Analysis of 22 Base-Neutral Pesticides and Pesticide Metabolites in Raw and Treated Waters Using Liquid Chromatography and Positive Ion Electrospray-Mass Spectrometry

Application

Water Analysis

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Abstract

A method was defined for the quantitative analysis of 22 pesticides and pesticide metabolites in raw and treated drinking water. The method used solid-phase extraction of the water sample, liquid chromatography and electrospray-mass spectrometry. Results were shown to meet UK regulatory requirements of recovery, sensitivity, accuracy, and precision.

Introduction

Due to the widespread use of agricultural pesticides and the consequential drainage into public waterways and underground water reservoirs, these types of compounds must be monitored for health and safety reasons, and in order to comply with governmental regulations and standards. There are numerous and varied methods for the analysis of certain classes of pesticides. Some examples include: a) separate analyses of triazines by gas chromatography (GC) [1], b) analysis of thermally unstable urons using liquid chromatography (LC) with UV diode array detection (DAD) [1], and c) an online method using solid-phase extraction (SPE) with LC and DAD, and briefly by LC/MS (LC/mass spectrometry) [2]. We needed a robust, high-throughput analytical method for varied pesticides, capable of validation to UKAS (United Kingdom Accreditation Service) and DWI (Drinking Water Inspectorate) requirements. To develop an appropriate compound list, we combined pesticide data for the Wessex region, obtained from Central Science Lab (CSL, York), with stability and leaching data to predict problem pesticides. We decided to develop an analysis based on LC/MS, because of its potential to handle varied pesticide types, provide positive identifications, yield high sensitivity, and avoid possible GC-induced thermal degradations.

Experimental Conditions and Results

Although acetonitrile (ACN) is an often used LC solvent, we decided to compare its performance, for our purposes, against methanol. We found that methanol permitted higher temperature separations resulting in generally superior chromatographic selectivity, good sensitivity for all target compounds, and superior sensitivity for diuron, linuron, and monolinuron. See Figure 1 and Table 1. Additionally, the good solubility of ammonium acetate in methanol facilitated the LC operation, and the high temperature reduced the viscosity of the methanol/aqueous mobile phase.

