High Through-put Analysis of Contaminants in Drinking Water or Surface Water with Integrated Sample Clean-up and Spectral Confirmation

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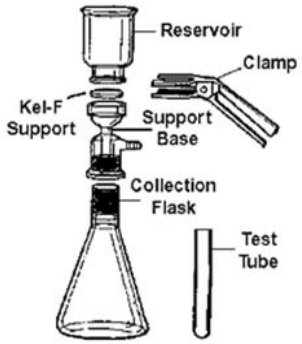
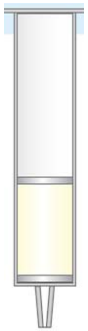
Typical sample preparation for water

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

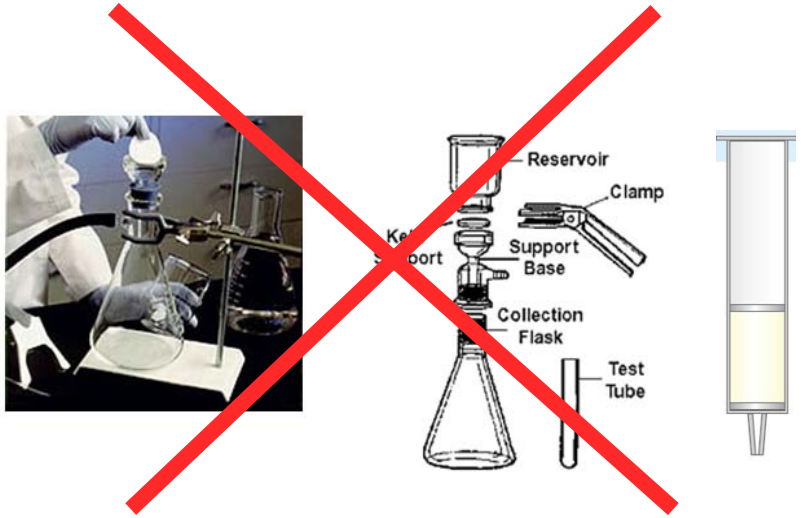
Solid Phase Extraction (SPE)





- 1-5 liters transported to the lab
- Trace enrich onto a cartridge or disk
- Elute with few mLs solvent, dry, reconstitute
- Amenable LC, % organic solvent
- 1-5 mL eluent loaded into autosampler vial
- 2-20 uL injected, HPLC with UV/MS detection

Solid Phase Extraction (SPE)



Approaches:

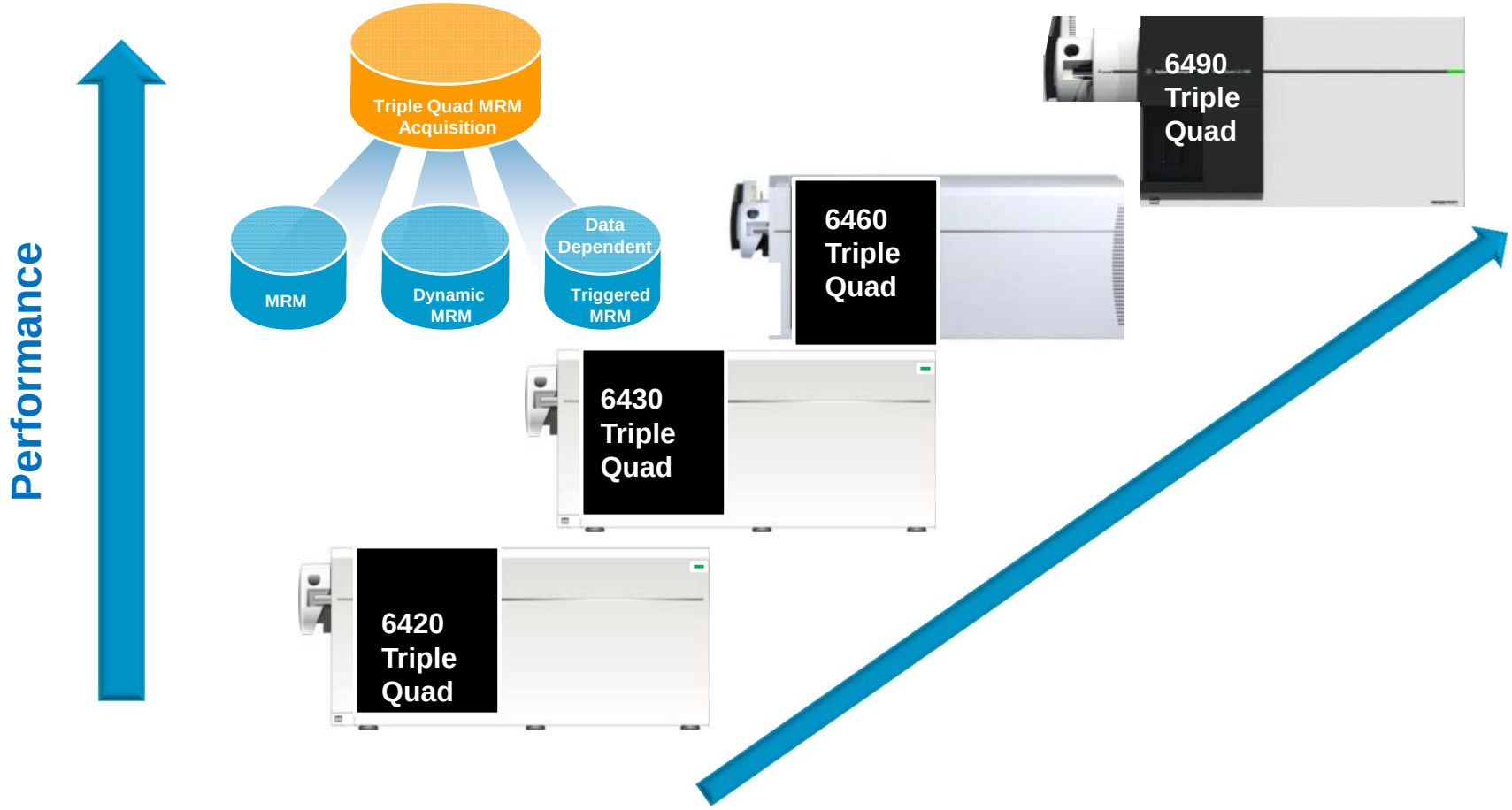
1. **Automate SPE in LCMS workflow**
2. **Bypass SPE and direct injection**

-both reduce sample size and solvent volumes.

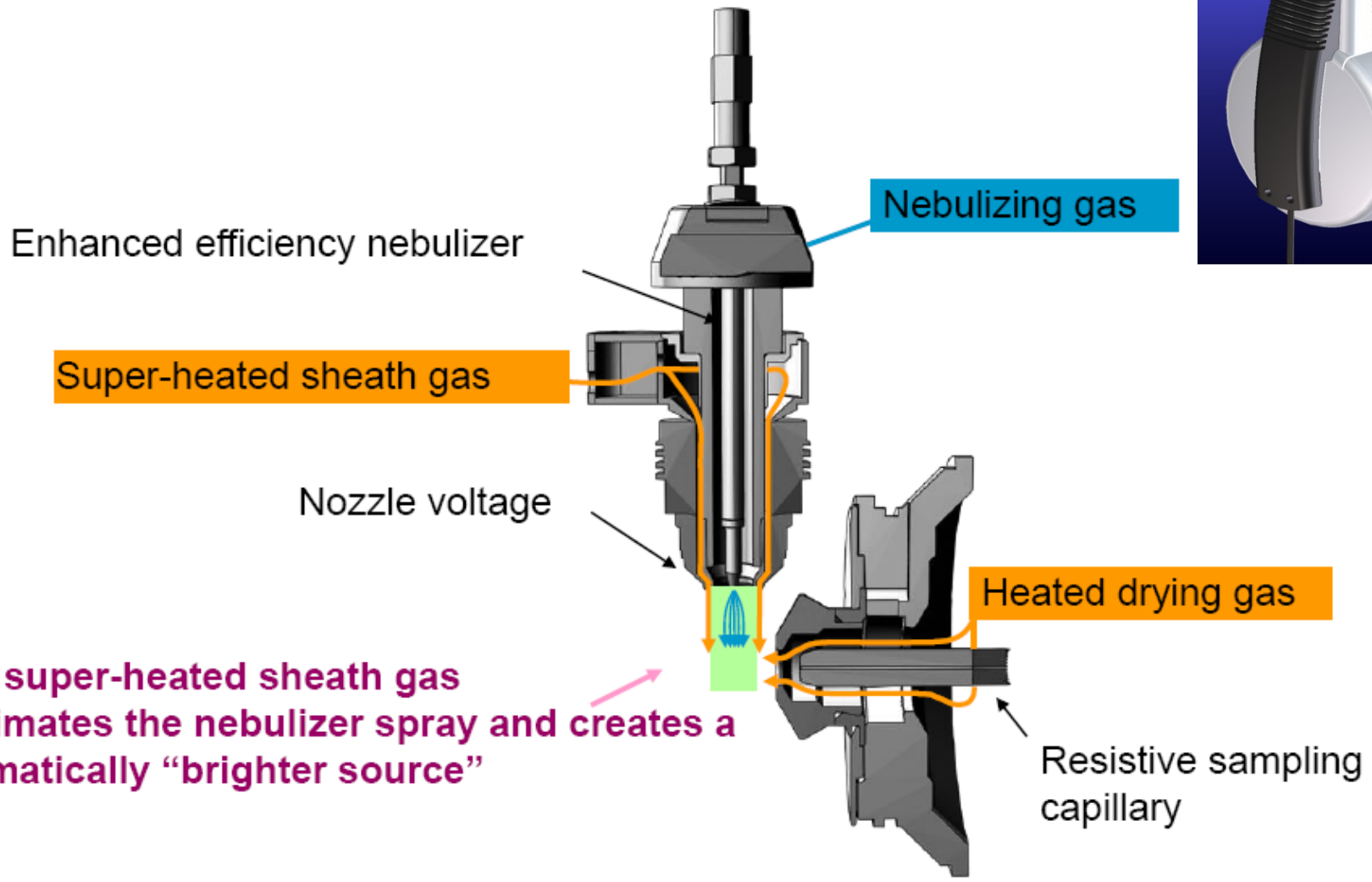
Agenda

- Instrumentation and system configurations
- Method comparison
  - Direct injection (Agilent 6490 QQQ)
  - Online SPE (Agilent 6460 QQQ)
- Spectral Confirmation
- Conclusion

