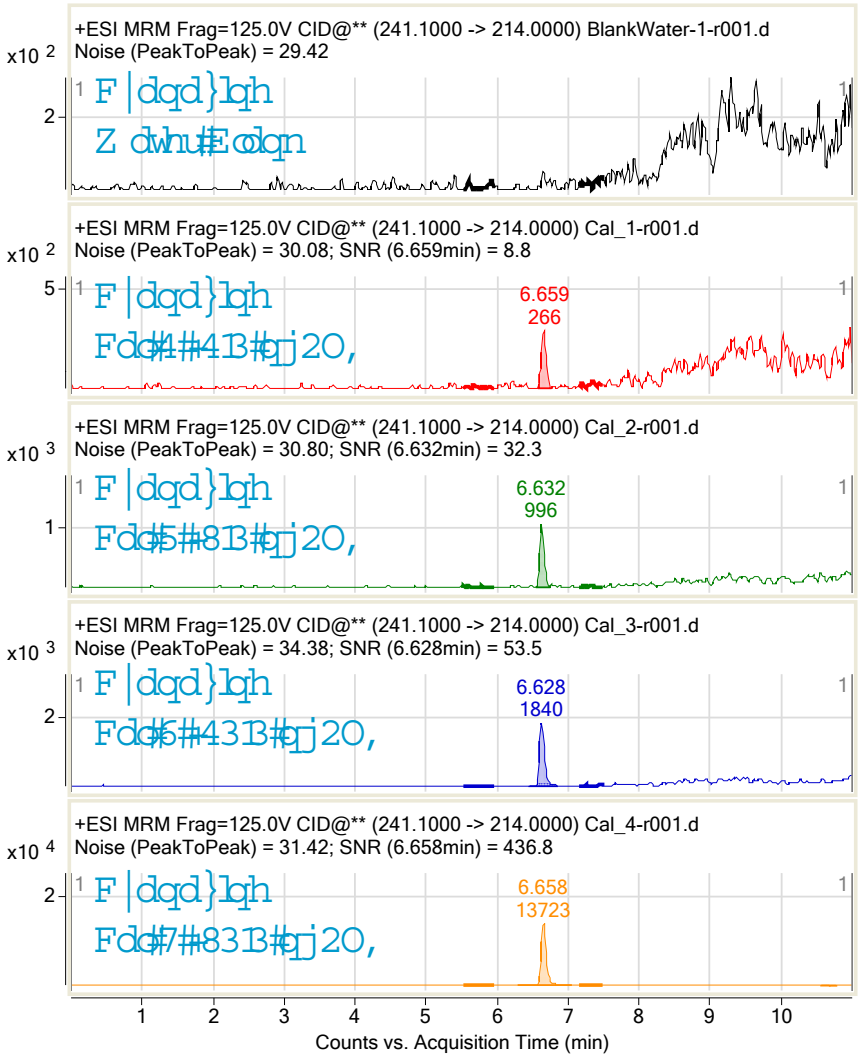
- F|dqd}lqh

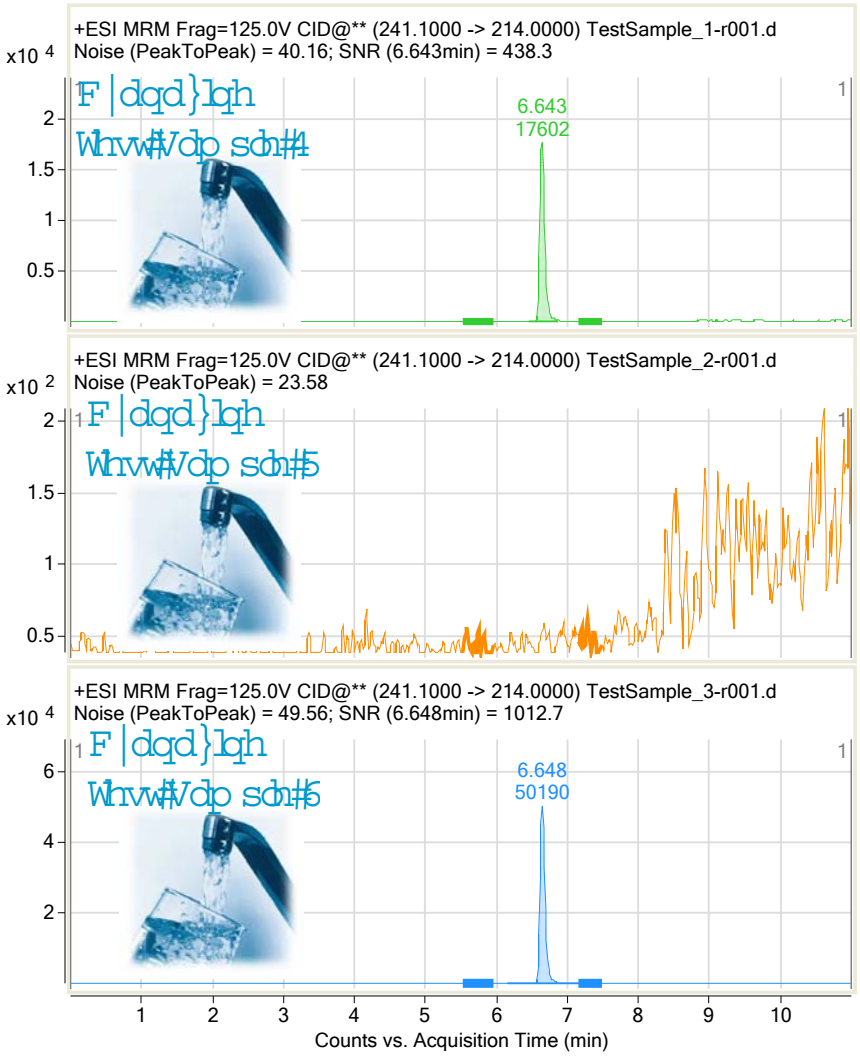


- Quantifier: 241.1 → 214.0
- Qualifier: 241.1 → 68.0
- Peak width: 16.0 s
- Data points: 18
- Linear range: 1 ng/L to 5 µg/L

