Waters™

□□□□

Multi-Residue Analysis of Priority Pollutants in Surface Waters Using Exact Mass GC-TOF-MS

Peter Hancock, Keith Worrall

Waters Corporation

Abstract

A method has been presented for the targeted confirmation of more than one hundred priority pollutants using Oasis SPE cartridges and the GCT Premier with the TargetLynx Application Manager.

Benefits

- Majority of the pollutants can be confirmed to concentration levels of <0.1 µg/L in surface waters using a single injection technique and exact mass chromatograms
- Exact mass chromatograms improve the selectivity available, allowing lower LODs to be reached
- ChromaLynx enables automatic peak detection, deconvolution, library searching and exact mass confirmation

Introduction

The Dangerous Substances Directive (76/464/EC)[1] lists 132 compounds that have legislated levels in drinking and surface waters, and more than 100 of these are amenable to GC analysis. This legislation is currently being integrated into the Water Framework Directive (2000/60/EC).[2] Water for human consumption has its own specific legislation in the Drinking Water Directive (98/83/EC)[3] where the reporting level for individual pollutants is 0.1 µg/L.

The EU list has many similarities with the lists from the U.S. EPA water quality methods such as 6254 and 82705. It should be noted that the list analyzed in this method is by no means an exhaustive one.

The compound groups analyzed here represent a wide range of polarities and types, and include benzidines, chloroanilines, chloronitrobenzenes, chloronitrotoluenes, chlorophenols, chlorotoluidines, OCs, OPs, PAHs, PCBs, phenylureas, triazines and volatile amines. Many of these compound groups will typically have their own dedicated analytical method that requires specific extraction/clean-up and final analysis. Combining these groups into a single method would allow the laboratory to significantly increase sample throughput. A method has previously been published6 employing single quadrupole mass spectrometry using selected ion recording (SIR) for screening of the GC amenable compounds. SIR allows for targeted screening of a finite number of compounds to be achieved. However, much of the chemical information is discarded so full spectrum techniques are still required in so-called “open” or untargeted screening environments.

To establish a suitable untargeted screening technique there are a number of requirements that would need to be met to extract, detect, locate and identify all components. These include: minimal non-selective sample preparation for a wide range of compound groups with different polarities; simple high resolution GC separation to minimize matrix interference while maintaining resolution of critical pairs; and automated peak detection and deconvolution of all components in the sample.

Exact mass Time-of-Flight mass spectrometry (ToF MS) is a full-spectrum technique capable of both the targeted and the untargeted screening approaches.

A method will be introduced for the targeted screening of 107 GC-amenable compounds in surface waters down to the legislated concentration of 0.1 µg/L.

A method will also be introduced for the untargeted screening of other GC amenable compounds in surface waters using automatic peak detection, deconvolution and library searching with exact mass confirmation.

Waters Micromass GCT Premier ToF MS System.

Experimental

Extraction Method

200 mL filtered water was adjusted to pH 4.0 with 1 N HCl. The sample was spiked with 500 ng internal standards (d_5-nitrophenol, 2-fluorobiphenyl, p-terphenyld_{14}). A Waters Oasis HLB 60 mg, 3cc SPE cartridge was conditioned with 6 mL dichloromethane, 6 mL acetonitrile and 6 mL water. The sample was loaded at approximately 6 mL/min. The cartridge was washed with 1 mL water and dried under nitrogen for 20 min. The elution was performed with 2 x 2.5 mL dichloromethane. The volume was then adjusted to 0.5 mL under nitrogen at ambient temperature. 500 ng recovery standard was added (d_{10}-anthracene) for a 400-fold concentration step during the extraction.

GC Method

System:	Agilent 6890N GC with CTC CombiPal autosampler
Column:	J & W Scientific DB-17ms 30 m x 0.25 mm i.d., 0.25 µm
Flow rate:	1.0 mL/min helium constant flow
Temp. ramp:	40 °C (Hold 1 min) 310 °C @ 15 °C/min (Hold 11 min)
Total run time:	30 min
Injection method:	Pulsed split less injection, 1 µL 4mm i.d. double taper liner 140 kPa pulse for 1.1 min

GC-MS Method

The Waters Micromass GCT Premier ToF MS was used in electron ionization (EI+) mode. The ion source was operated at 200 ºC with an electron energy of 70 eV and a trap current of 200 µA. The temperature of the transfer line was held at 280 °C during the run. Spectra were acquired between 50 and 550 Da in a time of

0.09s and a delay of 0.01 s (10 spectra/s). Exact mass spectra were obtained using a single-point lock mass (2,4,6-tris-(trifluoromethyl)-1,3,5-triazine, *m/z* = 284.9949) infused into the ion source continuouslyduring the chromatographic run. The resolution of the instrument was greater than 7000 full width half maximum (FWHM).

The pollutants and their respective exact masses are listed in Table 1.