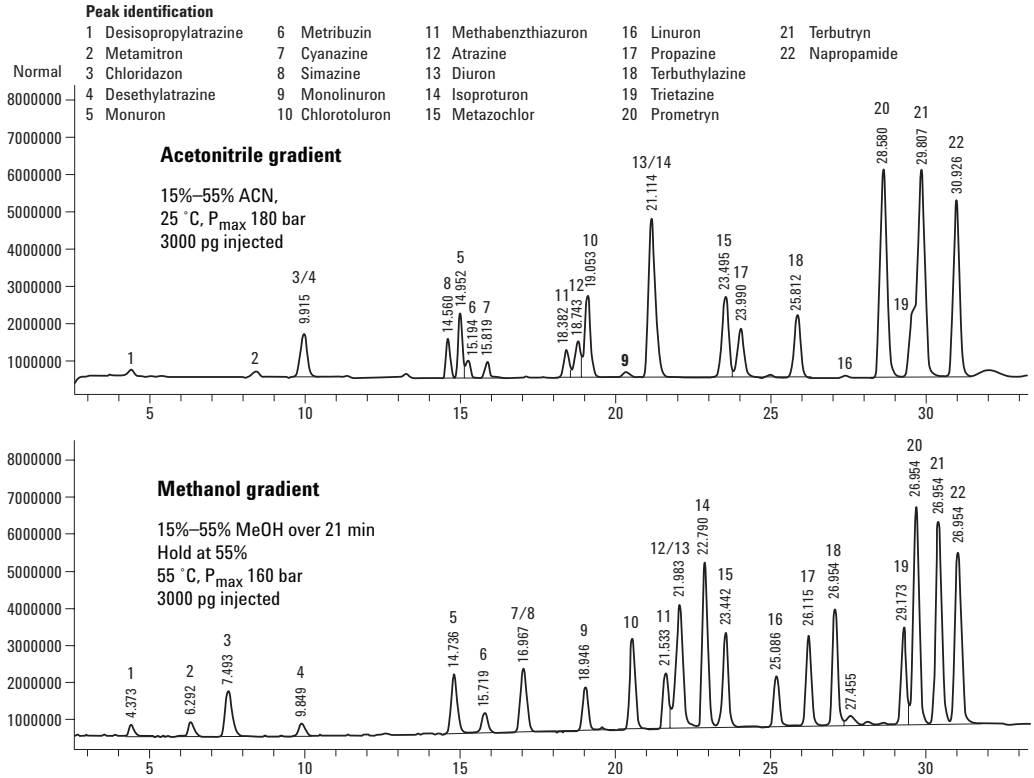

Figure 1. A comparison of LC/MS scan mode total ion chromatograms (TICs) of target pesticides and pesticide metabolites, using methanol and ACN gradients. Note the greatly improved resolution and sensitivity, for compounds 1, 4, 9, 13, and 16 using the methanol gradient.

Table 1. Peak Area Comparisons for the Target Compounds Using the Two Solvent Gradients. Major Differences are Highlighted. Desisopropylatrazine (#1) and Desethylatrazine (#4) are Pesticide Metabolites

Peak number	Analyte	MeOH peak area	ACN peak area
1	**Desisopropylatrazine**	**2,443,420**	1,283,000
2	Metamitron	2,963,500	2,305,730
3	Chloridazon	3,814,240	3,830,330
4	**Desethylatrazine**	**3,480,230**	1,788,900
5	Monuron	11,398,200	9,353,810
6	Metribuzin	5,985,140	3,804,690
7	Cyanazine	3,438,040	2,156,270
8	Simazine	9,868,370	6,781,130
9	**Monolinuron**	**8,178,430**	1,369,500
10	Chlorotoluron	15,567,400	15,549,300
11	Methabenzthiazuron	10,896,600	6,809,840
12	Atrazine	14,109,900	9,804,800
13	**Diuron**	**7,718,180**	2,977,630
14	Isoproturon	32,433,700	36,313,200
15	Metazochlor	16,100,900	15,900,100
16	**Linuron**	**6,436,780**	494,439
17	Propazine	18,913,200	12,811,700
18	Terbuthylazine	23,836,100	16,087,500
19	Trietazine	21,209,100	18,607,900
20	Prometryn	56,223,800	67,664,500
21	Terbutryn	54,657,100	66,395,700
22	Napropamide	40,470,800	44,921,000

Using methanol gradient, the greatly improved sensitivity for compounds 1, 4, 9, 13, and 16 far exceeds the slightly diminished sensitivity encountered for compounds 14, 20, 21, and 22. Given the advantages of the methanol gradient, a selective ion monitoring (SIM) method was developed. This required optimization of several experimental variables.

Chromatographic optimization

LC Conditions	
Analytical column	C18 Hypersil BDS, 10 cm × 2 mm id × 3 µm
Guard column	Same type; 1 cm long
Solvent	5 mM NH_4OAc, pH 6 buffer/methanol gradient 15%–55%, then 80%
Injection	40 µL sample
Flow	300 µL/min

MS Optimization

Electrospray - MS Conditions	
Nebulizer pressure	55 psig
Drying gas	Nitrogen
Temperature	350 °C
Flow rate	10 L/min
V_{cap} (positive)	2000 V
V_{cap} (negative)	–2000 V
Fragmentor voltages	Customized

The exact slope of solvent gradient and temperature were optimized to maximize the resolution of all 22 herbicide peaks, and minimize co-elution with known interferences. NBBS (n-butylbenzenesulphonamide), a plasticizer, has a parent ion at 214 *m/z*, and a significant ion at 216 *m/z* interfering with atrazine, thereby requiring that the separation between NBBS and atrazine be maximized. The internal standard (ISTD), d_6-desethylatrazine, was used for the first four analytes and a second ISTD, d_5-terbuthylazine, for the remaining 18. See Figure 2.