Example 1: High Sensitivity Analysis of Pesticides

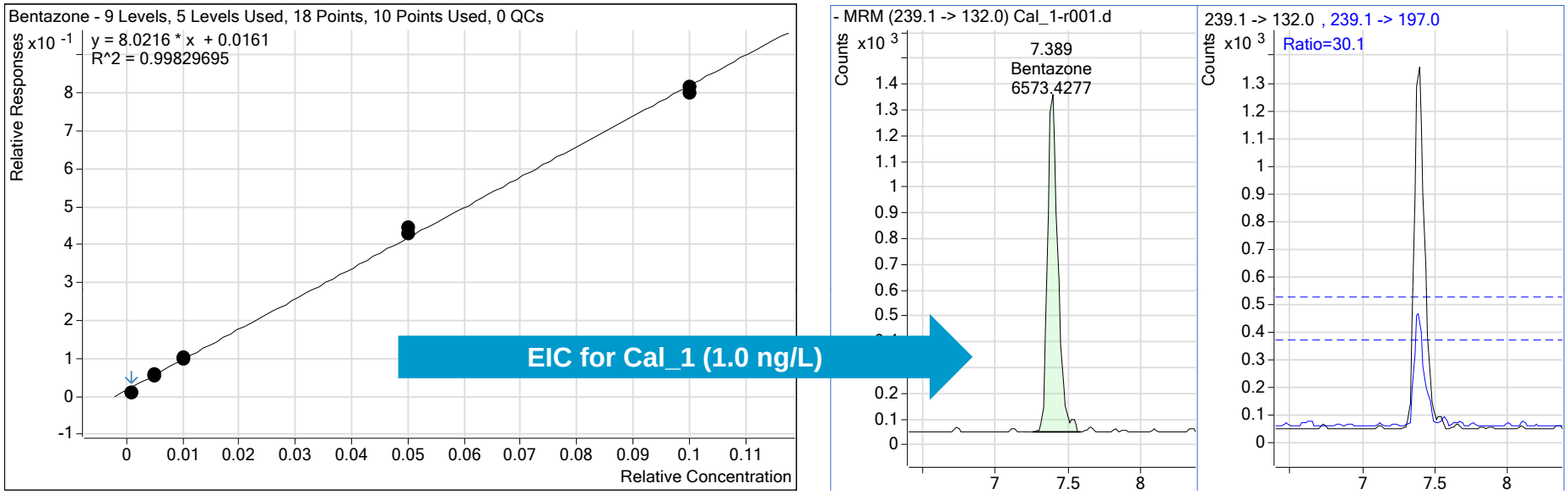
- F|dqd}lqh +V2Q#fdcf1#shdn(wr(shdn,





Example 1: High Sensitivity Analysis of Pesticides

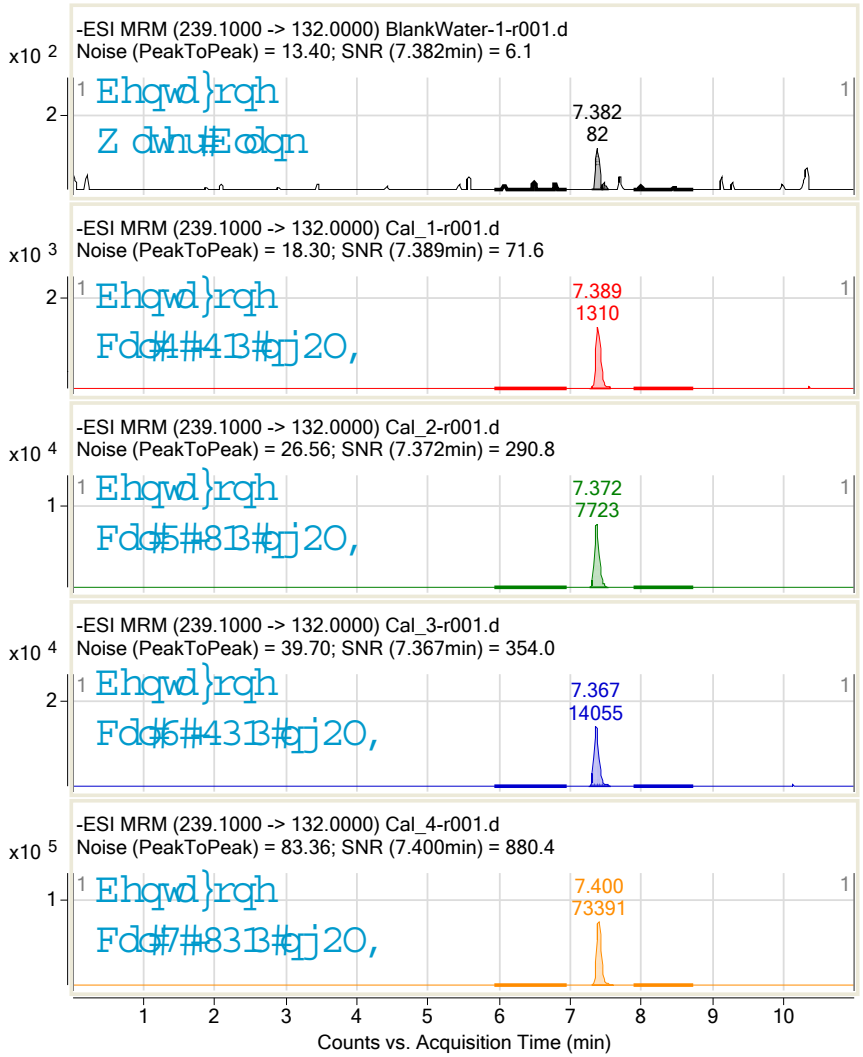