Triple Quadrupoles from Agilent

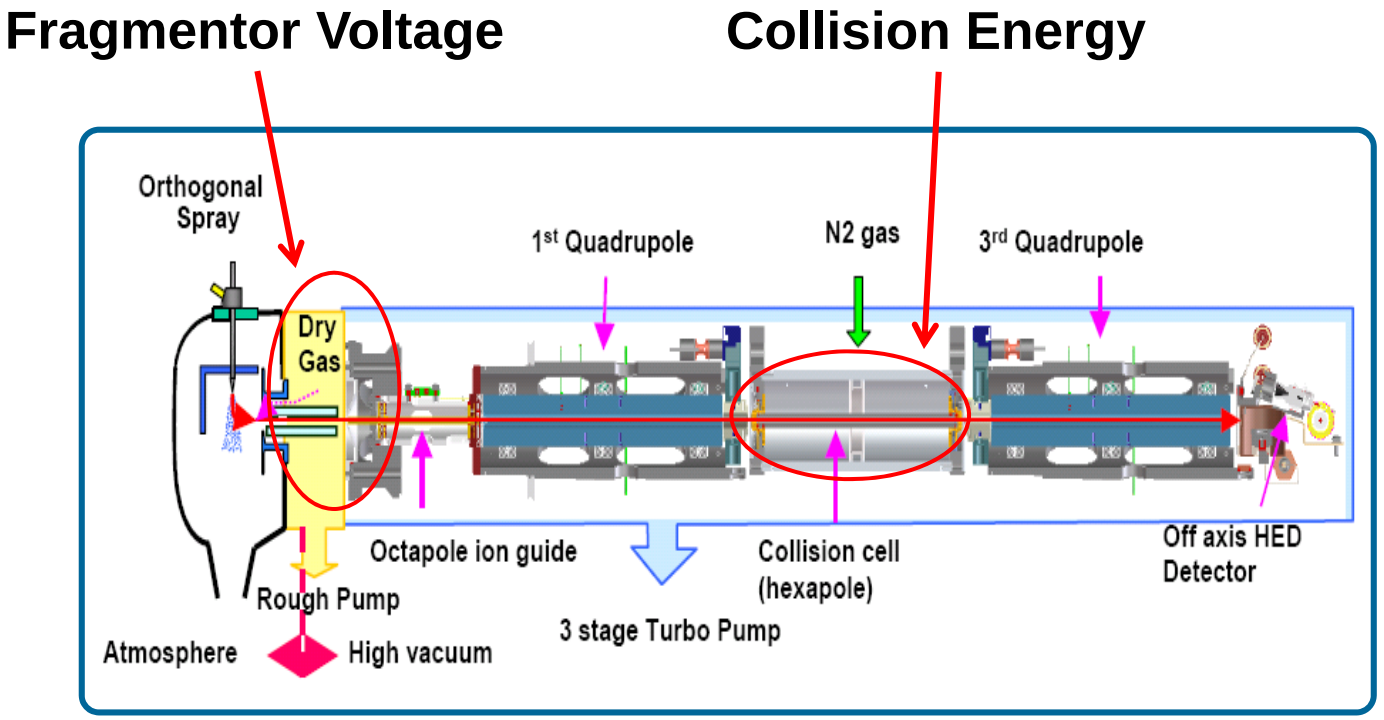


Agilent Jet Stream



The super-heated sheath gas collimates the nebulizer spray and creates a dramatically "brighter source"

QQQ Analyte Optimization: 2 key settings.



Experiment

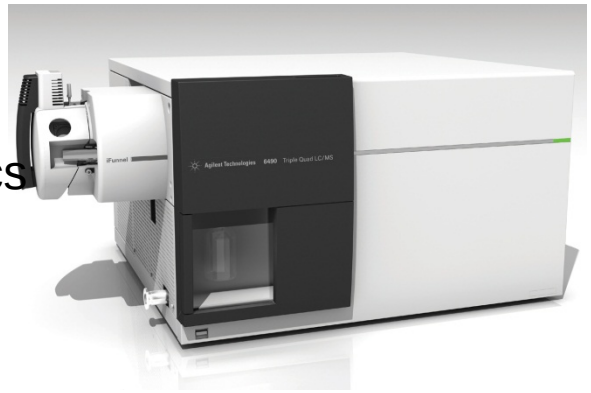
- 28 pesticides spiked into samples of drinking water, ground water and surface water (Part of a round robin from German government)
- Analyzed via direct injection with Agilent 6490 QQQ
- Analyzed via on-line SPE with Agilent 6460 QQQ with Flex cube

Instrumentation and system configuration

Direct injection

- **Infinity 1290 UHPLC system** consisting of G4220A binary pump, G4226A High Performance sampler equipped with G4216A 1290 large volume injection kit, G1316C column compartment
- **Zorbax Eclipse Plus C-18 RRHD**, 100 x 2.1 mm, 1.8 µm

- **G6490A QQQ** mass spectrometer with Agilent Jet Stream ionization source and dual ion funnel ion optics
- **Dynamic MRM** acquisition

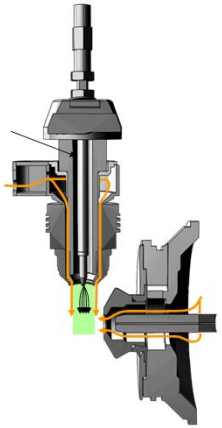


Agilent iFunnel technology

Captures 6 to 10 times more ions

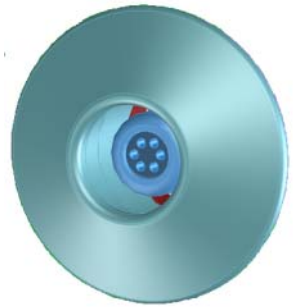
Agilent Jet Stream

- Thermal confinement of ESI plume to create ion rich zone
- Efficient desolvation to create gas phase ions
- Effective ionization across broad range of analyte classes including many APCI compounds



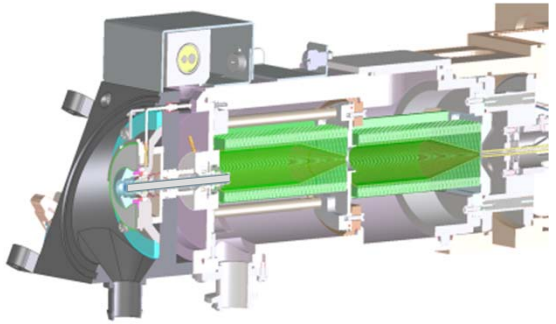
Hexabore Capillary

- Six bores and half the length means much less restriction
- Samples 6 to 10 times more ion rich gas from the source with 6 capillaries
- Captures the majority of the gas from the source region



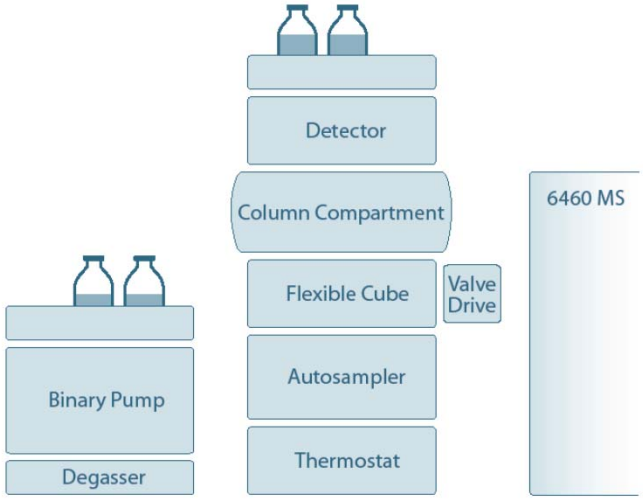
Dual Ion Funnel

- Removes the gas but captures the ions
- Makes skimmer and one compound dependant parameter obsolete
- Removes neutral noise
- Low capacitance design allows for fast polarity switching



Instrumentation and system configuration

Online SPE



- **Infinity HPLC system** consisting of G1312B binary pump, G1329B autosampler with 900 µL sample head, G4227A Flexible Cube with two G4232B 2-position/10-port valves, G1316C column compartment
- 2 x **PLRP-S cartridges**, 12.5 x 2.1 mm in Guard Column Hardware Kit for trapping
- **Zorbax Eclipse Plus C-18**, 150 x 2.1 mm, 3.5 µm

- **G6460A QQQ** mass spectrometer with Agilent Jet Stream ionization source
- **Dynamic MRM** acquisition

Online SPE with the Flex Cube

Instrumentation and system configuration

G4227A Flexible Cube

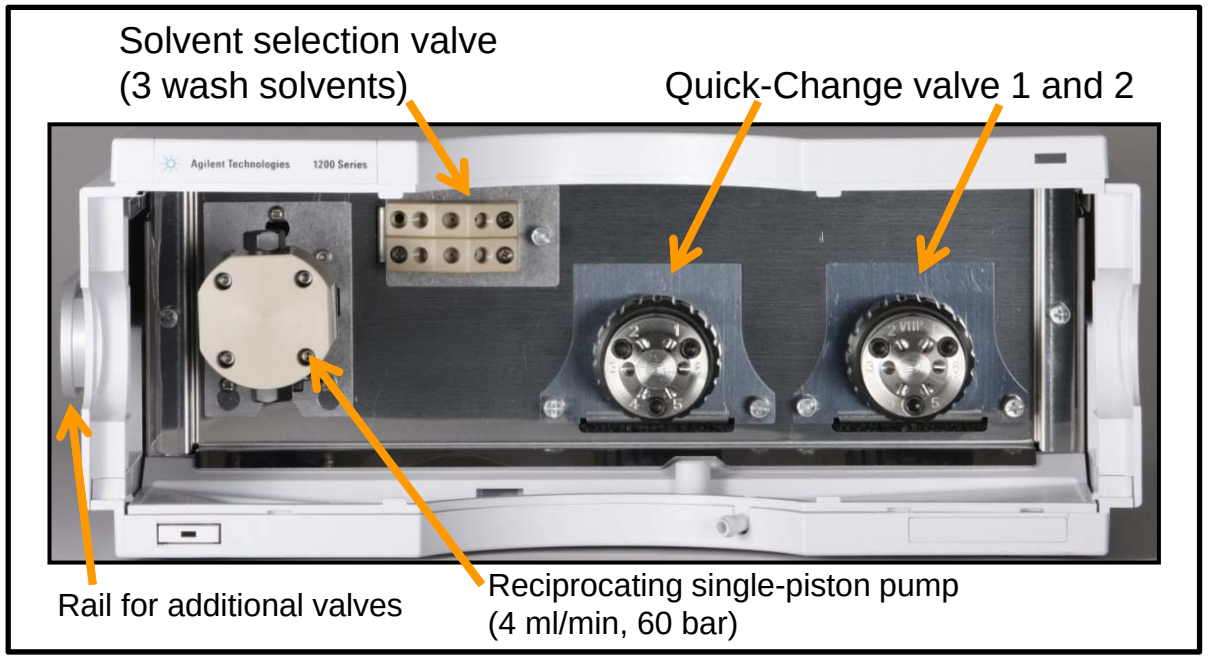
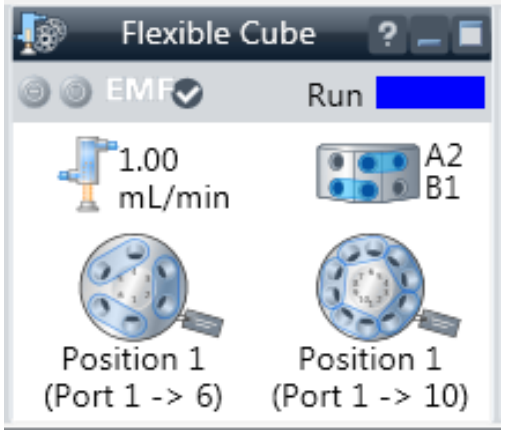


Figure 1) The Agilent 1290 Infinity Flexible Cube is an additional module to the 1290/1260 Infinity LC system hosting up to two 1200 Infinity Series Quick-Change valves.