Pollutant	Retention Time	Quantification Ion	Confirmation Ion 1	Confirmation Ion 2
Cumene	5.02	105.0704	120.0926	
2-Chlorophenol	6.31	128.0029	129.9999	
Hexachlorobutadiene	8.15	224.8413	222.8443	226.8384
2,4-Dichlorophenol	8.29	161.9639	163.9610	
2-Chloroaniline	8.33	127.0189	129.0159	
3-Chlorophenol	8.57	128.0029	129.9999	
Naphthalene	8.75	128.0626	127.0548	
4-Chlorophenol	8.69	128.0029	129.9999	
3-Chloroaniline	9.23	127.0189	129.0159	
2-Chloro-4-toluidine	9.25	141.0345	140.0267	
4-Chloroaniline	9.27	127.0189	129.0159	
2,6-Dichloroaniline	9.33	160.9799	162.9770	
1-Chloro-3-nitrobenzene	9.32	156.9931	127.0189	
Dichlorvos	9.33	109.0055	184.9770	
1-Chloro-4-nitrobenzene	9.55	156.9931	127.0189	
1-Chloro-2-nitrobenzene	9.69	156.9931	127.0189	
4-Chloro-3-methylphenol	9.62	142.0185	144.0156	107.0497
2,3,5-Trichlorophenol	9.93	195.9249	197.9220	
4-Chloro-2-nitrotoluene	9.96	154.0060	126.0111	156.0030
2-Chloro-6-nitrotoluene	10.00	154.0060	126.0111	156.0030
3,5-Dichloronitrobenzene	10.13	190.9540	144.9612	192.9511
2,4,6-Trichlorophenol	10.19	195.9249	197.9220	
2,4,5-Trichlorophenol	10.23	195.9249	197.9220	
2,4-Dichloroaniline	10.37	160.9799	162.9770	
2,5-Dichloroaniline	10.43	160.9799	162.9770	
2-Fluorobiphenyl (I.S.)	10.40	172.0688		
2,3,4-Trichlorophenol	10.50	195.9250	197.9220	
Biphenyl	10.63	154.0783	153.0704	
2,3,6-Trichlorophenol	10.66	195.9250	197.9220	
4-Chloro-3-nitrotoluene	10.62	171.0087	173.0058	
2-Chloro-3-nitrotoluene	10.66	171.0087	173.0058	
2,3-Dichloroaniline	10.72	160.9799	162.9770	
2,5-Dichloronitrobenzene	10.67	190.9540	144.9612	192.9511
2,4-Dichloronitrobenzene	10.83	190.9540	144.9612	192.9511
3,4-Dichloronitrobenzene	10.92	190.9540	144.9612	192.9511
2,3-Dichloronitrobenzene	11.16	190.9540	144.9612	192.9511
Mevinphos (Z)	11.23	127.0160	192.0188	

Table 1. Retention times, quantification and confirmation ions for each pollutant.

Pollutant	Retention Time	Quantification Ion	Confirmation Ion 1	Confirmation Ion 2
3,4-Dichloroaniline	11.18	160.9799	162.9770	
Mevinphos (E)	11.47	127.0160	192.0188	
Trifluralin	11.85	306.0702	264.0232	
1,2-Dichloronaphthalene	12.55	195.9847	197.9817	
Tributylphosphate	12.16	98.9847	155.0473	211.1099
Demeton-O	12.43	88.0347	89.0425	171.0211
3,4,5-Trichlorophenol	12.65	195.9250	197.9220	
1-Chloro-2,4-dinitrobenzene	12.87	201.9781	203.9752	
Hexachlorobenzene	13.25	283.8102	285.8072	
4-Chloro-2-nitroaniline	13.20	172.0040	126.0111	174.0010
Demeton-s-methyl	13.10	88.0347	109.0055	141.9854
Alpha-HCH	13.49	218.9116	180.9379	216.9145
Omethoate	13.45	156.0010	110.0133	
Pentachlorophenol	13.86	265.8441	263.8470	267.8411
Monolinuron	13.91	61.0528	214.0509	126.0111
Atrazine	13.88	200.0703	215.0938	
Lindane	14.11	218.9116	180.9379	216.9145
Simazine	14.01	201.0781	186.0546	173.0468
Disulfoton	14.09	88.0347	89.0425	274.0285
d10-Anthracene (I.S.)	14.49	188.1410		
Phenanthrene	14.47	178.0783	176.0626	
Anthracene	14.53	178.0783	176.0626	
Beta-HCH	14.41	218.9116	180.9379	216.9145
Dimethoate	14.43	87.0143	124.9826	93.0105
Heptachlor	14.58	271.8102	273.8072	269.8131
PCB 28	14.64	255.9613	257.9584	
Delta-HCH	14.88	218.9116	180.9379	216.9145
Aldrin	15.06	262.8570	260.8599	264.8540
Propanil	14.99	160.9799	162.9770	
Parathion-methyl	15.15	263.0017	124.9826	109.0055
Tetrachloronaphthalene	15.45	265.9038	263.9067	267.9008
Linuron	15.36	61.0528	248.0119	
Malathion	15.35	173.0814	127.0395	124.9826
PCB 52	15.49	291.9194	289.9224	293.9165
Fenitrothion	15.45	277.0174	260.0146	
Parathion-ethyl	15.49	291.0330	109.0055	96.9513
Isodrin	15.71	192.9379	194.9349	

Table 1. cont. Retention times, quantification and confirmation ions for each pollutant.