- Ehqwd}rqh

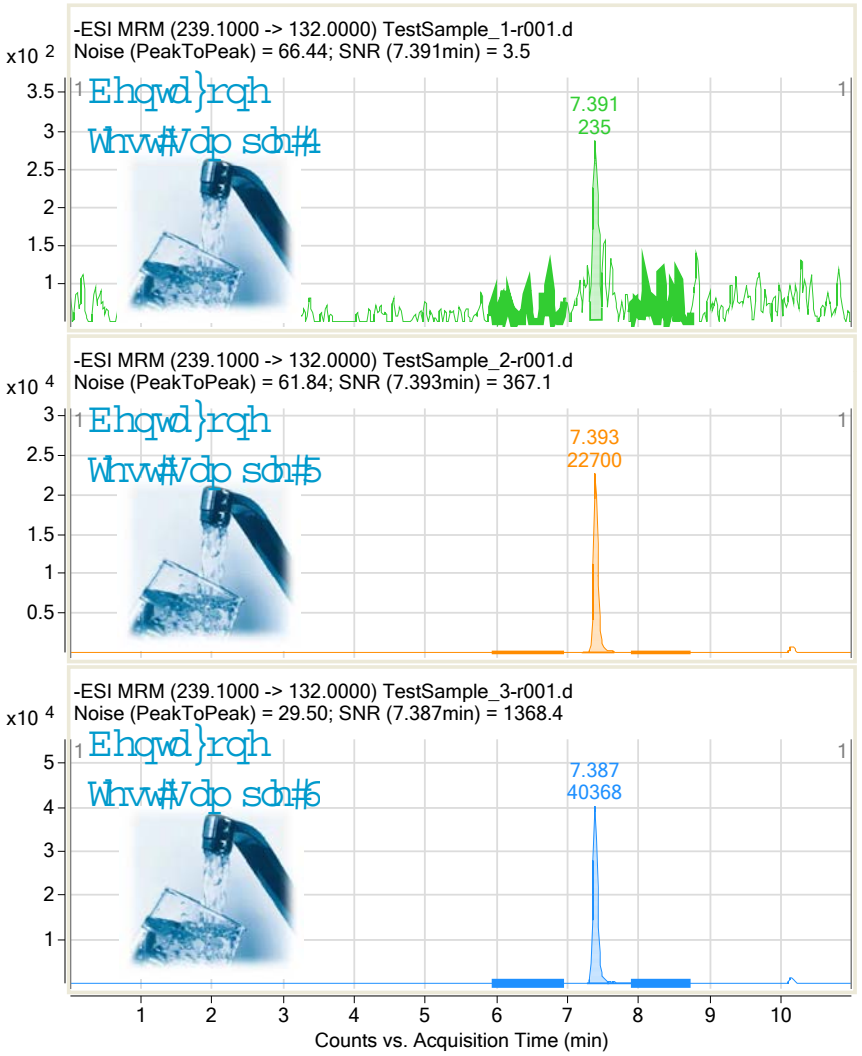


- Quantifier: 239.1 → 132.0
- Qualifier: 239.1 → 197.0
- Peak width: 19.8 s
- Data points: 23
- Linear range: << 1 ng/L to 0.1 µg/L