Stages in operation for ON-Line SPE LC/MS

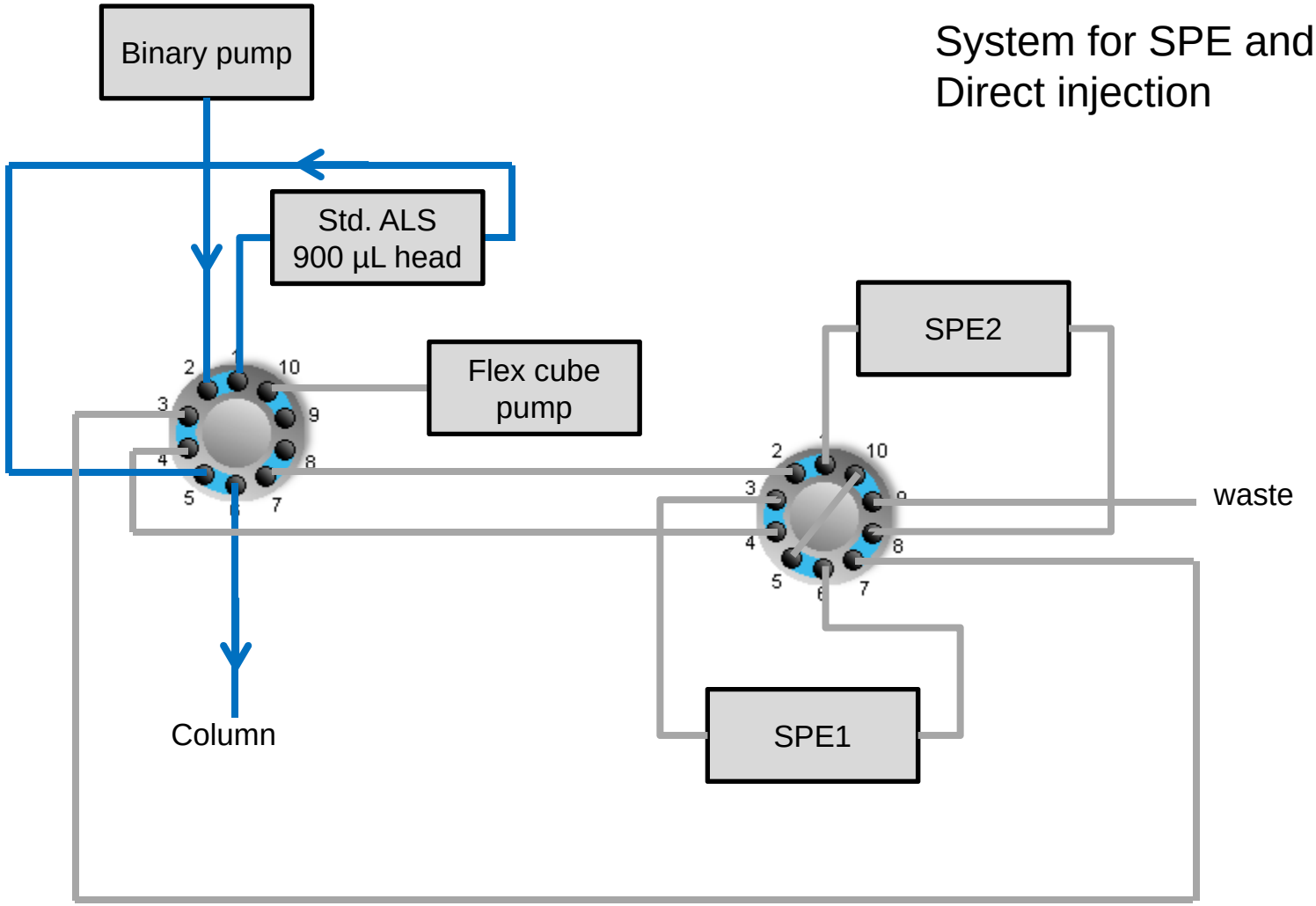
1. Load water sample (0.5 to 2.3 ml) on the enrichment cartridge.
2. Valve switch, ‘backflush‘ desorb using hplc gradient.
3. Separate on the analytical column and collect MRM tranistions on the QQQ.
4. Condition the second enrichment cartridge using the flexcube pump during analysis.
5. Ready for next sample after ~ 22 minutes.



Instrumentation and system configuration

Tubing and column switching (1)

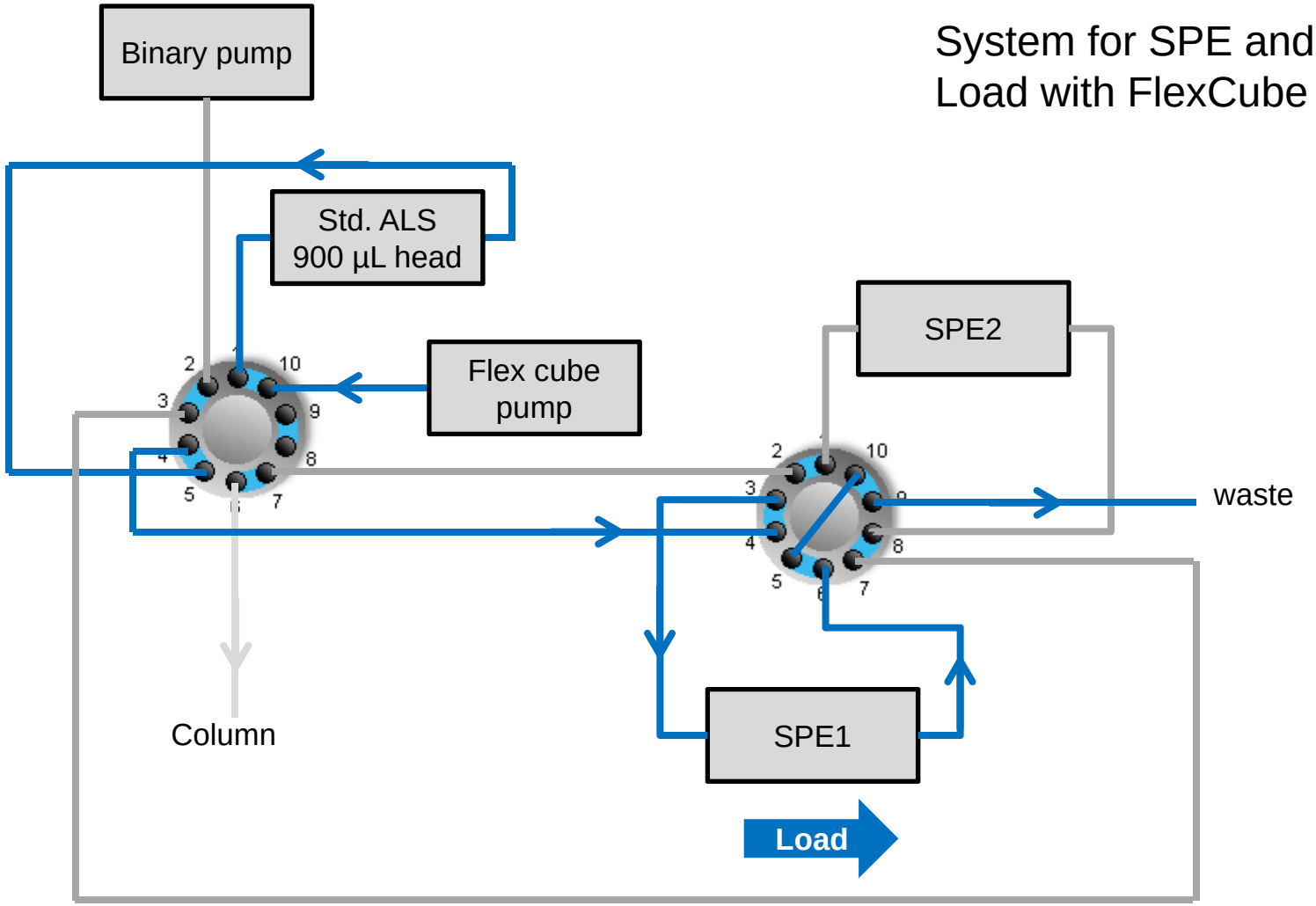
System for SPE and direct injection:
Direct injection



Instrumentation and system configuration

Tubing and column switching (2)

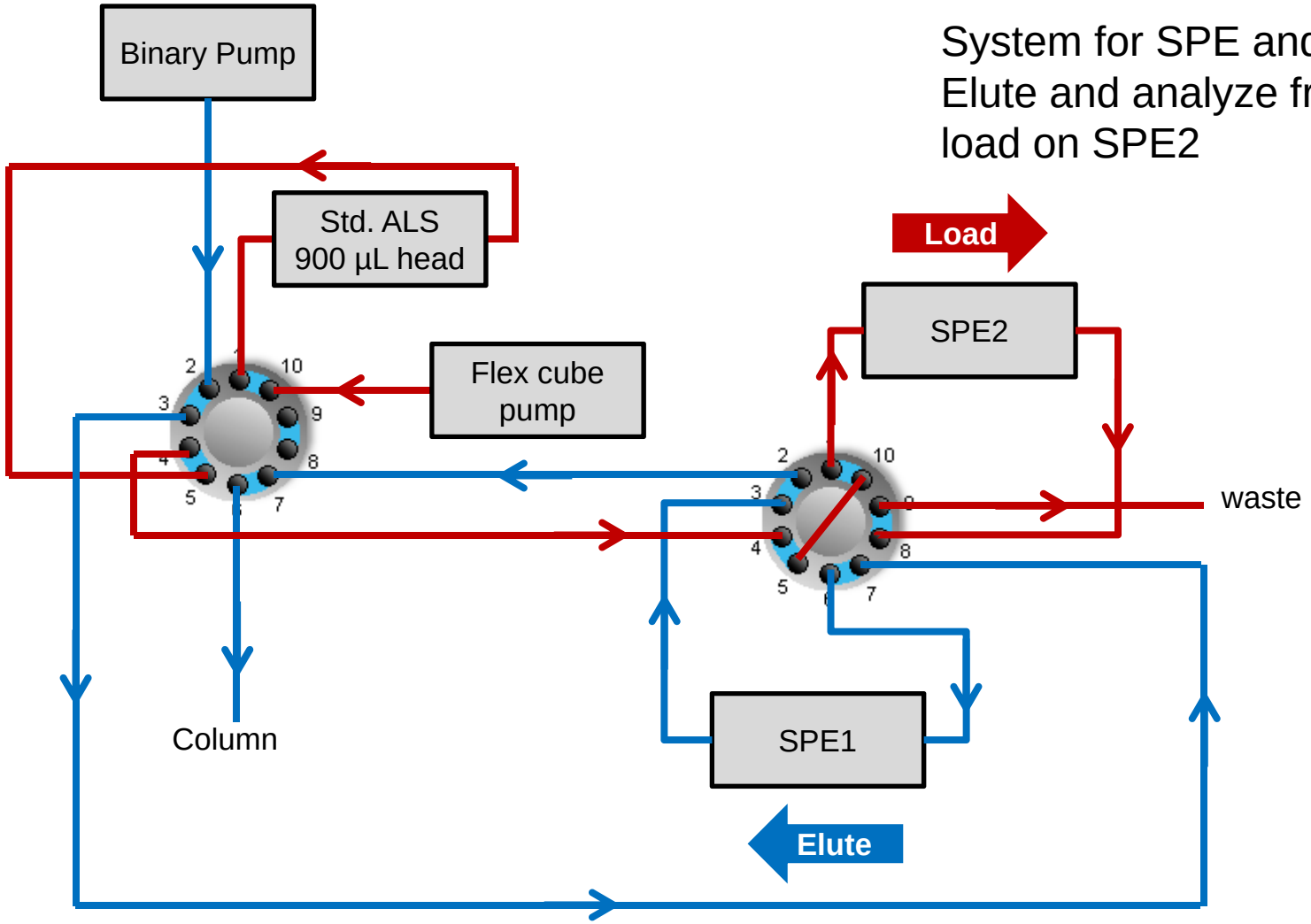
System for SPE and direct injection:
Load with FlexCube on SPE1



Instrumentation and system configuration

Tubing and column switching (3)

System for SPE and direct injection:
Elute and analyze from SPE1,
load on SPE2



LCMS method comparison

Direct injection method

Parameter	Values
Injection volume:	100 µl
Gradient program:	0.00 min – 2% B 1.00 min – 2% B 2.00 min – 25% B 13.0 min – 100% B 15.0 min – 100% B 15.1 min – 2 % B Post time: 2 min
Mobile phase:	5 mM ammonium formate in water (A) and methanol (B)
MS parameters:	28 compounds with 2 transitions for each compound Dynamic MRM acquisition, Minimum Dwell time 20 ms
Spray chamber conditions:	Gas temp.: 120°C Dry gas: 14 l/min Nebulizer: 35 psi Sheath gas temp: 350°C Sheath gas flow: 12 l/min CapVoltage: 3000 V Nozzle voltage 300 V
Calibration:	External calibration in drinking water (1 – 5 –) 10 – 20 – 50 – 100 – 200 – 500 – 1000 ng/L; QC 100 ng/L
Sample pretreatment:	Samples were centrifuged for 5 min @ 15,000rpm