Pollutant	Retention Time	Quantification Ion	Confirmation Ion 1	Confirmation Ion 2
Fenthion	15.77	278.0200	169.0146	
Gamma-chlordane	16.14	372.8260	374.8230	
o,o'-DDE	16.11	246.0003	247.9974	
PCB 101	16.16	325.8804	323.8834	327.8775
Alpha-chlordane	16.31	372.8260	374.8230	
o,p'-DDE	16.27	246.0003	247.9974	
Alpha-endosulfan	16.41	240.9015	236.8427	238.8498
Fluoranthene	16.58	202.0783	200.0626	
Bentazone	16.42	198.0099	119.0371	
p,p'-DDE	16.62	246.0003	247.9974	
Dieldrin	16.83	262.8570	260.8599	264.8540
PCB 77	17.06	325.8804	323.8834	327.8775
o,p-DDD	16.96	235.0081	237.0052	
p-Terphenyl-d14 (I.S.)	17.05	244.1974		
PCB 118	17.61	325.8804	323.8834	327.8775
Endrin	17.31	262.8570	260.8599	264.8540
PCB 153	17.21	359.8415	361.8385	357.8444
o,p'-DDT	17.35	235.0081	237.0052	
p,p-DDD	17.41	235.0081	237.0052	
Benzidine	17.55	184.1000	183.0922	
Beta-Endosulfan	17.63	240.9015	236.8427	238.8498
PCB 138	17.77	359.8415	361.8385	357.8444
p,p'-DDT	17.79	235.0081	237.0052	
PCB 126	17.90	325.8804	323.8834	327.8775
PCB 180	17.80	393.8025	395.7995	
PCB 169	18.33	359.8415	361.8385	357.8444
Pyrazon	19.29	221.0356	220.0278	
3,3'-Dichlorobenzidine	19.42	252.0221	254.0192	
Azinphos-methyl	20.08	160.0511	132.0449	
Azinphos-ethyl	20.27	160.0511	132.0449	
Coumaphos	20.34	362.0145	225.9855	
Benzo(b)fluoranthene	21.49	252.0939	250.0783	
Benzo(k)fluoranthene	21.55	252.0939	250.0783	
Benzo(a)pyrene	22.75	252.0939	250.0783	
Indeno(1,2,3-cd)pyrene	27.25	276.0939	274.0783	
Benzo(ghi)perylene	29.19	276.0939	274.0783	

Table 1. cont. Retention times, quantification and confirmation ions for each pollutant.

Acquisition and Processing Methods

The data were acquired using Waters MassLynx Software and processed using either the TargetLynx or ChromaLynx Application Managers.

Results and Discussion

The chromatography was optimized for sensitivity, speed and separation. The results from the final conditions are illustrated in Figure 1 with baseline resolution obtained for three critical pairs, 3- and 4-chlorophenol (a), E and Z mevinphos (b) and o,p'- DDT and p,p'-DDD (c), using the DB-17ms column. A typical reconstructed ion chromatogram (RIC) for all the pollutants is illustrated in Figure 2.

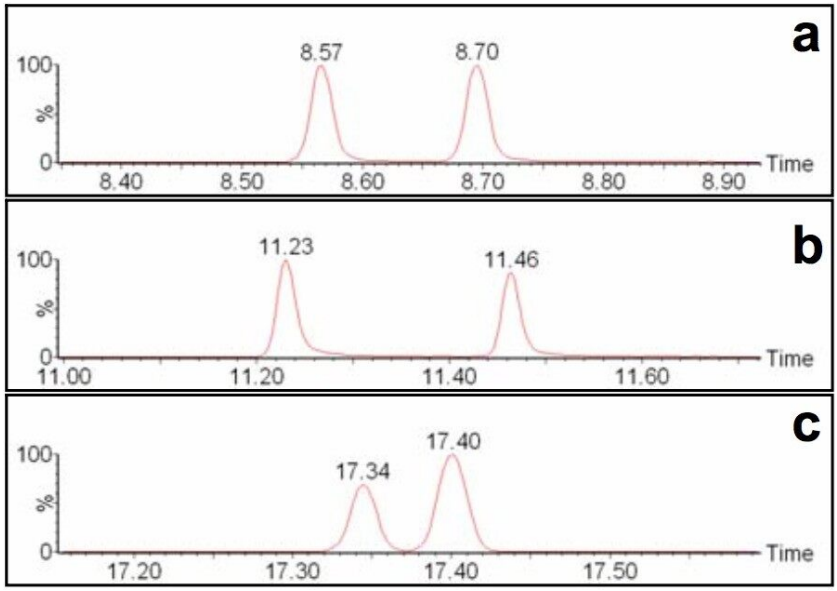

Figure 1. Baseline resolution of critical pairs.

