For trace contaminants in drinking water and wastewater

On-Line SPE LC/MS/MS Applications for Environmental Analysis

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The Measure of Confidence

Agenda Online SPE Solution

- Solid phase extraction
- Setup of automated online SPE
- Example 1: Polycyclic Aromatic Hydrocarbons
- Example 2: Pesticide
- Conclusion

Sample Preparation
Goals for liquid Chromatography

Matrix removal (dissolved and non-dissolved constituents)
- Protects analytical columns
- Increases method selectivity and robustness
- Improves quantitation

Analyte enrichment
- Improves trace analysis performance
- Makes analysis feasible, if original concentration is below limit of detection (LOD)

Sample solvent adaptation (if limited compatibility with LC method)
- Makes analysis feasible
- Improves resolution, LOD, robustness

Sample Preparation
Techniques

Most common techniques
- Solid phase extraction (SPE)
- Liquid-liquid extraction (LLE)
- Precipitation (e.g., proteins) and centrifugation
- Membrane filtration, ultra centrifugation

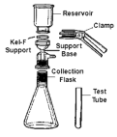
Disadvantages of offline SPE

Often performed manually, there are several disadvantages with this approach.

- Time-consuming, labor and cost intensive, and limits productivity
- Exposure to hazardous or infectious matrices (such as biofluids) raises safety concerns
- Batch to batch reproducibility of the recovery of the analyte can vary

Typical sample preparation for water

- For water screening and quantitation in the 10 ppt range usually requires solid phase extraction (SPE) to pre-concentrate the target compounds in the sample solution



10 ng/L	1 ug/L	10 pg o.c.
1000 mL water sample	1 mL organic extract	Injection volume: 10 µL

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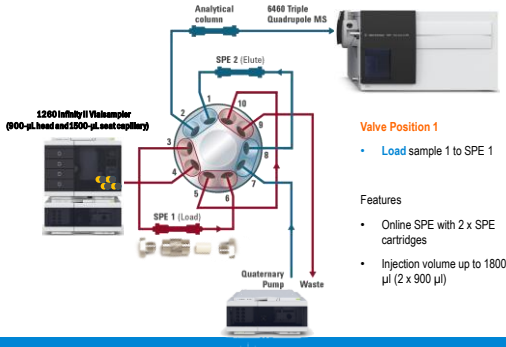
Online SPE

Online Solid Phase Extraction

Automating this SPE process removes the limitations of the manual SPE process:

- Enables direct injection of untreated samples
- Automates sample cleanup and analyte enrichment
- Automation allows for a faster overall workflow
- higher batch to batch reproducibility
- Samples can be run unattended.

Online SPE Process



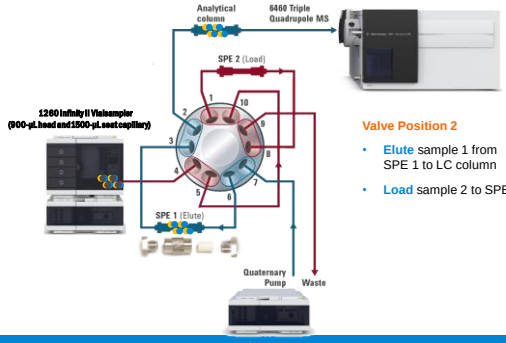
Valve Position 1

- **Load** sample 1 to SPE 1

Features

- Online SPE with 2 x SPE cartridges
- Injection volume up to 1800 µl (2 x 900 µl)

Online SPE Process



Valve Position 2

- **Elute** sample 1 from SPE 1 to LC column
- **Load** sample 2 to SPE 2

Application 1

Sensitive Determination of Polycyclic Aromatic Hydrocarbons in Tap Water by Online Solid Phase Extraction and UHPLC

Polycyclic Aromatic Hydrocarbons

- Formed by the incomplete burning and combustion of hydrocarbons.
- Adsorb onto flying particles which can form as rain drops and snow.
- Leaching from linings of water storage tanks and distribution lines.
- 7 PAHs are classified as probable human carcinogens by the Environmental Protection Agency (US EPA)

Polycyclic Aromatic Hydrocarbons Analysis

EPA / EU Regulations

According to EPA (method 550, 550.1 and 610) and the European Union legislation, 16 parent PAHs should be monitored.