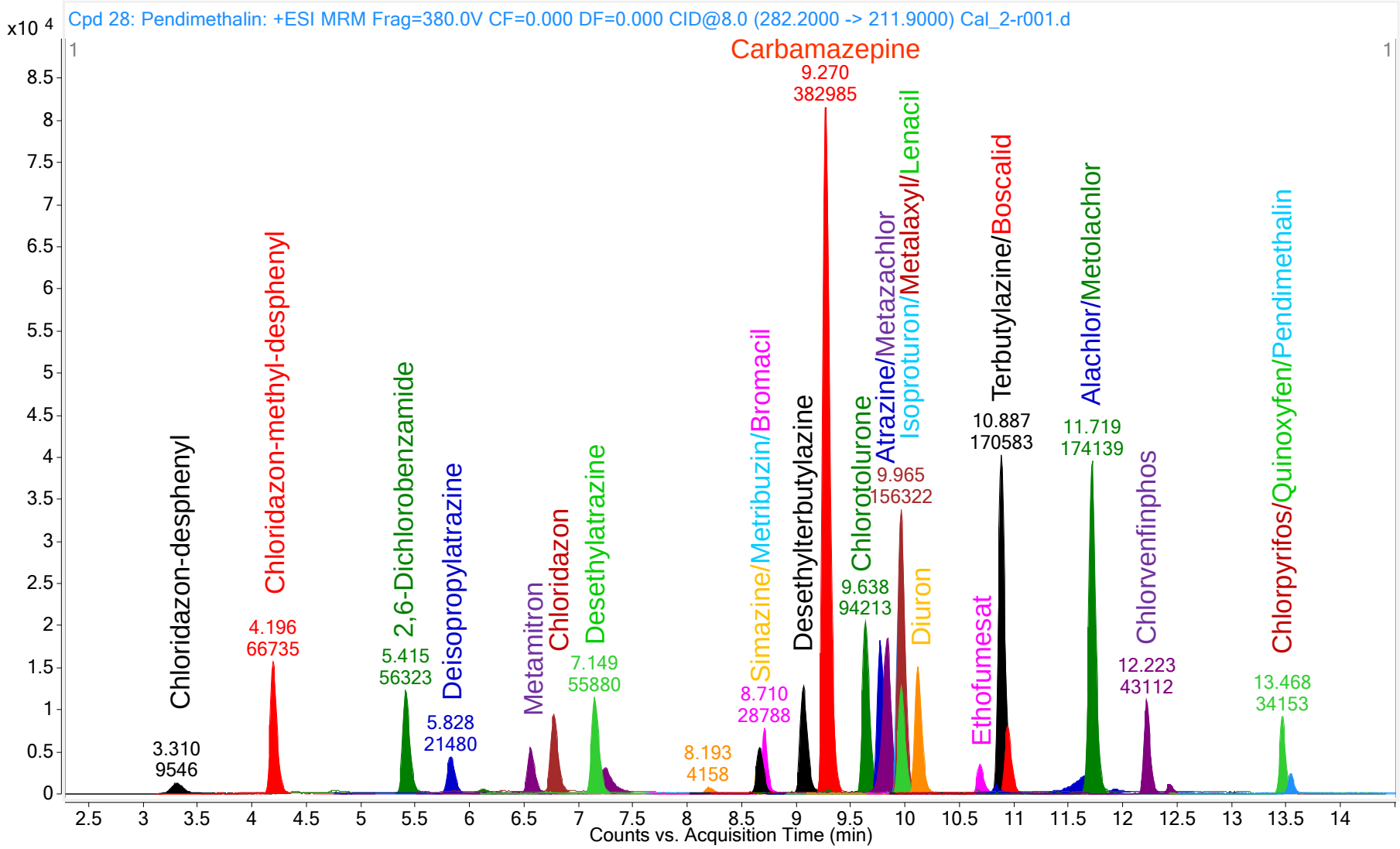
LCMS method comparison

Online SPE method

Parameter	Values	
Injection volume:	900 µl	
Gradient program:	0.00 min – 2% B 2.00 min – 2% B 2.50 min – 25% B 12.0 min – 100% B 22.0 min – 100% B 22.1 min – 2 % B Post time: 10 min	<u>Loading:</u> 0.00 min – Pump 60 s, flow 1 mL/min, Channel A1 (water) 2.00 min – Switch Valve 2.10 min – Pump 180 s, flow 1.5 mL/min, Channel A2 (acetonitrile) 6.00 min – Pump 300 s, flow 1.5 mL/min, Channel A1 (water)
Mobile phase:	0.01% formic acid and 5 mM ammonium formate in water (A) and methanol (B)	
MS parameters:	28 compounds with 2 transitions for each compound Dynamic MRM acquisition, Minimum Dwell time 17.5 ms	
Spray chamber conditions:	Gas temp.: 260°C Dry gas: 9 l/min Nebulizer: 45 psi Sheath gas temp: 300°C Sheath gas flow: 12 l/min CapVoltage: 3500 V Nozzle voltage 500 V	
Calibration:	External calibration in drinking water 10 – 25 – 50 – 100 – 200 – 500 ng/L; QC 100 ng/L	
Sample pretreatment:	Samples were centrifuged for 5 min @ 15,000rpm	

Results direct injection

MRM traces for target compounds



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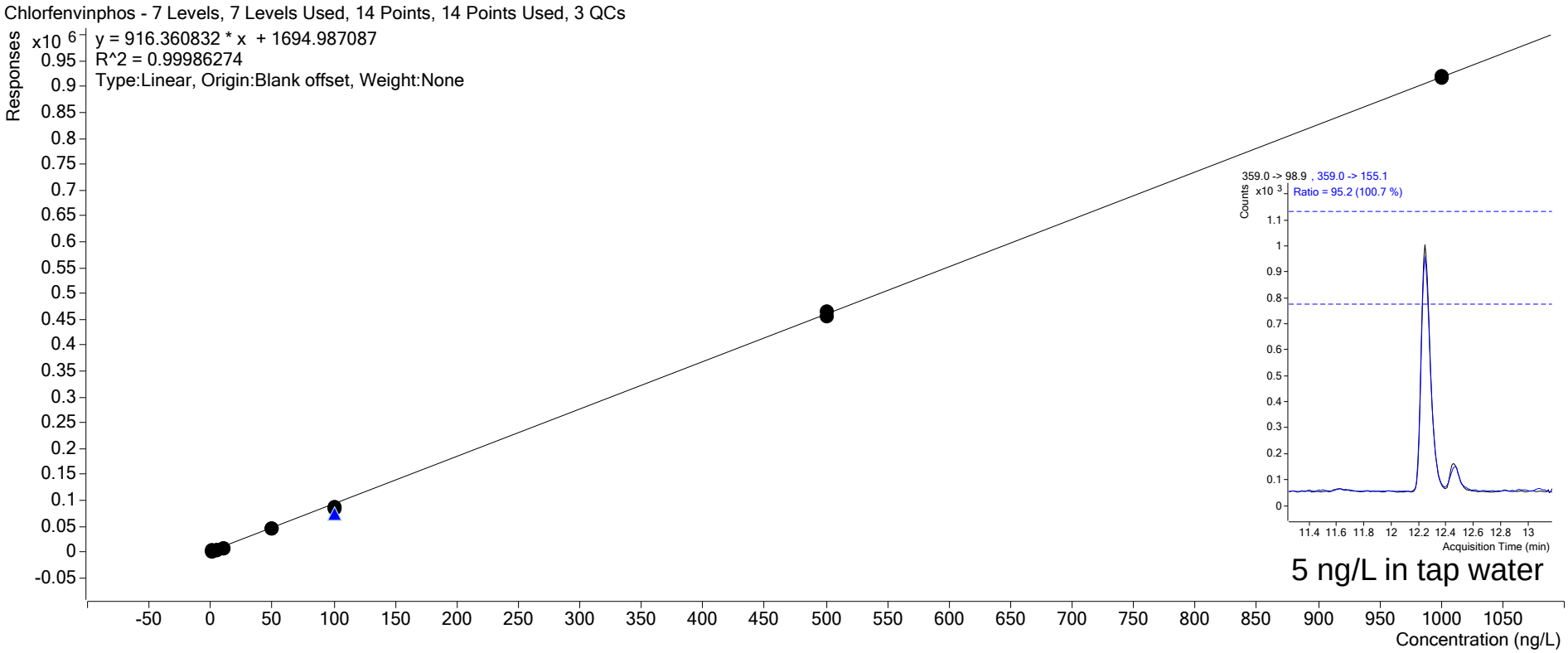
Results direct injection

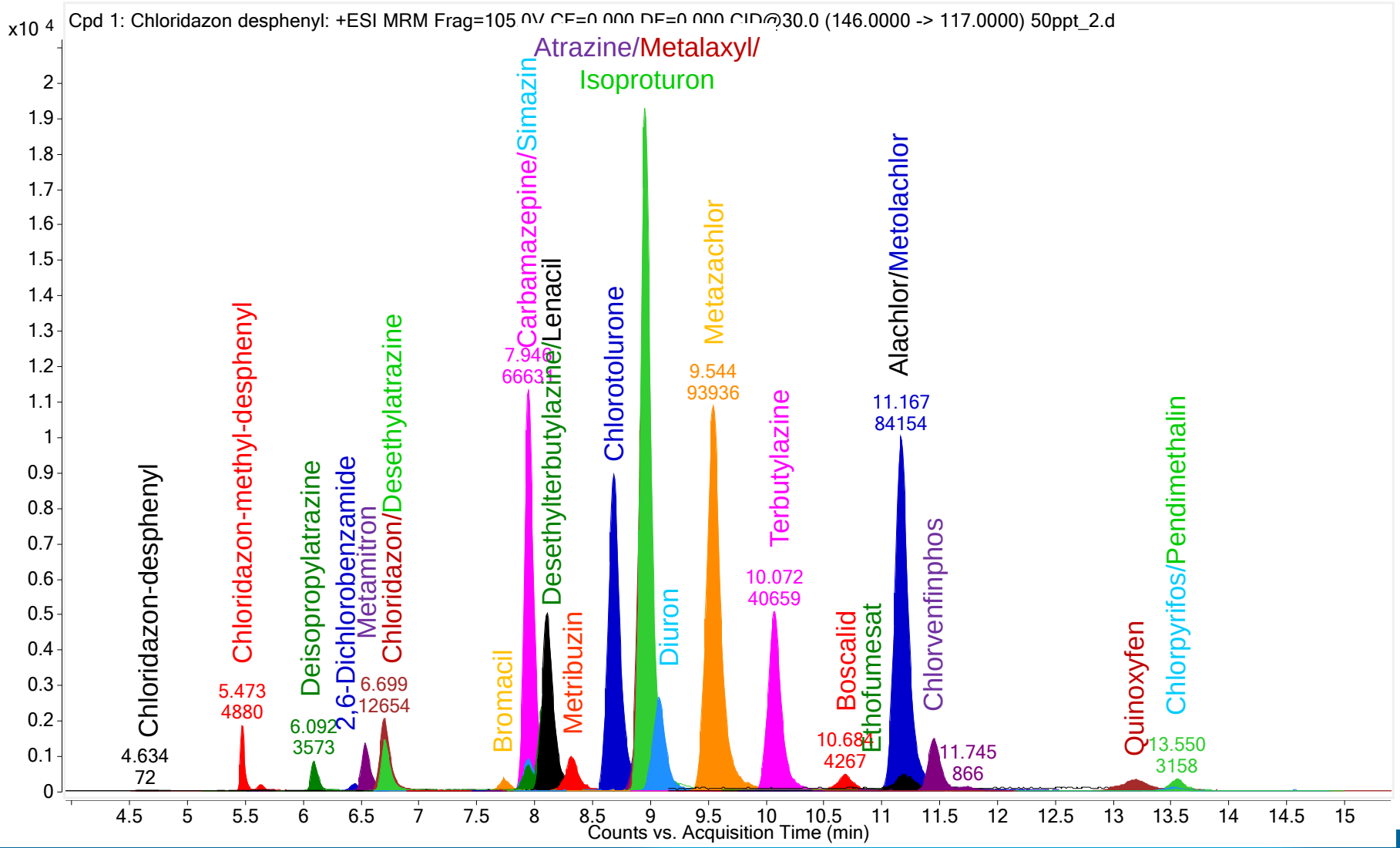
Chlorfenvinphos (RT 12.2 min)