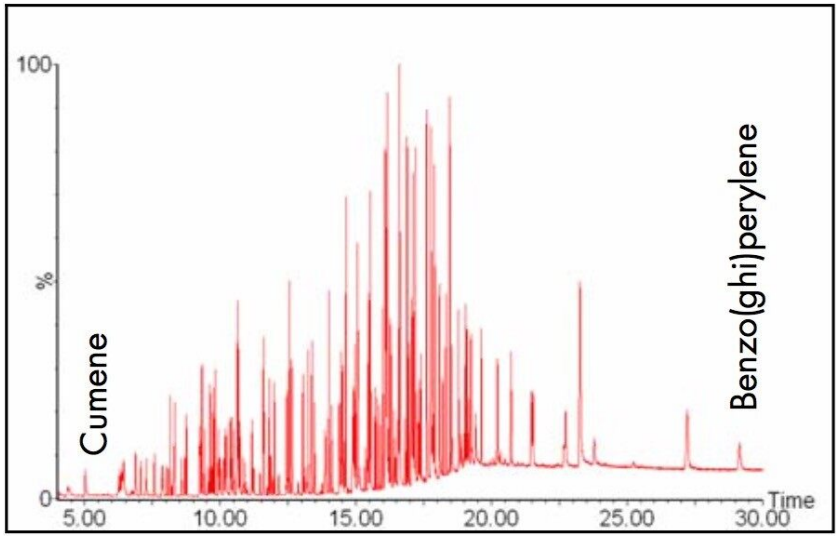

Figure 2. RIC for all the pollutants.

Targeted Screening Results

The 0.5 µg/L spiked drinking water samples were analyzed to determine the recovery for each pollutant using a single SPE sorbent. Table 2 summarizes the recoveries achieved from five replicate extractions using the described method.

Mean Recovery	Number of Compounds (%)
70 - 120%	79 (72%)
50 - 70%	9 (8 %)
< 50%	14 (13%)
>120%	8 (7%)

Table 2. Extraction recovery for pollutants spiked at 0.5 µg/L (n = 5) from water.

Dichloromethane elution gave good overall performance with 72% of pollutants recovered within the range 70–120%. The compounds recovered at less than 50% included compounds such as disulfoton, which undergoes rapid degradation in aqueous solution, and bentazone and the benzidines, which are more suitable for LC determination, or require derivatization prior to GC based analysis. The recoveries of any hydrogen-bonding donors could be improved by adding 5% methanol to the eluent but this was not attempted in this instance.

Overall, the distribution of recoveries for such a wide range of polarities, boiling points, pKa's and water/octanol partition coefficients (K_{ow}) using a single SPE sorbent is excellent.

The limits of detection (LODs) were assessed for confirmation (two exact mass chromatograms per compound) and screening (one exact mass chromatogram per compound). A summary of the instrumental and method LODs is listed in Table 3. All LODs are based upon a peak-to-peak, signal-tonoise (S/N) ratio of 3:1. The instrumental LODs are based upon the lowest concentration standard injection. The method LODs are based upon the average LOD obtained from five replicate extractions of 0.5 µg/L spiked water samples. The LODs reported are excellent for such a wide range of compounds with a single generic extraction method.

			Number of Compounds < 0.1 µg/L	Number of Compounds > 0.1 µg/L
Instrument		Screening	102	1
		Confirmation	101	2
Method	Drinking Water	Screening	101	2
		Confirmation	98	5
	Surface Water	Screening	101	2
		Confirmation	99	4

Table 3. Retention times, quantification and confirmation ions for each pollutant.

The chlorophenols (0.1 µg/L) were chosen to illustrate the improvement in selectivity offered by exact mass chromatograms. The nominal mass chromatogram (1 Da, *m/z* 128), illustrated in Figure 3, shows seven intense peaks which could lead to interference when using automatic integration. In the exact mass chromatogram (20 mDa, *m/z* 128.003) the three chlorophenols have little or no interference, improving the selectivity of the method.