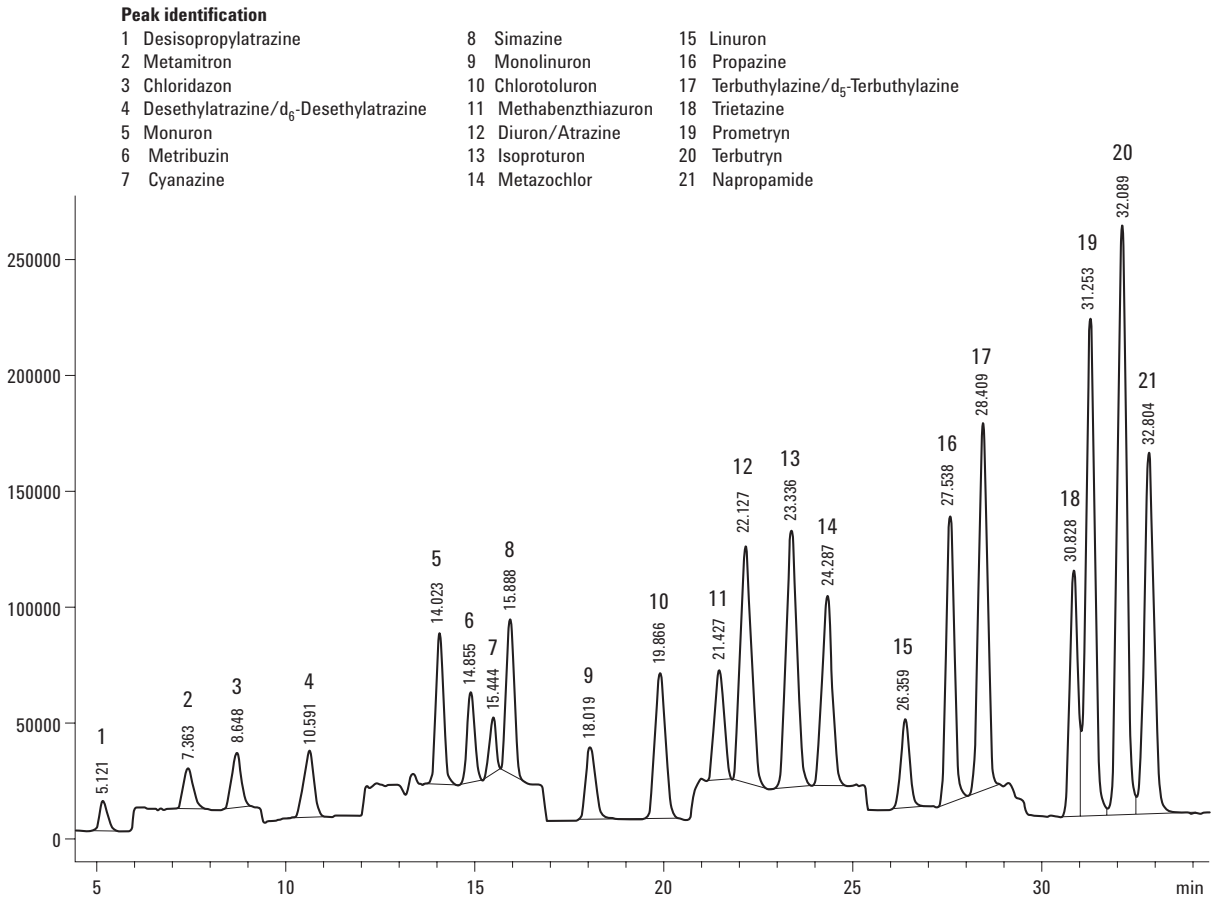

Figure 2. TIC of optimized SIM method for the target herbicides and ISTDs. The only co-elution is with diuron and atrazine; each with distinctive ions at 233/235 and 216/218/174, respectively.

Fragmentor voltages were optimized in both positive and negative modes, and were determined chromatographically. As an example, the effects observed for the first five compounds are shown in Table 2.

Table 2. Effects of Fragmentor Voltages for First Five Compounds

Peak	Compound		Positive ions	Fragmentor voltage	Count		Negative ions	Fragmentor voltage	Count
1	Desisopropylatrazine	M+H	174	110	24800				
			176	110	8000				
2	Metamitron	M+H	203	110	26300	M-H	201/202	90	13500
			204	110	<3000	frag	185	150	2000
		M+Na	225	90	12000				
		M+K	241	90	5000				
		Frag	175	170	6500				
3	Chloridazon	M+H	222	110	30000	M-H	220	110	28000
			224	110			222	110	8000
		M+Na	244/246	90	20000				
		M+59	280/282	90	73000				
		Note 224 is in background, giving 224 low sensitivity.							
4	Desethylatrazine	M+H	188	110	27000				
			190	110	9000				
5	Monuron	M+H	199	110	89000	M-H	197	110	81000
			201	110	30000		199	110	25000
		M+Na	222/224	90–110	30000				

The fragmentor voltage resulting in the highest ion count for each significant ion was chosen for each target compound. The chosen ions for this method are summarized in Table 3.