1, 5, 10, 50, 100, 500, 1000 ng/l – 7 level

Results Online-SPE

MRM traces for target compounds



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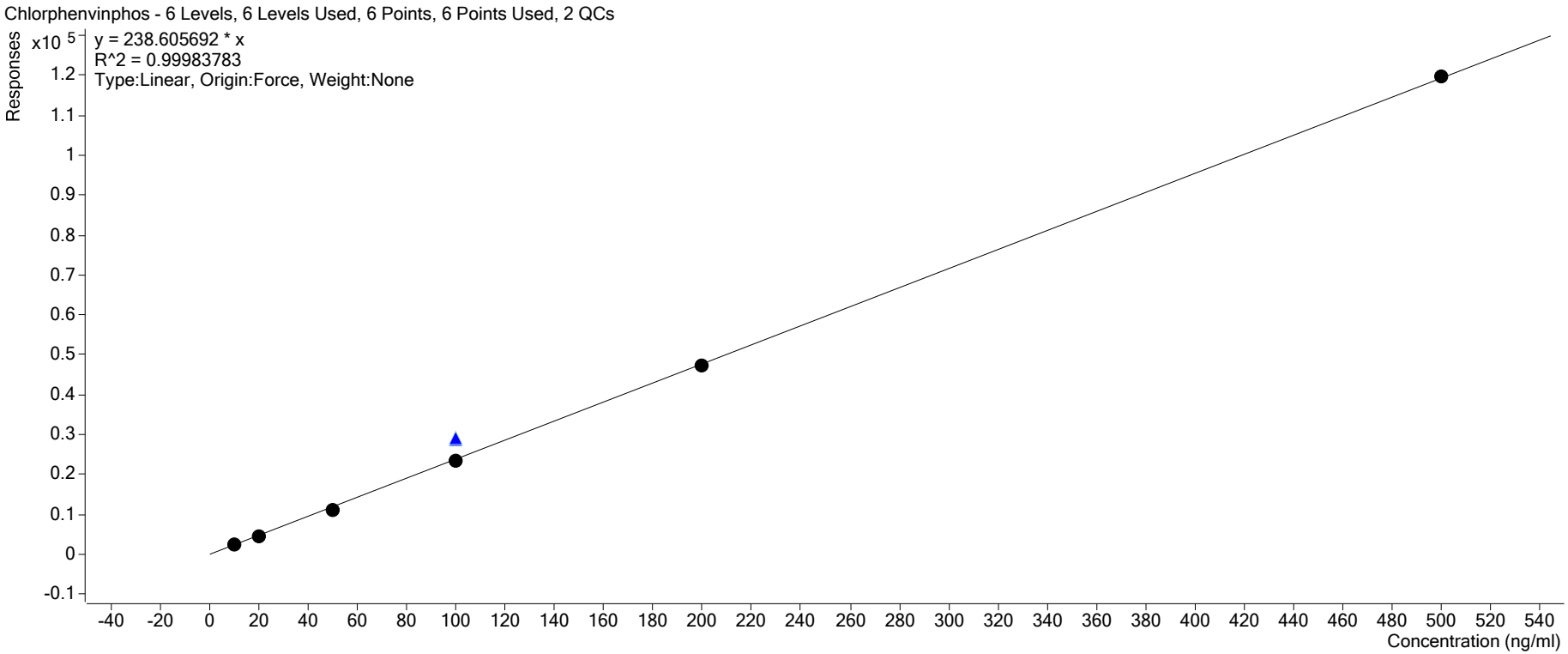
Results Online-SPE

Chlorfenvinphos (RT 11.3 min)



10, 20, 50, 100, 200, 500 ng/l – 6 level

Direct injection (6490 QQQ) vs Online SPE Sample 1 (6460 QQQ) (Tap water)

	Average	SD	Reference	Accuracy
Chloridazon-desphenyl	0.0478	0.0004	0.05	95.5
Chloridazon-methyldesphenyl	0.0773	0.0003	0.08	96.6
2,6-Dichlorbenzamid	0.0459	0.0002	0.05	91.8
Atrazin-desisopropyl	0.1037	0.0004	0.1	103.7
Metamitron	0.0834	0.0013	0.08	104.2
Chloridazon	0.0996	0.0005	0.1	99.6
Atrazin-desethyl	0.0524	0.0003	0.05	104.9
Metribuzin	0.0799	0.0004	0.08	99.9
Bromacil	0.1043	0.0015	0.1	104.3
Simazin	0.0788	0.0012	0.08	98.5
Carbamazepin	0.0973	0.0008	0.1	97.3
Terbuthylazin-desethyl	0.0527	0.0009	0.05	105.3
Chlortoluron	0.0794	0.0016	0.08	99.2
Metazachlor	0.0818	0.0008	0.08	102.3
Metalaxyl	0.0475	0.0007	0.05	95.0
Atrazin	0.0874	0.0013	0.08	109.3
Lenacil	0.0515	0.0010	0.05	102.9
Isoproturon	0.1256	0.0015	0.12	104.6
Diuron	0.0816	0.0014	0.08	102.0
Ethofumesat	0.0997	0.0018	0.1	99.7
Boscalid	0.0750	0.0003	0.08	93.8
Terbuthylazin	0.1105	0.0021	0.1	110.5
Alachlor	0.0731	0.0010	0.08	91.4
Metolachlor	0.0744	0.0003	0.08	92.9
Chlorfenvinphos	0.0338	0.0006	0.05	67.6
Chlorpyrifos	0.0379	0.0015	0.12	31.6
Quinoxyfen	0.0507	0.0012	0.08	63.4
Pendimethalin	0.0716	0.0018	0.1	71.6

	Average	SD	Reference	Accuracy
Chloridazon-desphenyl			0.05	
Chloridazon-methyldesphenyl	0.0889	0.0039	0.08	111.1
2,6-Dichlorbenzamid	0.0502	0.0025	0.05	100.5
Atrazin-desisopropyl	0.1116	0.0019	0.1	111.6
Metamitron	0.0921	0.0017	0.08	115.1
Chloridazon	0.0975	0.0011	0.1	97.5
Atrazin-desethyl	0.0530	0.0005	0.05	106.0
Metribuzin	0.0920	0.0018	0.08	115.0
Bromacil	0.1292	0.0026	0.1	129.2
Simazin	0.0903	0.0014	0.08	112.9
Carbamazepin	0.1063	0.0004	0.1	106.3
Terbuthylazin-desethyl	0.0581	0.0005	0.05	116.2
Chlortoluron	0.0907	0.0008	0.08	113.3
Metazachlor	0.0954	0.0003	0.08	119.3
Metalaxyl	0.0558	0.0006	0.05	111.7
Atrazin	0.1043	0.0017	0.08	130.4
Lenacil	0.0554	0.0006	0.05	110.8
Isoproturon	0.1390	0.0012	0.12	115.9
Diuron	0.0948	0.0008	0.08	118.5
Ethofumesat	0.1044	0.0071	0.1	104.4
Boscalid	0.0972	0.0010	0.08	121.5
Terbuthylazin	0.1274	0.0007	0.1	127.4
Alachlor	0.0959	0.0005	0.08	119.8
Metolachlor	0.0919	0.0003	0.08	114.9
Chlorfenvinphos	0.0621	0.0008	0.05	124.1
Chlorpyrifos	0.0424	0.0029	0.12	35.3
Quinoxyfen	0.0767	0.0068	0.08	95.8
Pendimethalin	0.1390	0.0120	0.1	139.0

Direct injection (6490 QQQ) vs Online SPE Sample 1 (6460 QQQ) (Tap water)

	Average	SD	Reference	Accuracy
Chloridazon-desphenyl	0.0478	0.0004	0.05	95.5
Chloridazon-methyldesphenyl	0.0773	0.0003	0.08	96.6
2,6-Dichlorbenzamid	0.0459	0.0002	0.05	91.8
Atrazin-desisopropyl	0.1037	0.0004	0.1	103.7
Metamitron	0.0834	0.0013	0.08	104.2
Chloridazon	0.0996	0.0005	0.1	99.6
Lenacil	0.0515	0.001		
Isoproturon	0.1256	0.001		
Diuron	0.0816	0.001		
Ethofumesat	0.0997	0.001		
Boscalid	0.0750	0.000		
Terbuthylazin	0.1105	0.002		
Alachlor	0.0731	0.001		
Metolachlor	0.0744	0.000		
Chlorfenvinphos	0.0338	0.000		
Chlorpyrifos	0.0379	0.0015	0.12	31.6
Quinoxyfen	0.0507	0.0012	0.08	63.4
Pendimethalin	0.0716	0.0018	0.1	71.6

	Average	SD	Reference	Accuracy
Chloridazon-desphenyl			0.05	
Chloridazon-methyldesphenyl	0.0889	0.0039	0.08	111.1
2,6-Dichlorbenzamid	0.0502	0.0025	0.05	100.5
Atrazin-desisopropyl	0.1116	0.0019	0.1	111.6
Metamitron	0.0921	0.0017	0.08	115.1
Chloridazon	0.0975	0.0011	0.1	97.5
Atrazin-desethyl	0.0530	0.0005	0.05	106.0
	0.0920	0.0018	0.08	115.0
	0.1292	0.0026	0.1	129.2
	0.0903	0.0014	0.08	112.9
epin	0.1063	0.0004	0.1	106.3
zin-desethyl	0.0581	0.0005	0.05	116.2
on	0.0907	0.0008	0.08	113.3
or	0.0954	0.0003	0.08	119.3
	0.0558	0.0006	0.05	111.7
	0.1043	0.0017	0.08	130.4
				0.8
				5.9
				8.5
				4.4
				1.5
				7.4
				9.8
				4.9
				4.1
Chlorpyrifos	0.0424	0.0029	0.12	35.3
Quinoxyfen	0.0767	0.0068	0.08	95.8
Pendimethalin	0.1390	0.0120	0.1	139.0

Chloridazon-desphenyl	0.0478	0.0004	0.05	95.5
Chloridazon-methyldesphenyl	0.0773	0.0003	0.08	96.6
2,6-Dichlorbenzamid	0.0459	0.0002	0.05	91.8
Atrazin-desisopropyl	0.1037	0.0004	0.1	103.7
Metamitron	0.0834	0.0013	0.08	104.2
Chloridazon	0.0996	0.0005	0.1	99.6

Chloridazon-desphenyl		0.05	
Chloridazon-methyldesphenyl	0.0889 ± 0.0039	0.08	111.1
2,6-Dichlorbenzamid	0.0502 ± 0.0025	0.05	100.5
Atrazin-desisopropyl	0.1116 ± 0.0019	0.1	111.6
Metamitron	0.0921 ± 0.0017	0.08	115.1
Chloridazon	0.0975 ± 0.0011	0.1	97.5

Direct injection (6490 QQQ) vs Online SPE (6460 QQQ) Sample 3 (Surface water)

	Average	SD	Reference	Accuracy
Chloridazon-desphenyl	0.2317 ±	0.0013	0.182	127.3
Chloridazon-methyldesphenyl	0.2868 ±	0.0019	0.319	89.9
2,6-Dichlorbenzamid	0.0914 ±	0.0005	0.1	91.4
Atrazin-desisopropyl	0.1407 ±	0.0013	0.16	87.9
Metamitron	0.0432 ±	0.0002	0.05	86.5
Metazachlor	0.1672 ±	0		
Metalaxyl	0.0940 ±	0		
Atrazin	0.3033 ±	0		
Lenacil	0.1758 ±	0		
Isoproturon	0.1128 ±	0		
Diuron	0.2940 ±	0		
Ethofumesat	0.0468 ±	0		
Boscalid	0.1477 ±	0		
Terbuthylazin	0.0530 ±	0.0002	0.05	106.0
Alachlor	0.2836 ±	0.0030	0.3	94.5
Metolachlor	0.0468 ±	0.0004	0.05	93.6
Chlorfenvinphos	0.1293 ±	0.0012	0.16	80.8
Chlorpyrifos	0.0596 ±	0.0005	0.1	59.6
Quinoxyfen	0.0487 ±	0.0009	0.1	48.7
Pendimethalin	0.1024 ±	0.0006	0.16	64.0