Example 1: High Sensitivity Analysis of Pesticides

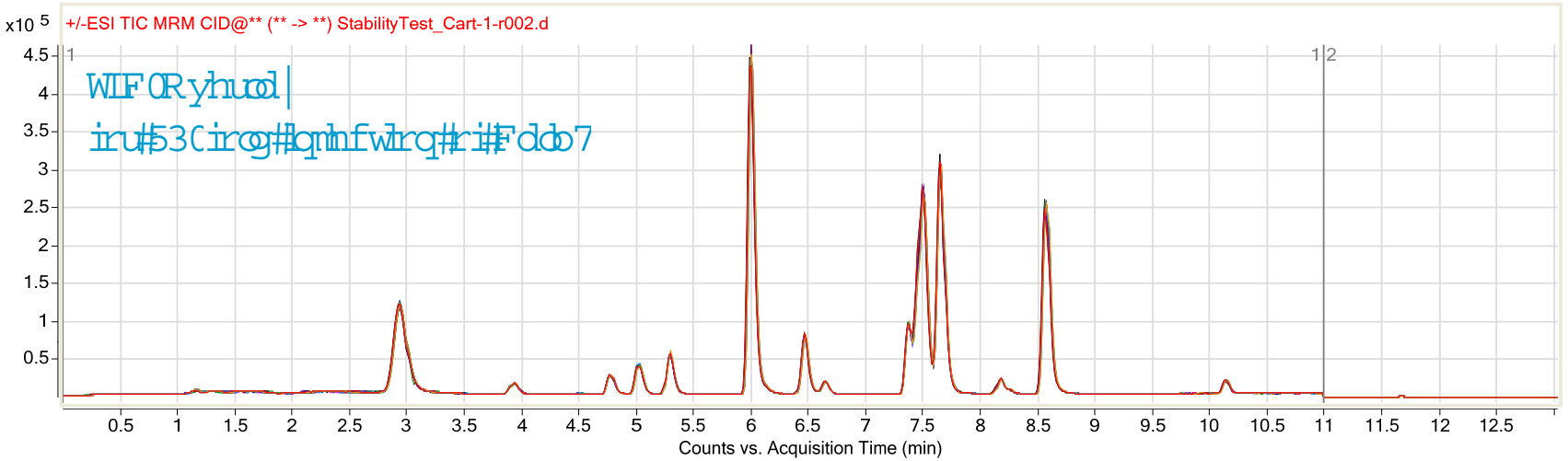
- Ehqwd}rqh +V2Q#Fdf1#shdn(wr(shdn,





Example 1: High sensitivity analysis of pesticides

- 53(irog#Lqmfwlrq#ri#Fdo7#83#qj20/#vdp h#fduwulgjh,



Compound	Average (µg/L)	SD	RSD
2OH-Atrazine	0.059	0.002	3.50
Atrazine-desisopropyl	0.051	0.001	2.60
2,6-Dichlorobenzamide	0.047	0.003	6.80
Metamitron	0.053	0.001	2.64
Atrazine-desethyl	0.051	0.002	3.43
Dimethoate	0.055	0.002	2.94
Hexazinone	0.060	0.002	2.56
Simazine	0.052	0.001	2.84
Cyanazine	0.043	0.001	2.79
Bentazone	0.057	0.002	3.32
Atrazine	0.053	0.001	2.34

Compound	Average (µg/L)	SD	RSD
Isoproturon	0.053	0.001	2.56
2,4-D	0.055	0.002	3.71
MCPA	0.046	0.003	5.97
DNOC	0.044	0.003	6.31
Mechlorprop	0.050	0.002	3.98
Dichlorprop	0.047	0.003	5.62
Terbuthylazin	0.053	0.001	2.45
Dinoseb	0.039	0.002	4.55
Pendimethalin	0.040	0.002	4.54