1	Naphthalene	5	Phenantrene	9	Benzo(a)anthracene	13	Benzo(a)pyrene
2	Acenaphthylene	6	Anthracene	10	Chrysene	14	Dibenzo(a,h)anthracene
3	Acenaphtene	7	Fluoranthene	11	Benzo(b)fluoranthene	15	Benzo(g,h,i)perylene
4	Fluorene	8	Pyrene	12	Benzo(k)fluoranthene	16	Indeno(1,2,3-cd)pyrene

- based on offline SPE or LLE
- require **high sample volumes (1 L)**
- are **labor intensive and time-consuming**
- have **run times greater than 60 minutes**

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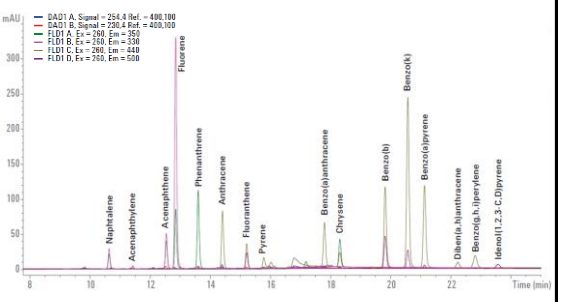
Chromatographic Conditions

Parameter	Value
Analytical column	Agilent ZORBAX Eclipse PAH 4.6 × 150 mm, 3.5 µm (p/n 959961-918)
Column temperature	25 °C
Gradient	A) Water, B) Acetonitrile 0 minutes, 40 %B at 1.3 mL/min 5 minutes, 40 %B 20 minutes, 100 %B 30 minutes, 100 %B 30.1 minutes, 40 %B Posttime 6 minutes
Injection volume	1,800 µL (online SPE), needle wash 3 seconds with acetonitrile
Autosampler temperature	18 °C
Online SPE enrichment column	Polaris 5 C18-A, 3.0 × 50 mm (p/n A2000050X030)
Online SPE enrichment method	A) Water, B) Acetonitrile 0 minutes, 20 %B at 1.3 mL/min 3 minutes, 20 %B 4 minutes at 8 mL/min 5 minutes, 100 %B at 1 mL/min 9 minutes, 100 %B 9.1 minutes, 20 %B at 1 mL/min 15 minutes, 20 %B 15.1 minutes, 20 %B at 0 mL/min
Diode array detector	230 nm, bandwidth 4 nm, reference 400 nm, reference band width 100 nm, 10 Hz
Fluorescence detector	Multisignal acquisition, set at λ_{ex} = 260 nm and λ_{em} = 350 nm (FLD A), 330 nm (FLD B), 440 nm (FLD C), 500 nm (FLD D), 18.51 Hz, PMT 13

Overlay of Chromatograms with UV and FLD

16 PAHs in drinking water (1 µg/L)



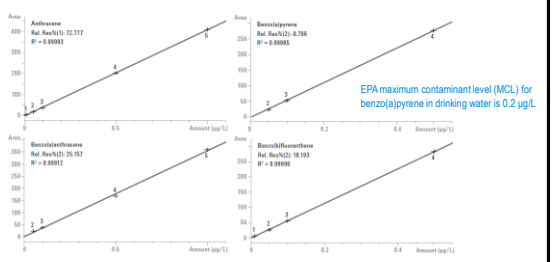
Chromatographic Results

RT, RT & Area RSDs, Detection

Elution order	Compound	Retention time (min)	Detection (nm)	RT RSD (%) (n = 7)	Area RSD (%) (n = 7)
1	Naphthalene	10.6	UV 230/Ref 400 Ex 260, Em 330	0.028	3.8
2	Acenaphthylene	11.4	UV 230/Ref 400	0.025	0.9
3	Acenaphtene	12.5	Ex 260/Em 330	0.029	1.0
4	Fluorene	12.8	Ex 260/Em 330	0.031	0.5
5	Phenantrene	13.5	Ex 260/Em 350	0.035	0.9
6	Anthracene	14.3	Ex 260/Em 440	0.040	0.6
7	Fluoranthene	15.1	Ex 260/Em 440	0.050	0.3
8	Pyrene	15.7	Ex 260/Em 440	0.050	6.0
9	Benzo(a)anthracene	17.7	Ex 260/Em 440	0.070	2.3
10	Chrysene	18.2	Ex 260/Em 350	0.071	0.8
11	Benzo(b)fluoranthene	19.7	Ex 260/Em 440	0.080	1.3
12	Benzo(k)fluoranthene	20.5	Ex 260/Em 440	0.090	1.1
13	Benzo(a)pyrene	22.1	Ex 260/Em 440	0.080	2.5
14	Dibenzo(a,h)anthracene	22.2	Ex 260/Em 440	0.011	2.0
15	Benzo(g,h,i)perylene	22.8	Ex 260/Em 440	0.013	1.4
16	Indeno(1,2,3-cd)pyrene	23.5	Ex 260/Em 500	0.015	3.9