	Average	SD	Reference	Accuracy
Chloridazon-desphenyl	0.2317 ±	0.0013	0.182	127.3
Chloridazon-methyldesphenyl	0.2868 ±	0.0019	0.319	89.9
2,6-Dichlorbenzamid	0.0914 ±	0.0005	0.1	91.4
Atrazin-desisopropyl	0.1407 ±	0.0013	0.16	87.9
Metamitron	0.0432 ±	0.0002	0.05	86.5

	Average	SD	Reference	Accuracy
Chloridazon-desphenyl			0.182	
Chloridazon-methyldesphenyl	0.2767 ±	0.0104	0.319	86.7
2,6-Dichlorbenzamid	0.1089 ±	0.0036	0.1	108.9
Atrazin-desisopropyl	0.1673 ±	0.0015	0.16	104.6
Metamitron	0.0509 ±	0.0011	0.05	101.9
	0.0471 ±	0.0009	0.05	94.2
	0.1322 ±	0.0024	0.1	132.2
	0.1845 ±	0.0029	0.18	102.5
	0.0550 ±	0.0029	0.05	110.0
	0.1704 ±	0.0034	0.16	106.5
	0.1653 ±	0.0019	0.137	120.7
	0.0917 ±	0.0019	0.08	114.6
	0.0745 ±	0.0004	0.068	109.6
Terbuthylazin	0.0636 ±	0.0005	0.05	127.1
Alachlor	0.3727 ±	0.0054	0.3	124.2
Metolachlor	0.0600 ±	0.0007	0.05	120.1
Chlorfenvinphos	0.1956 ±	0.0029	0.16	122.2
Chlorpyrifos	0.0938 ±	0.0018	0.1	93.8
Quinoxyfen	0.0591 ±	0.0028	0.1	59.1
Pendimethalin	0.1389 ±	0.0030	0.16	86.8

Chloridazon-desphenyl			0.182	
Chloridazon-methyldesphenyl	0.2767 ±	0.0104	0.319	86.7
2,6-Dichlorbenzamid	0.1089 ±	0.0036	0.1	108.9
Atrazin-desisopropyl	0.1673 ±	0.0015	0.16	104.6
Metamitron	0.0509 ±	0.0011	0.05	101.9

Results direct injection

QC samples (100 ng/L)

Chlorfenvinphos

Sample								Chlorfenvi...	Chlorfenvinphos Results						Qualifier...	
		Name	Data File	Type	Level	Acq. Date-Time	Vol.	Exp. Conc.	RT	Resp.	MI	Calc. Conc.	Final Conc.	Accuracy	Ratio	MI
		QC_100ppt	QC-100-r001.d	QC	4	1/22/2013 2:07 PM	100.00	100.0000	12.2...	174549		83.9786	83.9786	84.0	96.6	
		QC_100ppt	QC-100-r002.d	QC	4	1/22/2013 4:53 PM	100.00	100.0000	12.2...	173339		83.3952	83.3952	83.4	99.5	
		QC_100ppt	QC-100-r002a.d	QC	4	1/22/2013 8:05 PM	100.00	100.0000	12.2...	170729		82.1368	82.1368	82.1	95.1	
		QC_100ppt	QC-100-r003.d	QC	4	1/22/2013 10:51 PM	100.00	100.0000	12.2...	168019		80.8306	80.8306	80.8	95.7	
		QC_100ppt	QC-100-r004.d	QC	4	1/23/2013 1:37 AM	100.00	100.0000	12.2...	166022		79.8681	79.8681	79.9	96.0	
		QC_100ppt	QC-100-r005.d	QC	4	1/23/2013 4:23 AM	100.00	100.0000	12.2...	159904		76.9189	76.9189	76.9	96.3	
		QC_100ppt	QC-100-r006.d	QC	4	1/23/2013 6:54 AM	100.00	100.0000	12.2...	159141		76.5509	76.5509	76.6	95.9	
		QC_100ppt	QC-100-r007.d	QC	4	1/23/2013 9:55 AM	100.00	100.0000	12.2...	153389		73.7781	73.7781	73.8	94.5	
		QC_100ppt	QC-100-r008.d	QC	4	1/23/2013 12:41 PM	100.00	100.0000	12.2...	144916		69.6939	69.6939	69.7	96.6	

Chlorpyrifos

Sample								Chlorpyrif...	Chlorpyrifos Results						Qualifier...	
		Name	Data File	Type	Level	Acq. Date-Time	Vol.	Exp. Conc.	RT	Resp.	MI	Calc. Conc.	Final Conc.	Accuracy	Ratio	MI
		QC_100ppt	QC-100-r001.d	QC	4	1/22/2013 2:07 PM	100.00	100.0000	13.4...	26977		42.3430	42.3430	42.3	102...	
		QC_100ppt	QC-100-r002.d	QC	4	1/22/2013 4:53 PM	100.00	100.0000	13.4...	25817		40.5051	40.5051	40.5	102...	
		QC_100ppt	QC-100-r002a.d	QC	4	1/22/2013 8:05 PM	100.00	100.0000	13.4...	22149		34.6910	34.6910	34.7	100...	
		QC_100ppt	QC-100-r003.d	QC	4	1/22/2013 10:51 PM	100.00	100.0000	13.4...	23822		37.3425	37.3425	37.3	99.9	
		QC_100ppt	QC-100-r004.d	QC	4	1/23/2013 1:37 AM	100.00	100.0000	13.4...	23762		37.2465	37.2465	37.2	100...	
		QC_100ppt	QC-100-r005.d	QC	4	1/23/2013 4:23 AM	100.00	100.0000	13.4...	23227		36.3984	36.3984	36.4	103...	
		QC_100ppt	QC-100-r006.d	QC	4	1/23/2013 6:54 AM	100.00	100.0000	13.4...	22293		34.9191	34.9191	34.9	98.0	
		QC_100ppt	QC-100-r007.d	QC	4	1/23/2013 9:55 AM	100.00	100.0000	13.4...	20457		32.0074	32.0074	32.0	99.0	
		QC_100ppt	QC-100-r008.d	QC	4	1/23/2013 12:41 PM	100.00	100.0000	13.4...	18150		28.3510	28.3510	28.4	103...	

Results direct injection

QC samples (100 ng/L)

Quinoxyfen

Sample								Quinoxyfe...	Quinoxyfen Results							Qualifier...	
		Name	Data File	Type	Level	Acq. Date-Time	Vol.	Exp. Conc.	RT	Resp.	MI	Calc. Conc.	Final Conc.	Accuracy	Ratio	MI
		QC_100ppt	QC-100-r001.d	QC	4	1/22/2013 2:07 PM	100.00	100.0000	13.4...	124136		88.8906	88.8906	88.9	65.4	
		QC_100ppt	QC-100-r002.d	QC	4	1/22/2013 4:53 PM	100.00	100.0000	13.4...	120239		86.0604	86.0604	86.1	65.9	
		QC_100ppt	QC-100-r002a.d	QC	4	1/22/2013 8:05 PM	100.00	100.0000	13.4...	113455		81.1346	81.1346	81.1	65.9	
		QC_100ppt	QC-100-r003.d	QC	4	1/22/2013 10:51 PM	100.00	100.0000	13.4...	114088		81.5941	81.5941	81.6	66.5	
		QC_100ppt	QC-100-r004.d	QC	4	1/23/2013 1:37 AM	100.00	100.0000	13.4...	113279		81.0070	81.0070	81.0	65.2	
		QC_100ppt	QC-100-r005.d	QC	4	1/23/2013 4:23 AM	100.00	100.0000	13.4...	111501		79.7161	79.7161	79.7	66.0	
		QC_100ppt	QC-100-r006.d	QC	4	1/23/2013 6:54 AM	100.00	100.0000	13.4...	109279		78.1023	78.1023	78.1	65.8	
		QC_100ppt	QC-100-r007.d	QC	4	1/23/2013 9:55 AM	100.00	100.0000	13.4...	107069		76.4974	76.4974	76.5	65.5	
		QC_100ppt	QC-100-r008.d	QC	4	1/23/2013 12:41 PM	100.00	100.0000	13.4...	103799		74.1229	74.1229	74.1	65.7	

Pendimethalin

Sample								Pendimet...	Pendimethalin Results							Qualifier...	
		Name	Data File	Type	Level	Acq. Date-Time	Vol.	Exp. Conc.	RT	Resp.	MI	Calc. Conc.	Final Conc.	Accuracy	Ratio	MI
		QC_100ppt	QC-100-r001.d	QC	4	1/22/2013 2:07 PM	100.00	100.0000	13.5...	24902		64.2658	64.2658	64.3	17.3	
		QC_100ppt	QC-100-r002.d	QC	4	1/22/2013 4:53 PM	100.00	100.0000	13.5...	25441		65.6633	65.6633	65.7	16.7	
		QC_100ppt	QC-100-r002a.d	QC	4	1/22/2013 8:05 PM	100.00	100.0000	13.5...	23296		60.0972	60.0972	60.1	17.1	
		QC_100ppt	QC-100-r003.d	QC	4	1/22/2013 10:51 PM	100.00	100.0000	13.5...	22557		58.1781	58.1781	58.2	16.9	
		QC_100ppt	QC-100-r004.d	QC	4	1/23/2013 1:37 AM	100.00	100.0000	13.5...	22682		58.5020	58.5020	58.5	16.8	
		QC_100ppt	QC-100-r005.d	QC	4	1/23/2013 4:23 AM	100.00	100.0000	13.5...	21763		56.1171	56.1171	56.1	17.4	
		QC_100ppt	QC-100-r006.d	QC	4	1/23/2013 6:54 AM	100.00	100.0000	13.5...	20827		53.6869	53.6869	53.7	17.4	
		QC_100ppt	QC-100-r007.d	QC	4	1/23/2013 9:55 AM	100.00	100.0000	13.5...	19455		50.1272	50.1272	50.1	18.1	
		QC_100ppt	QC-100-r008.d	QC	4	1/23/2013 12:41 PM	100.00	100.0000	13.5...	18443		47.4986	47.4986	47.5	17.0	

Spectral Confirmation: triggered MRM (tMRM)

Quantitation with Confirmation: Fingerprinting

<u>Full Scan Approach:</u>
Scan the entire fingerprint

<u>tMRM Approach:</u>
Focus on known fingerprint features

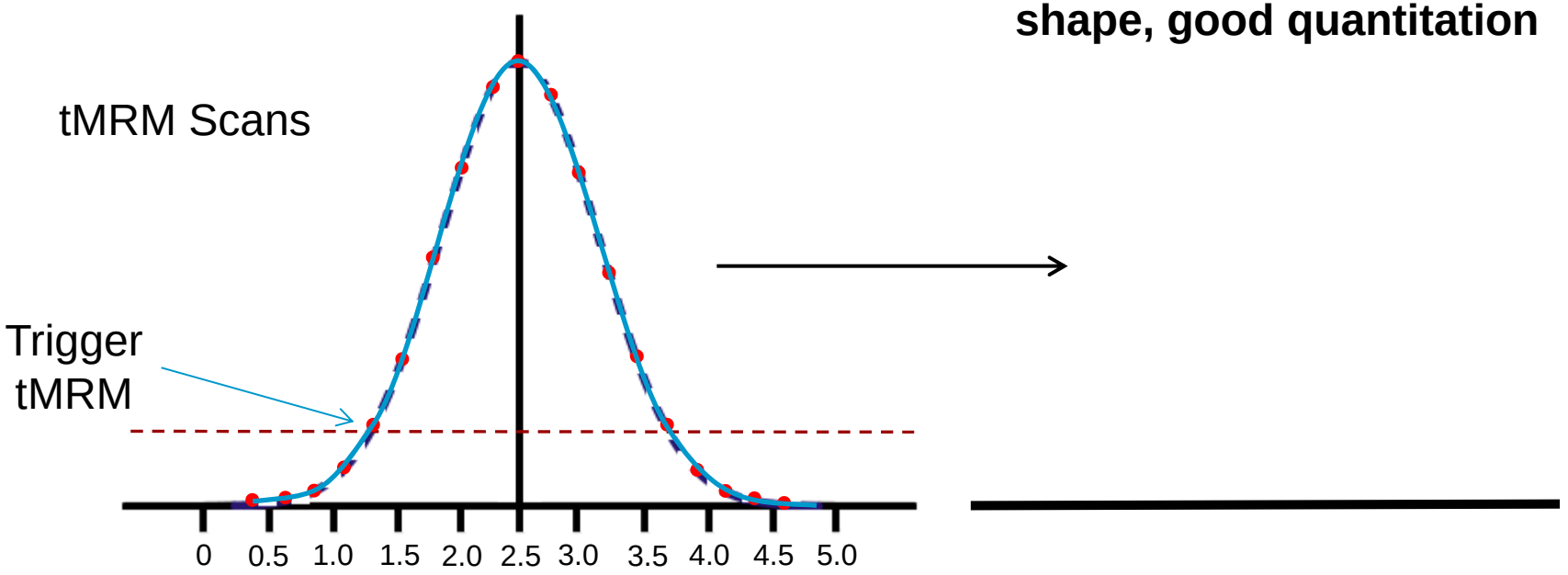
<u>Two possible scenarios:</u>

- Confirmation of positive findings with additional information (spectral matching)
- Elimination of potential false detects caused by matrix interferences