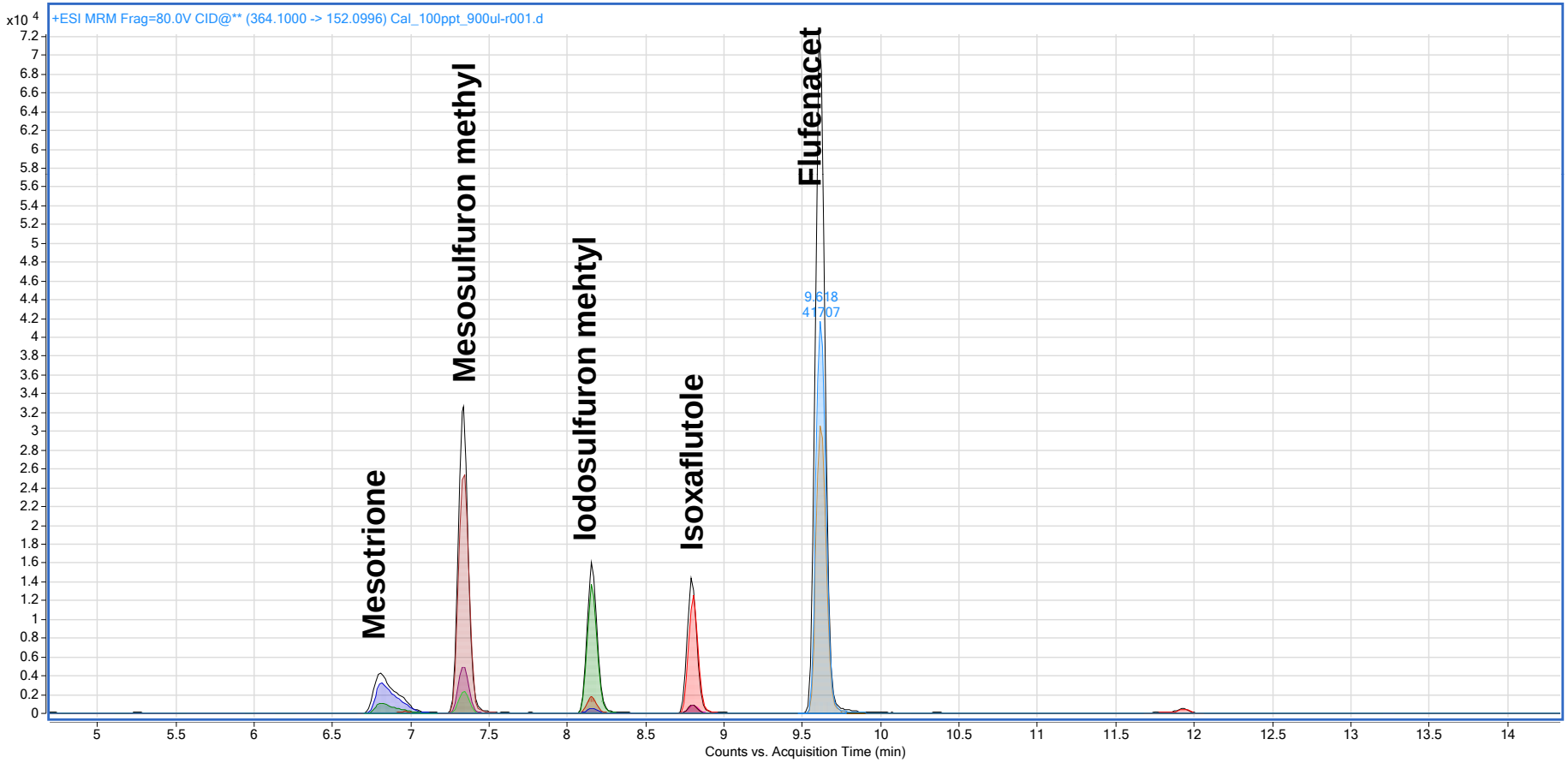
Example 2: Impact of Solvent and Sample Composition

❑ Uhtxluhp hqw

- In addition to method for the neutral herbicides an on-line SPE method for the following 5 compounds (LOQ < 10 ng/L):
  - Flufenacet
  - Iodsulfuron-methyl
  - Isoxaflutole
  - Mesosulfuron-methyl
  - Mesotrione
- Sample solution contains 80 mg/L Na_2SO_4 for sample conservation
- Compounds belong to group of acidic herbicides, Mesotrione shows broad peak in chromatography