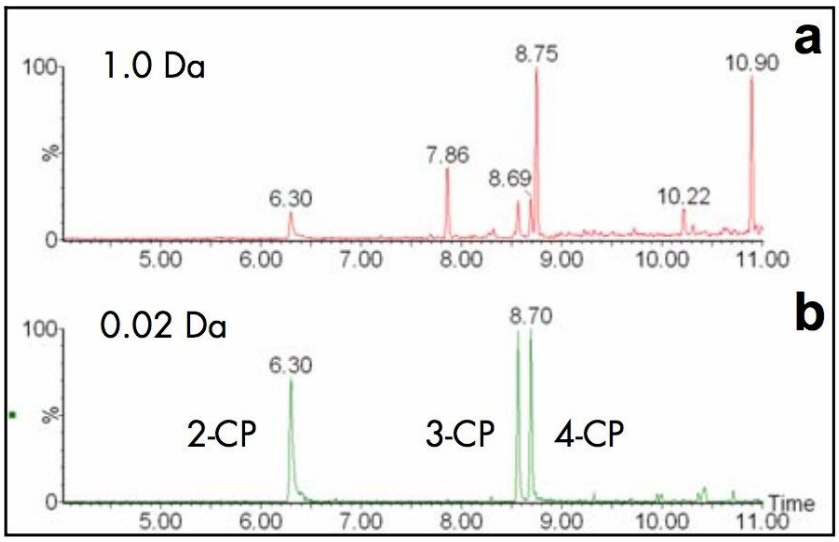

Figure 3. Selectivity offered by nominal mass (a) versus exact mass (b) chromatograms for chlorophenols.

This improvement in selectivity leads to an increase in the S/N ratio achieved. The confirmation ion for isodrin (0.1 µg/L) in surface water is illustrated in Figure 4. The nominal mass chromatogram (1 Da, *m/z* 195) results in a S/N ratio of 3:1. In the exact mass chromatogram (20 mDa, *m/z* 194.9349) the S/N ratio has been

improved to 57:1. The average difference between the nominal mass LOD and exact mass LOD was a factor of six for all the residues in the method. With nominal mass data, three ions would be required for confirmation as opposed to two for exact mass data and, this again, will further improve the LOD for confirmation.

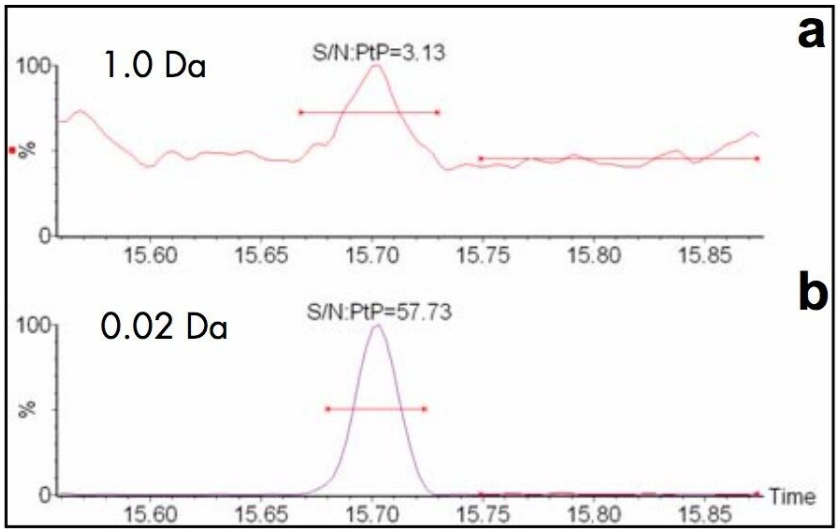

Figure 4. S/N offered by nominal mass (a) versus exact mass (b) chromatograms for isodrin.

The sensitivity of the method is illustrated in Figure 5, showing that dichlorvos can be screened and confirmed to a level below 0.1 µg/L in surface water. With a scanning instrument, increasing the number of ions, as in the case of confirmation, will decrease the overall sensitivity (S/N). With ToF, increasing the number of masses monitored does not decrease the sensitivity, as can be seen by the two peak areas (6.8). Moving from screening to confirmation does not affect the LOD with exact mass ToF, therefore, the number of confirmation ions can be increased without effect.

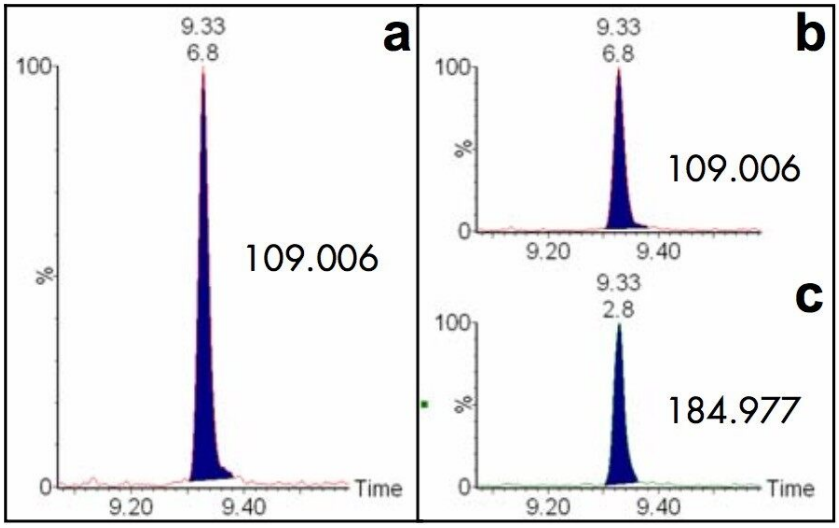

Figure 5. Sensitivity of screening (a) versus quantification (b) and confirmation (c) for 0.1 µg/L dichlorvos in surface water.