tMRM Scanning

tMRM Scan ≤ 50ms

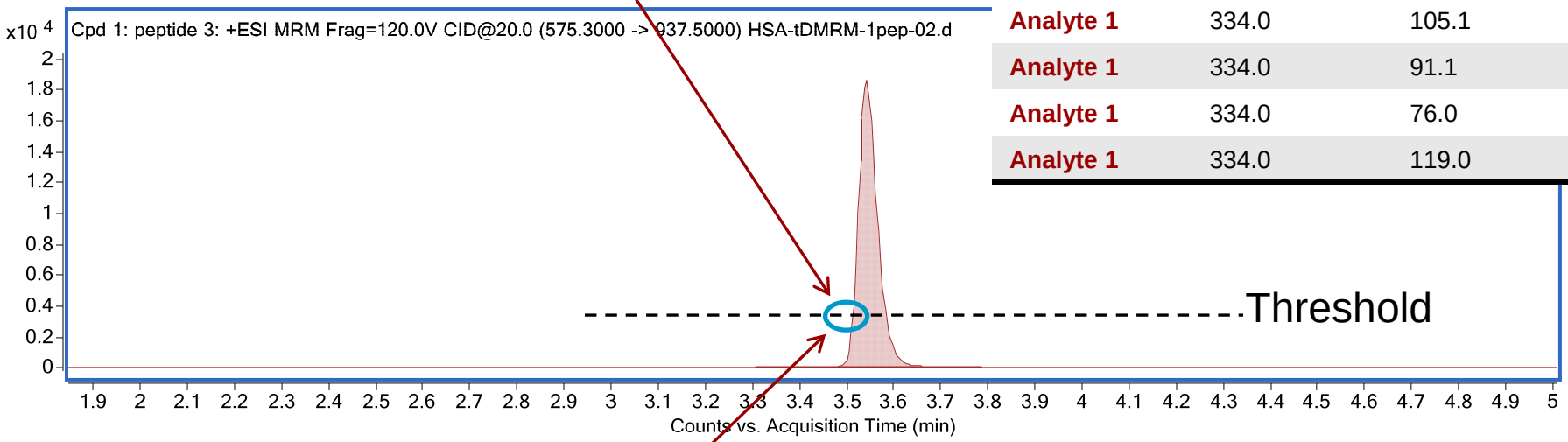
UHPLC compatible, good peak shape, good quantitation



Ideal situation: 1 Peak 5 sec wide, no Matrix

Triggered MRM (tMRM) Analysis

Secondary MRM
Transitions are “Triggered”



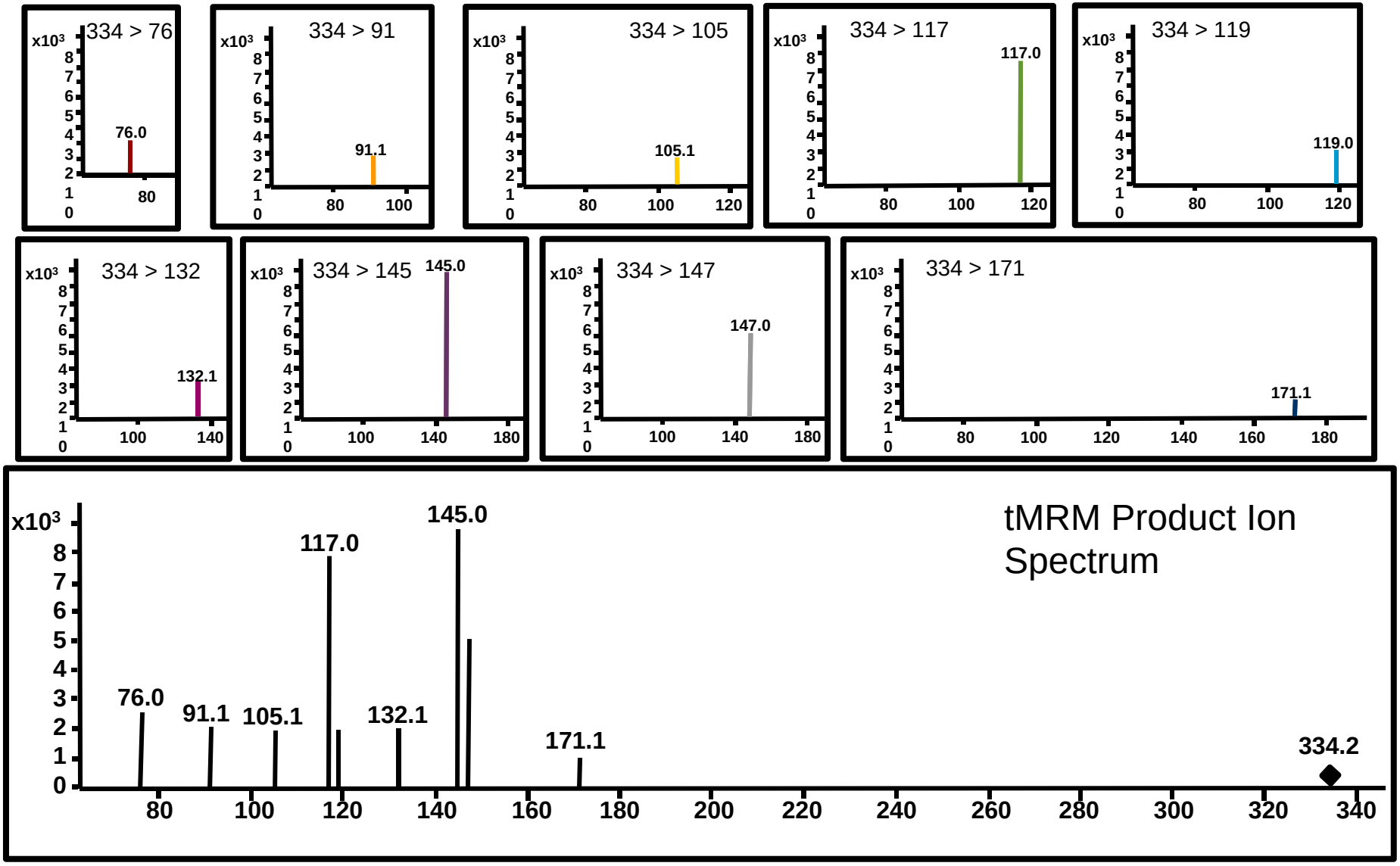
Triggered cycle (above threshold)

Compound	Precursor	Product
Analyte 1	334.0	145.0
Analyte 1	334.0	117.0
Analyte 1	334.0	132.1
Analyte 1	334.0	105.1
Analyte 1	334.0	91.1
Analyte 1	334.0	76.0
Analyte 1	334.0	119.0

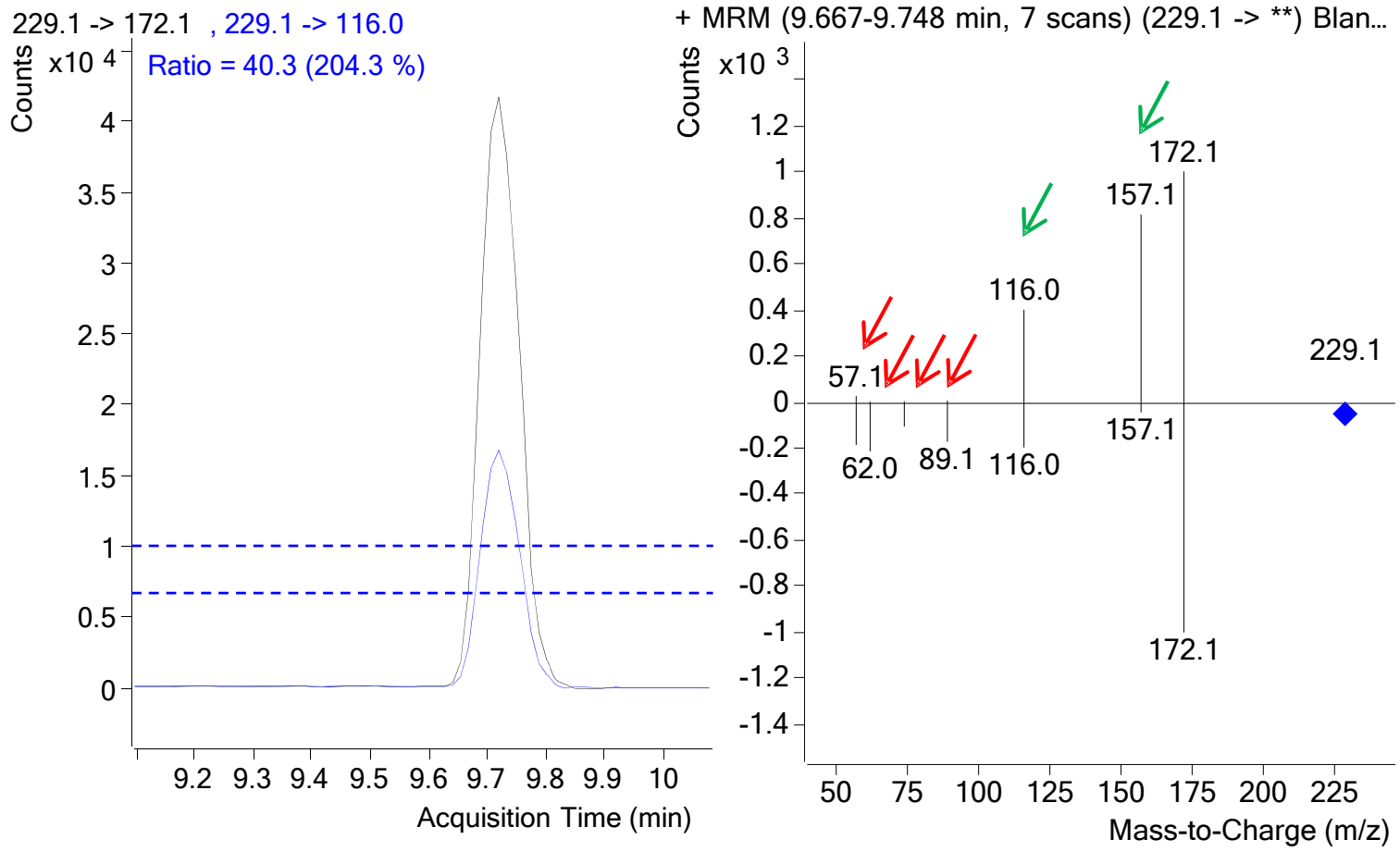
Primary cycle (below threshold)