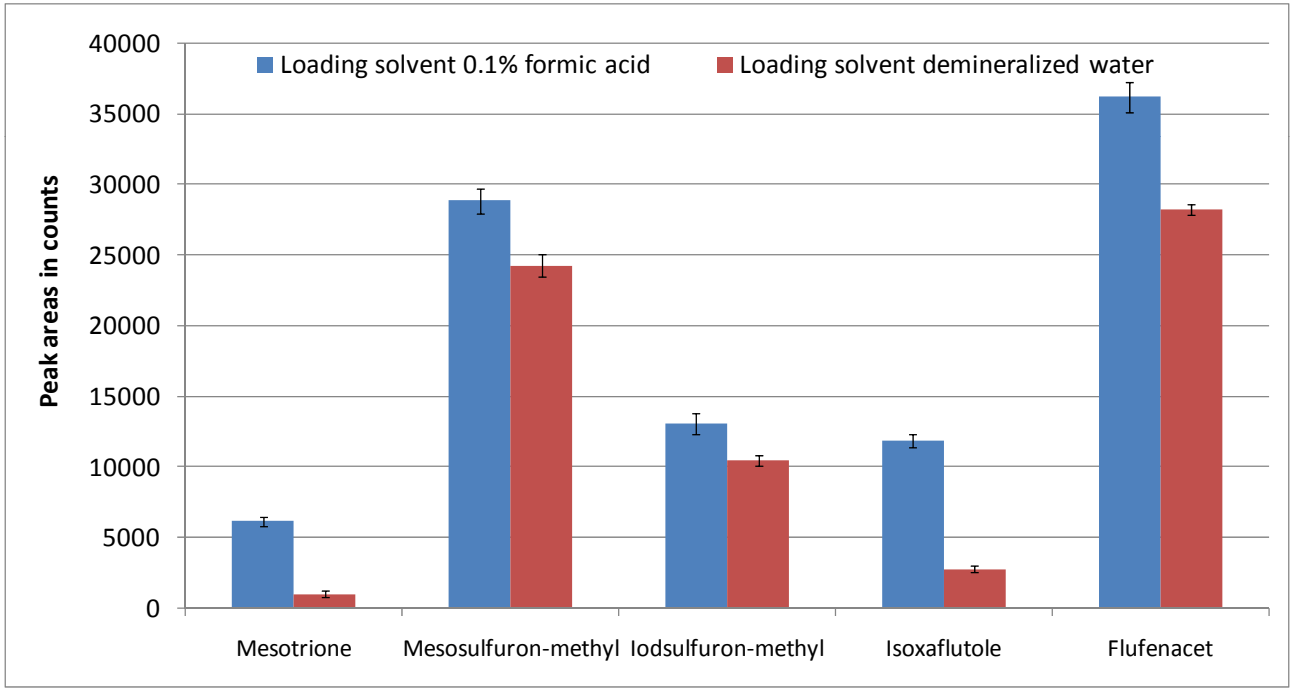
Example 2: Impact of Solvent and Sample Composition

- KSOF#fkurp dwrjudp
  - Calibration standard 5 (110 to 197 ng/L); injection volume 900 µL



Example 2: Impact of Solvent and Sample Composition

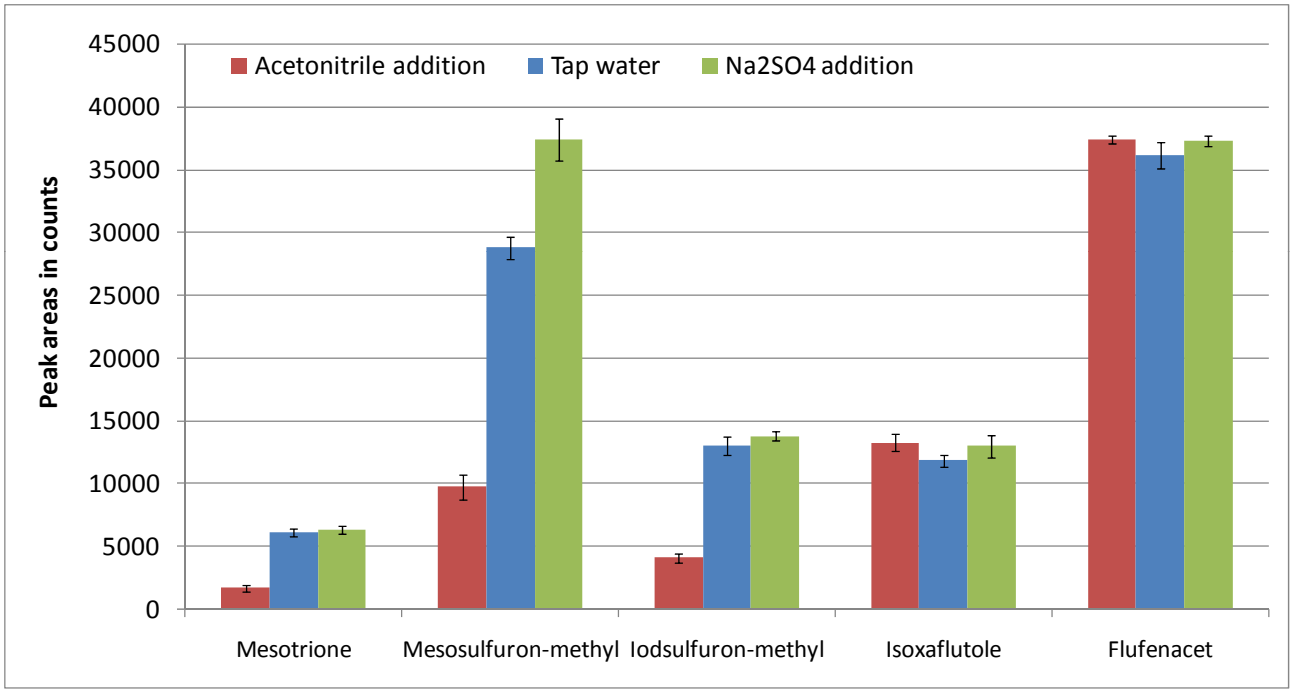
- RqCdqh#VSH#phwkrg#Ù frp sdulvrq#ri#bdglqj#frqglwlrqv#4,
  - Flow rate of the loading pump had no influence on loading efficiency and therefore a flow of 2 mL/min was chosen to reduce loading time.
  - Compared to the use of demineralized water as the loading solvent loading with 0.1% formic acid increased the response significantly.