Calibration Curves

Concentration ranges between 0.01 to 10 µg/L, injection volume of 1,800 µL

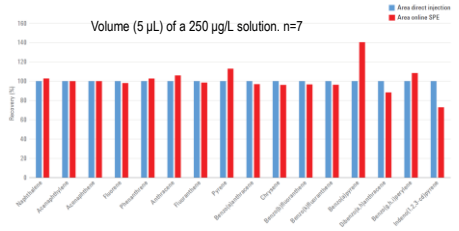


EPA maximum contaminant level (MCL) for benzo(a)pyrene in drinking water is 0.2 µg/L

LOQs for all compounds were between 1 and 38 ng/L except for acenaphthylene, with 77 ng/L (UV)
The online SPE system is **10 times more sensitive** with an injection volume of 1,800 µL

Recovery

Online SPE vs Direct Injection



Online SPE is perfectly suited for the analysis of PAHs in drinking water because no analyte is flushed out and lost during the enrichment process.

Application 2

Quantitative LC/MS/MS Analysis of 22 pesticides including Dichlorophenols in Water Using on-line SPE Enrichment.

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Results requirement

Name	LOQ µg/L	CV %	Recovery %
2,4-D	0,010	5	98-102
MCPA	0,010	5	98-102
Mechlorprop	0,010	5	98-102
Dinoseb	0,010	5	98-102
2,6-dichlorphenol *	0,010	5	98-102
Terbutylazin	0,010	5	98-102
Isoproturon	0,010	5	98-102
Atrazin	0,010	5	98-102
Bentazon	0,010	5	98-102
Pendimethalin	0,010	5	98-102
Cyanazin	0,010	5	98-102
2-Hydroxy-atrazin	0,010	5	98-102
Desethylatrazin	0,010	5	98-102
Hexazinon	0,010	5	98-102
DNOC	0,010	5	98-102
Dichlorprop	0,010	5	98-102
Desisopropylatrazin	0,010	5	98-102
2,6-dichlorbenzamid *	0,010	5	98-102
Dimethoat	0,010	5	98-102
Metamitron	0,010	5	98-102
2,4-dichlorphenol *	0,010	5	98-102
Simazin	0,010	5	98-102

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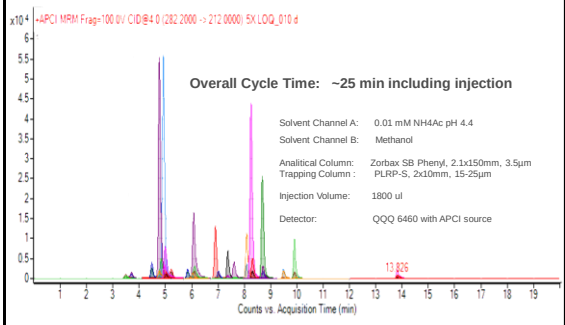
Level at 5 time the LOQ = 50 ng/l



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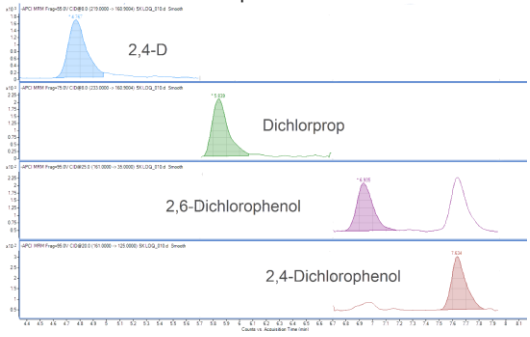
The difficult four compounds ….resolved



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Summary and Conclusions

- Online SPE/LC-QQQ simple, fast and reliable analysis at concentration of ng/L range.
- fully controlled and automate
- Good recovery reproducibilities
- time and labor savings compared to off line SPE

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Thank you for your Attention

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