Compound	Precursor	Product
Analyte 1	334.0	145.0
Analyte 1	334.0	117.0

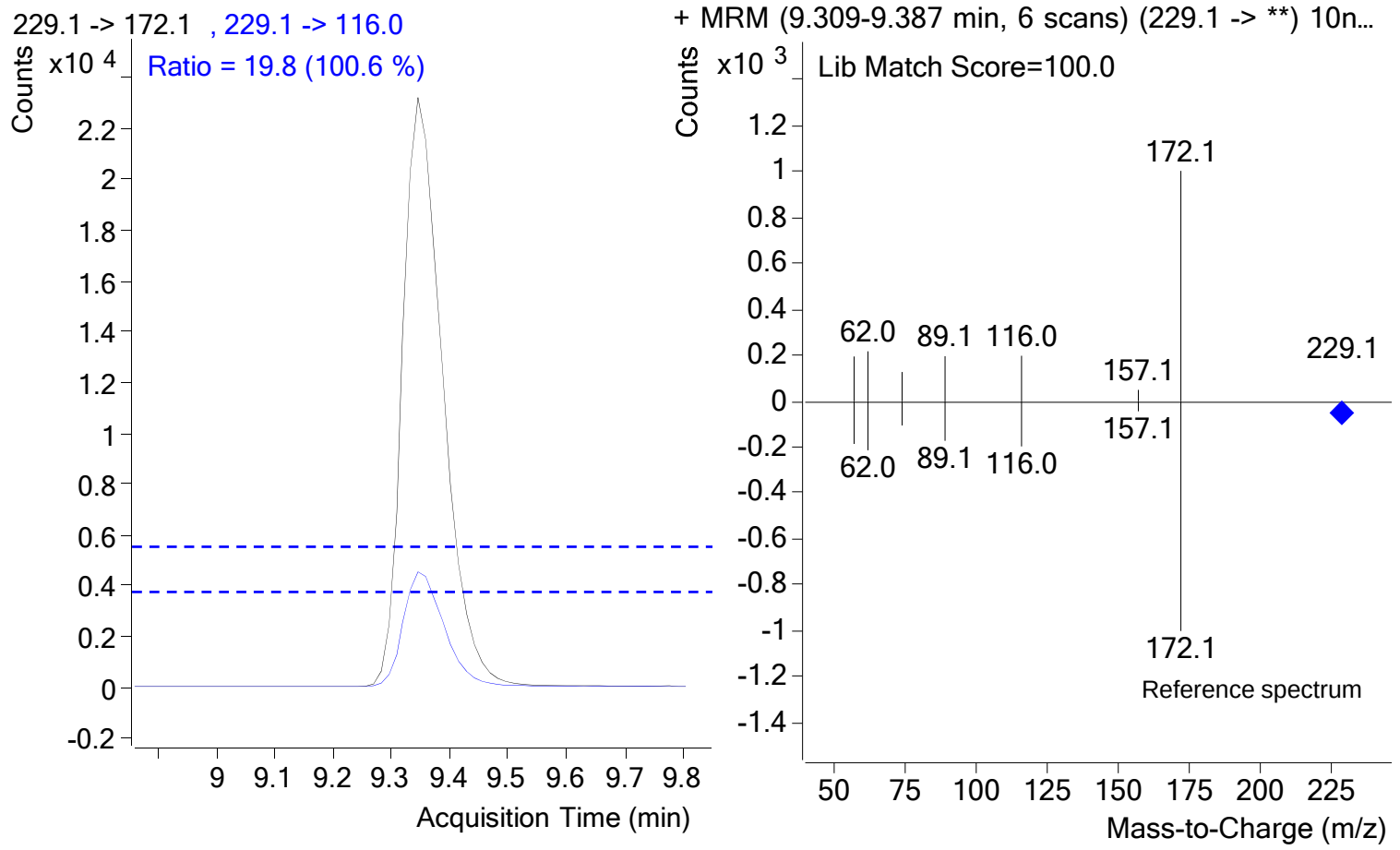
tMRM Product Ion Spectrum



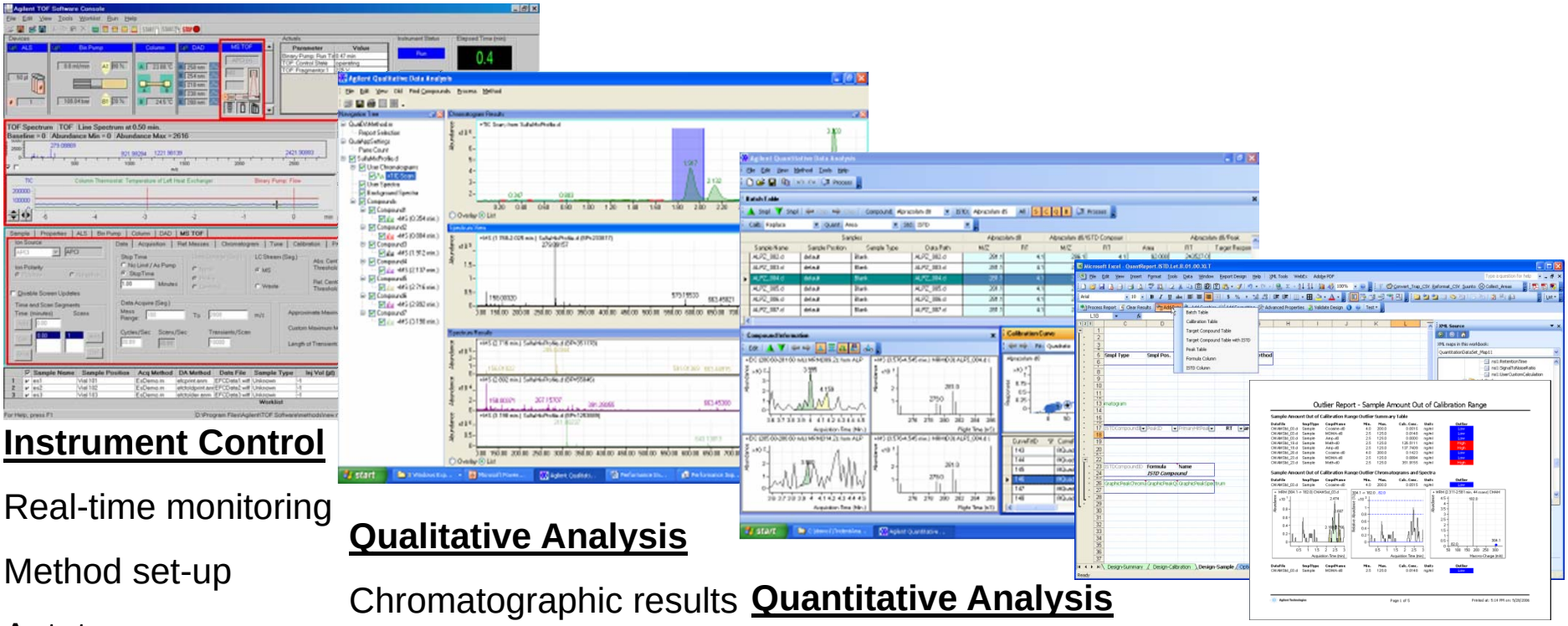
Real samples – Tebuthiuron in chamomile extract



Real samples – Tebuthiuron in chamomile extract



Agilent MassHunter Software



Instrument Control

Real-time monitoring

Method set-up

Autotune

Qualitative Analysis

Chromatographic results

Spectral results

Find compounds

Quantitative Analysis

User filters

Compound results

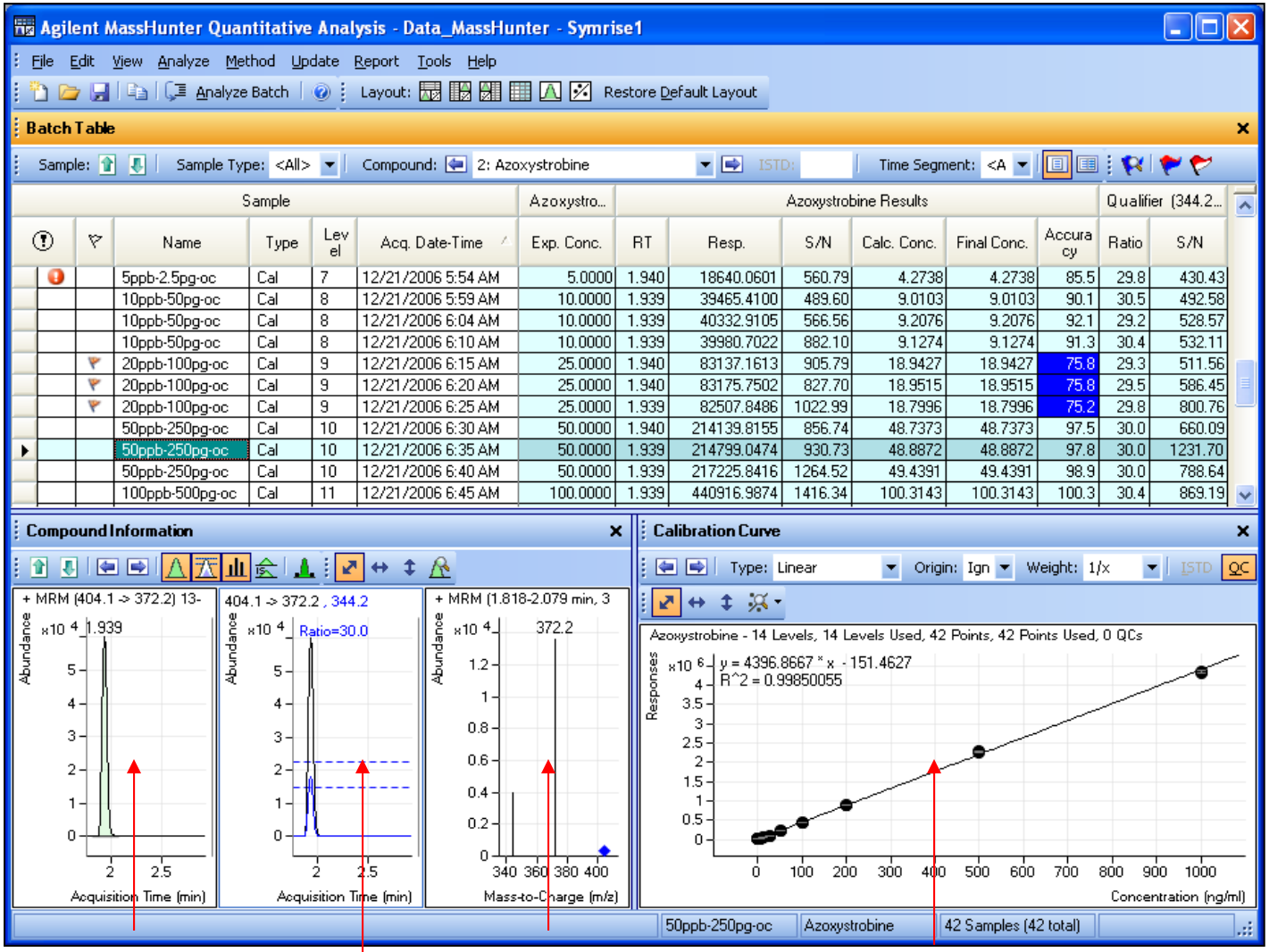
Calibration curve

Reporting

Easily Customisable

Based on Excel and XML technology

MassHunter Quantitative Software Batch-at-a-Glance



target compound

'spectrum'

calibration curve

overlaid quantifier and qualifier EICs

Agilent Technologies

Conclusions

- Direct injection resulted in good sensitivity and reproducibility for all compounds (LLOQ at or below 5 ng/L).
- Accuracy of most compounds within acceptable limits (± 20 %) except for the four most lipophilic compounds
- For these compounds successive loss in signal has been observed for the QC samples, indicating the adsorption of the compounds to the sample vials.
- Even for surface water no significant matrix effect has been observed.
- For Online-SPE all compounds except Chloridazon-desphenyl could be analyzed with acceptable recoveries
- Linear calibrations and good sensitivity has been observed for most compounds
- Accuracy for most compounds within acceptable limits (± 20 %) with slightly higher RSD values and deviations compared to the direct injection method.
- No systematic errors were observed eventually due to the use of silanized sample vials