Solvent standards were prepared in the concentration range 1–500 pg/µL, equivalent to 0.005–2.5 µg/L. 2-fluorobiphenyl was used as an internal standard to correct for any volumetric errors. The standards were injected in a typical batch analysis bracketing the drinking and surface water extracts. The resulting data was processed using Waters TargetLynx Application Manager. A representative curve for 2,5- dichloroaniline with a correlation coefficient of $r^2 = 0.9983$ is illustrated in Figure 6.

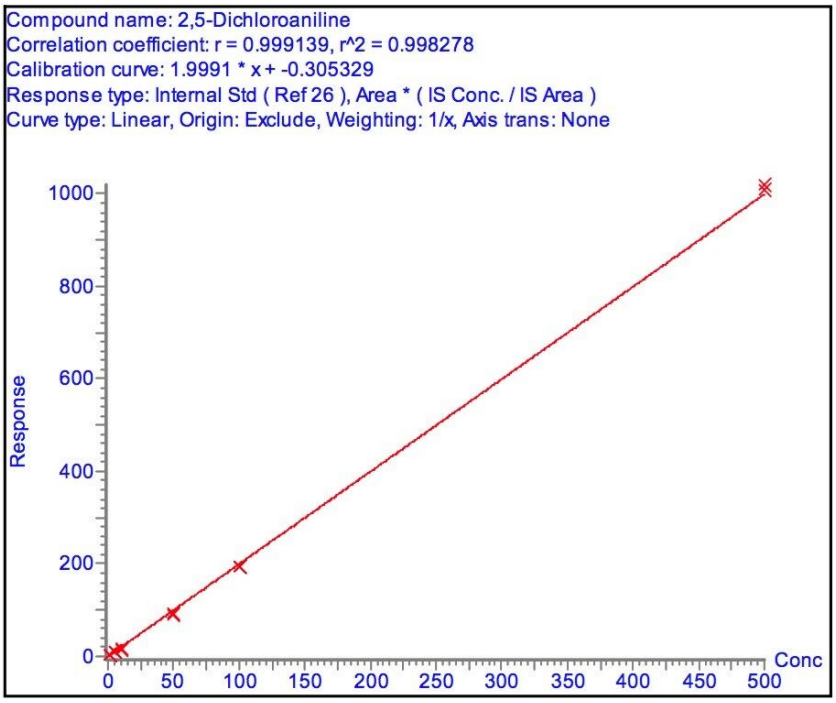

Figure 6. Representative calibration curve for 2,5-dichloroaniline, 0.005–2.5 µg/L.

The TargetLynx browser for 2-chloro-4-toluidine at a spiked concentration of 0.1 µg/L in surface water is illustrated in Figure 7. Two extracted exact masses are shown for confirmation. Ninety-nine residues could be confirmed using this method in surface waters to a concentration of 0.1 µg/L while 101 could be screened to the same concentration. This number is not absolute because more residues could be added as there will no affect on sensitivity. The results show that the GCT Premier can be used in a targeted environment.

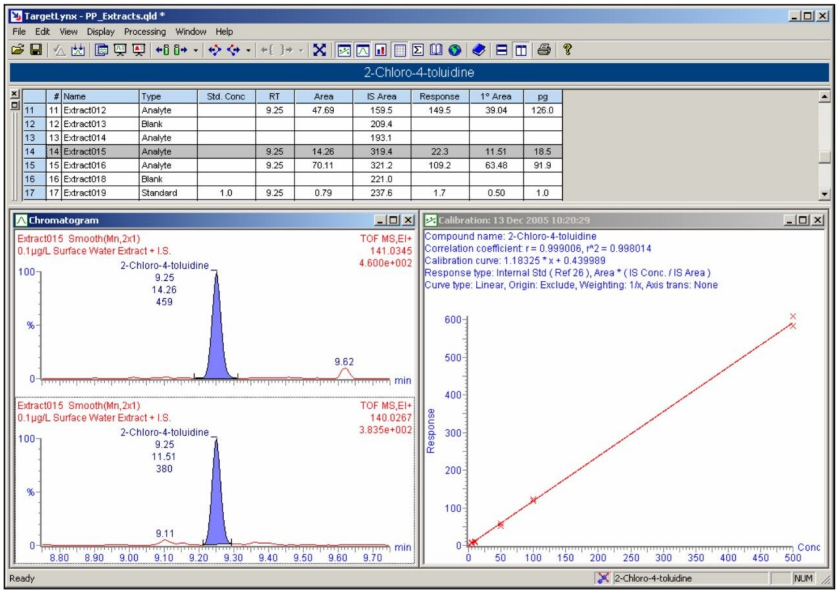

Figure 7. TargetLynx browser for 2-chloro-4-toluidine at a concentration of 0.1 µg/L in surface water.