  - For injection volumes > 900 µL the addition of 0.1% formic acid to the sample solution is strongly recommended.

Example 2: Impact of Solvent and Sample Composition

- RqCodqh#VSH#p hwkrg#Ù frp sdulvrq#r i#vdp sd#frp srvlwlrq+5,
  - The effect of the addition of acetonitrile and Na_2SO_4 (80 mg/L) to the sample solution on the on-line SPE recovery was tested

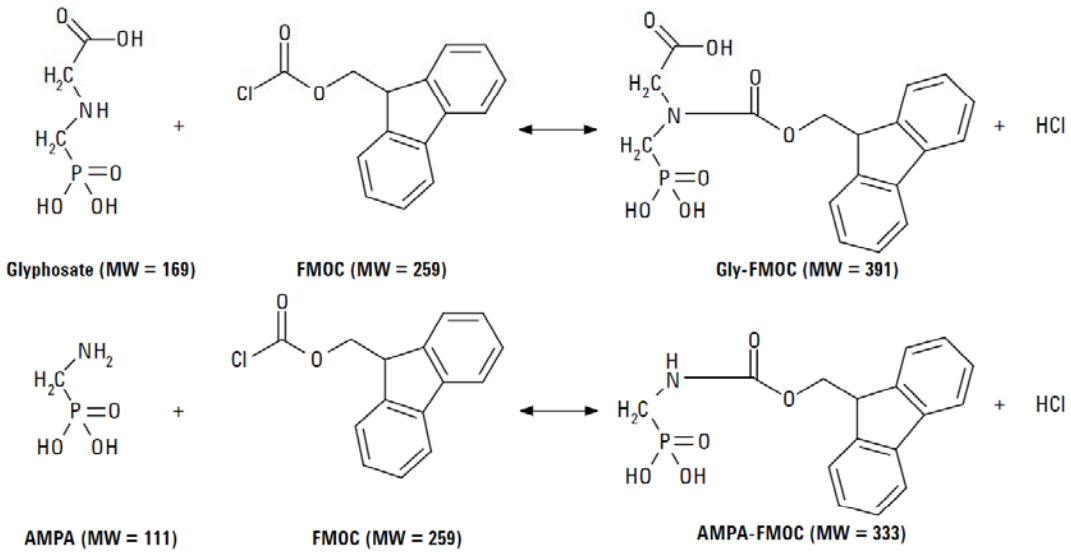


  - Acetonitrile addition was detrimental, the addition of Na_2SO_4 increased the mesosulfurone-methyl response. It is recommended to prepare samples and calibration standards identical.

Example 3: Robust Method for Glyphosate and AMPA

❑ Edfnjurxqg

- Glyphosate is a global herbicide which is widely used in agriculture and urban landscape management
- In the environment glyphosate is metabolized to its metabolite aminomethyl phosphonic acid (AMPA)
- Both compounds are extremely polar due to their bipolar structure
- For the analysis of both compounds a derivatization is widely accepted



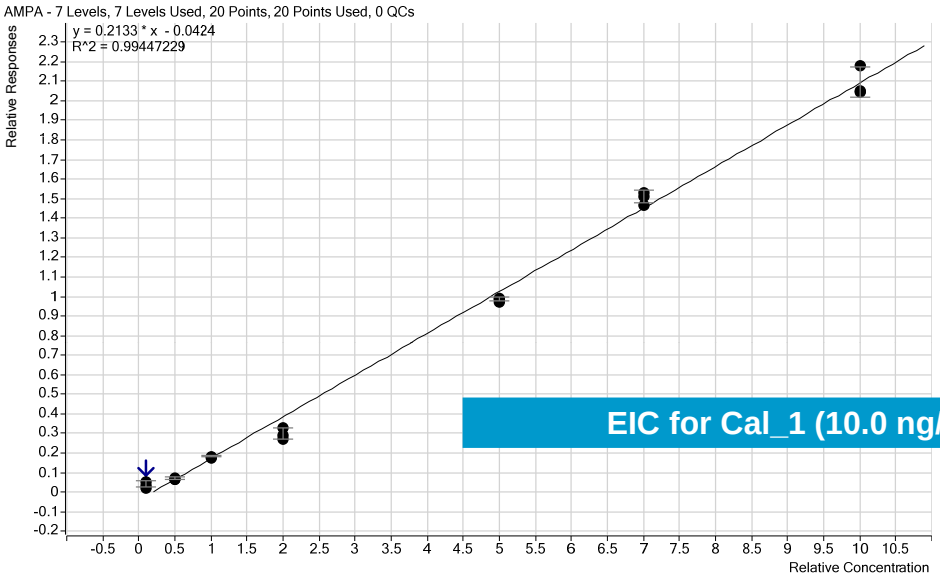
Example 3: Robust Method for Glyphosate and AMPA