Untargeted Screening Results

In the untargeted screening environment, there may be hundreds of peaks that need to be located, which would be very time consuming if performed manually. In this case, it would be useful to automatically process using a deconvolution package such as Waters ChromaLynx Application Manager.

ChromaLynx automatically plots the RICs of the eight most intense ions at any point in the chromatogram. If a peak is found to satisfy user-defined parameters, the software will display its deconvoluted mass spectrum. The spectrum can then be submitted to an automatic library search routine with the ability to confirm by exact mass scoring.

The importance of deconvolution for untargeted screening can be observed in Figure 8. Given the data shown, an analyst is likely to conclude there are five components in this 0.25 min section of the chromatogram at 9.23, 9.25, 9.27, 9.33, and 9.42 min.

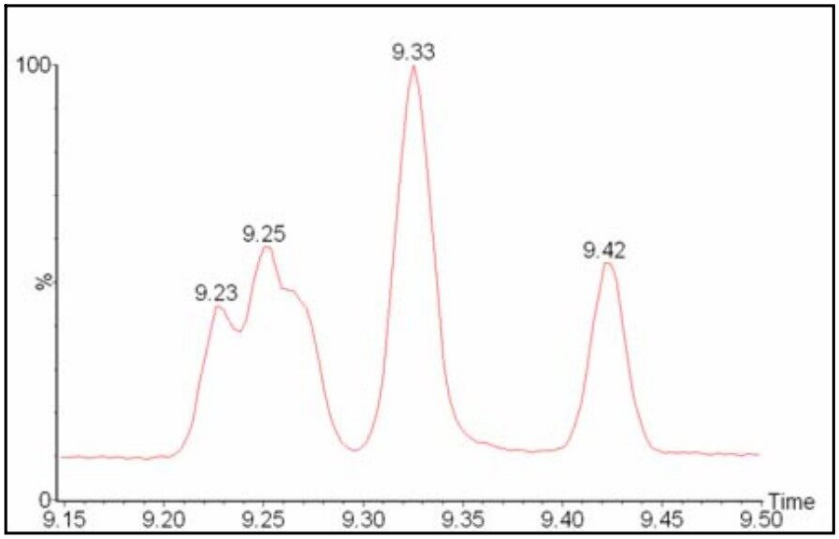

Figure 8. Chromatogram appears to indicate five components prior to deconvolution.

However, submitting the same section of the chromatogram to ChromaLynx results in the example browser displayed in Figure 9. Here, seven components, indicated by a pink triangle, were found with three peaks co-eluting at 9.33 min. The three components were successfully identified as 1-chloro-3- nitrobenzene, 2,6-dichloroaniline and dichlorvos.

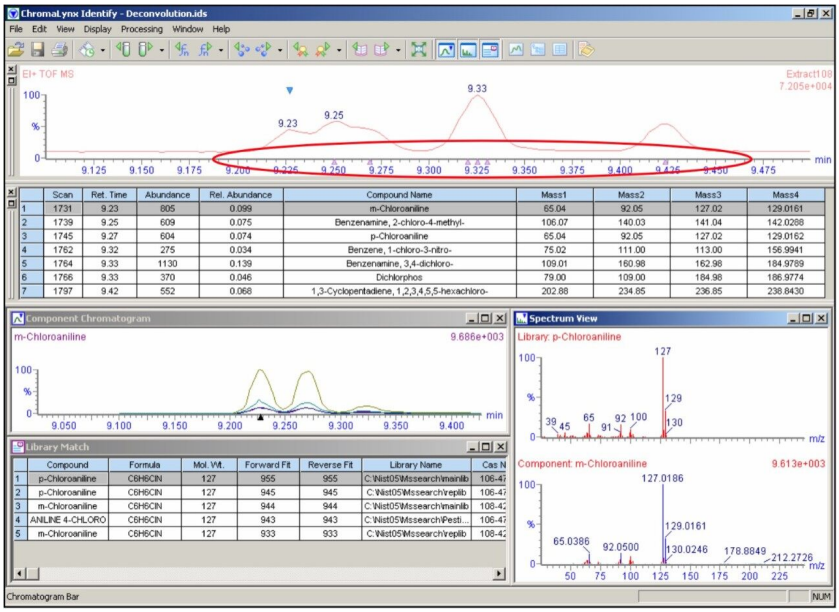

Figure 9. Example ChromaLynx browser for a 0.25 min section of the chromatogram. Figure 10.

ChromaLynx processing of the 0.5 µg/L spiked surface water extract located approximately 800 components in the whole chromatogram. One hundred eighty pollutants were spiked into the extract, of which 100 are in the targeted method. An example of one of the untargeted compounds (4-bromodiphenylether) that was found is illustrated in Figure 10. Other pollutants located with ChromaLynx included dibenzofuran, 2-chloronaphthalene, phenol and decachlorobiphenyl.

Figure 10. ChromaLynx browser for an untargeted pollutant in spiked surface water (0.5 µg/L).

Moving to the blank surface water, a few untargeted pollutants of interest were detected and identified with ChromaLynx. An example pollutant was 4-methylbenzenesulfonamide, which is illustrated in Figure 11. Other examples included naphthalene and 1-methylnaphthalene.

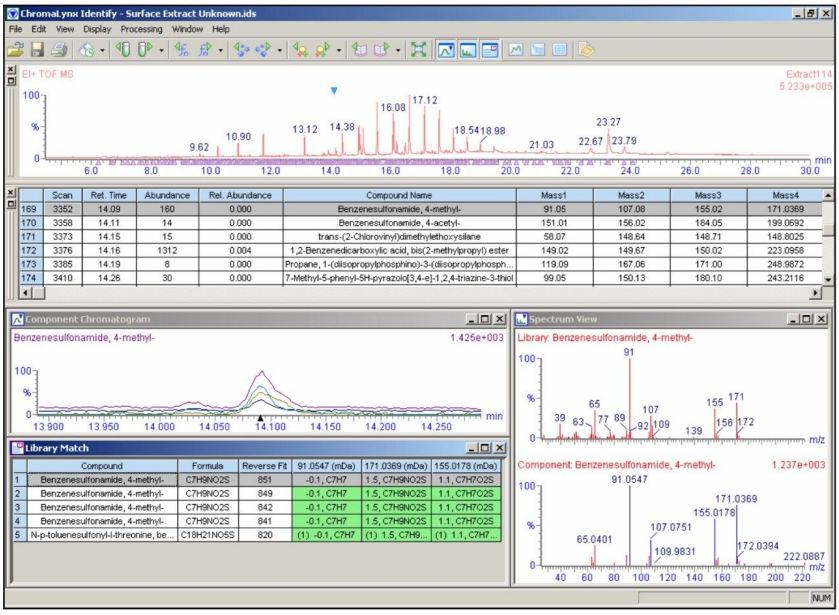

Figure 11. ChromaLynx browser with exact mass confirmation for an untargeted pollutant in surface water.

ChromaLynx automatically performs exact mass confirmation of the library search. The formula from the library hit is submitted to elemental composition and the “n” most intense ions are confirmed/rejected by exact mass. Green boxes indicate a good exact mass match, amber boxes indicate a tentative exact mass match and red boxes indicate no match.

Conclusion

A method has been presented for the targeted confirmation of more than 100 priority pollutants using Oasis SPE cartridges and the GCT Premier with the TargetLynx Application Manager.

A majority of the pollutants can be confirmed to concentration levels of <0.1 µg/L in surface waters using a single injection technique and exact mass chromatograms.

Exact mass chromatograms improve the selectivity available, allowing lower LODs to be reached.

The method can also be extended to larger numbers of residues without loss in sensitivity due to the full spectrum approach provided by ToF instruments.

This single injection can also be used to screen for untargeted residues in the same extract using the ChromaLynx Application Manager.

ChromaLynx enables automatic peak detection, deconvolution, library searching and exact mass confirmation.

References

1. Council Directive 76/464/EEC of 4th May 1976 on Pollution Caused by Certain Dangerous Substances Discharged into the Aquatic Environment of the Community, *Off. J. of the European Communities*, L129 (1976) 23.
2. http://europa.eu.int/comm/environment/water/ water-dangersub/76_464.htm#transition.
3. http://europa.eu.int/comm/environment/water/ water-drink/index_en.html.
4. USEPA method 625, Base/Neutral and Acid Organics in Wastewater, U.S. EPA National Exposure Research Laboratory (NERL), Microbiological and Chemical Exposure Assessment Research Division (MCEARD), Cincinnati, OH.
5. USEPA method 8270, Semivolatile Organic Compounds by GC-MS, U.S. EPA Office of Solid Waste (OSW) Methods Team, Ariel RiosBldg. (5307W), 1200 Pennsylvania Ave. NW, Washington, DC 20460.
6. Lacorte, S., Guiffard, I., Fraisse, D. Barceló D. *Anal. Chem*. 72 (2000) 1430.

Featured Products

MassLynx MS Software <https://www.waters.com/513662>

TargetLynx <https://www.waters.com/513791>

ChromaLynx <https://www.waters.com/513759>

720001521, March 2006