❑ Edfnjurxqg

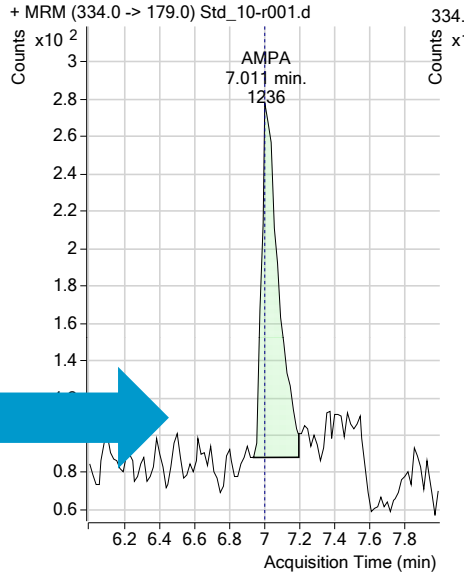
- Derivatization works at basic pH-values with high excess of FMOC and both reduces the lifetime of the chromatographic column and the robustness of the method
- Sensitivity of direct injection is just enough to detect 100 ng/L
- Online-SPE allows for clean-up and enrichment to comply with screening
- <u>Experimental:</u>
  - 1 mL of sample is fortified with 100 ng ISTD (Glyphosate 1,2 ^{13}C-^{15}N, AMPA ^{13}C)
  - Borate buffer (pH 10) and FMOC (5 mg/mL) is added and reaction takes place over night (> 4 h)
  - Acetic acid (58%) is added to neutralize sample
  - FMOC derivates are stable for > 48 hours

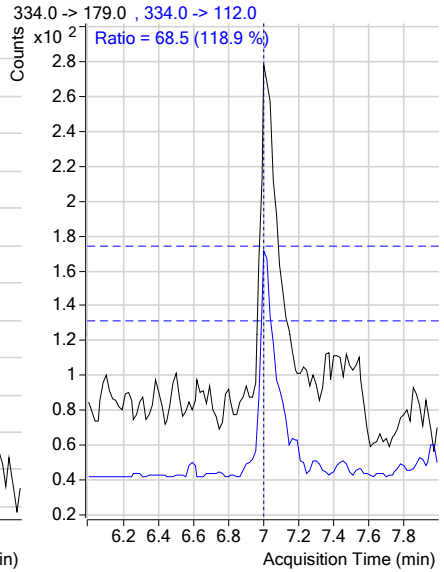
Example 3: Robust Method for Glyphosate and AMPA

❑ DP SD



EIC for Cal_1 (10.0 ng/L)





- Quantifier: 334.0 → 179.0
- Qualifier: 334.0 → 112.0
- Peak width: 15.4 s
- Data points: 15
- Linear range: 10 ng/L to 1.0 µg/L

Example 3: Robust Method for Glyphosate

❑ Uhvxow

- Use of internal standards needed to correct for derivatization yields in different water samples
- Online-SPE increases the sensitivity to the relevant concentration range (10 to 25 ng/L) for environmental samples
- Robustness of the method is substantially increased – run already > 1000 samples on the same chromatographic column with just 2 cartridges
- AMPA has been found in concentrations up to 2000 ng/L, Glyphosate just positive if AMPA concentrations are extremely high

Review: On-line versus Off-Line Trace Enrichment (SPE)

On-Line	Off-Line
Increased sensitivity-inject entire sample-analyzed	Only a fraction of concentrated sample injected 2-100 uL from 1 mL extract
Small Sample Volumes (mL)	Large sample volume 100mL-L
Increased sample throughput	Extraction and analysis are separate
Reduce error-sample handling	Increase chance of error-manual
No evaporation step	Concentration step needed
No reconstitution	Reconstitution
Integrated System	Standalone Instruments

Summary and Conclusions

- The on-line SPE combined with a LC-QQQ system allows a relatively simple, fast and reliable determination of herbicides in the low ng/L range in filtered water samples.
- The whole system is fully controlled with the MassHunter acquisition software.
- Adding a more sensitive QQQ system allows for even better sensitivity or compounds which are less weakly ionizable.
- Good recovery values and reproducibilities can be achieved even in complex samples and for very polar compounds.
- Online SPE not only increases the sensitivity but adds robustness to the method (e.g. for glyphosate)
- The chemistry remains the same – adding acid to the loading solvent increases the recovery for acidic compounds, adding salt to the sample solution increases the adsorption coefficient for some compounds.
- The use of online SPE with scanning instruments allows for unknown screening in complex environmental samples.

Thank You!
Any Questions?