Table 3. The Main and Qualifier Ions

Analyte	*m/z*	Qualifier ion (*m/z*)	Isotopic/Fragment	Rel. abundance (%)
Desisopropyl-atrazine	174.00	176.00	Isotopic	30.1
Metamitron	203.00	175.00	Fragment	26.2
Chloridazon	222.00	224.00	Isotopic	31.3
Desethylatrazine	188.00	190.00	Isotopic	29.8
d_6-Desethylatrazine	194.00	196.00	Isotopic	31.2
Monuron	199.00	201.00	Isotopic	31.7
Metribuzin	215.00	187.00	Fragment	13.9
Cyanazine	241.00	243.00	Isotopic	30.2
Simazine	202.00	204.00	Isotopic	31.6
Monolinuron	215.00	217.00	Isotopic	33.3
Chlorotoluron	213.00	215.00	Isotopic	31.1
Methabenzthiazuron*	222.00	165.00	Fragment	89.0
Diuron	233.00	235.00	Isotopic	61.0
Atrazine (1)	216.00	218.00	Isotopic	31.0
Atrazine (2)		174.00	Fragment	18.0
Isoproturon	207.00	165.00	Fragment	8.8
Metazachlor	278.00	210.00	Fragment	59.2
Linuron	249.00	251.00	Isotopic	63.0
Propazine	230.00	232.00	Isotopic	31.0
Terbuthylazine	230.00	232.00	Isotopic	31.9
d_5-Terbuthylazine	235.00	237.00	Isotopic	32.8
Trietazine	230.00	232.00	Isotopic	29.3
Prometryn	242.00	200.00	Fragment	17.9
Terbutryn	242.00	186.00	Fragment	54.6
Napropamide	272.00	171.00	Fragment	14.1

* Ions used for Sample Quantification are Main Ions, *except Methabenzthiazuron where the fragment ion is used for quantification, and the main ion for confirmation. Ions used for confirmation of positive results of 20 ng/L are Qualifier ions.

The main/qualifier ions were divided into eight SIM groups according to retention time. The SIM details are summarized in Table 4.

Table 4. SIM Specifications

Time	Group name	SIM ion	Fragmentor	Dwell
4.30	Group 1	174.00	110	589
		176.00	110	589
5.80	Group 2	175.00	170	294
		203.00	90	294
		222.00	110	294
		224.0	110	294
9.20	Group 3	188.00	110	294
		190.00	110	294
		194.00	110	294
		196.00	110	294
12.00	Group 4	187.00	170	146
		199.00	110	146
		201.00	110	146
		202.00	110	146
		204.00	110	146
		215.00	110	146
		241.00	110	146
		243.00	110	146
15.90	Group 5	213.00	110	392
		215.00	110	392
		217.00	110	392
19.30	Group 6	165.00	170	128
		174.00	170	116
		207.00	110	58
		210.00	130	116
		216.00	110	128
		218.00	110	116
		222.00	110	128
		233.00	110	128
		235.00	110	116
		278.00	90	128
24.20	Group 7	230.00	120	176
		232.00	120	176
		235.00	120	176
		237.00	120	176
		249.00	110	234
		251.00	110	234
28.75	Group 8	171.00	190	167
		186.00	190	167
		200.00	210	167
		230.00	130	167
		232.00	130	167
		242.00	120	167
		272.00	110	167

Gain and Resolution settings for Groups 1–8 are set to 3.0 and Low, respectively.

The LC samples were prepared using the SPE method described below.

1. Use 3 mL 100 mg IST 101 PSDVB, (polystyrene-divinylbenzene) cartridges
2. Prepare with 3 mL each of acetone, ethyl acetate, and pH8 borate buffer (pH8 is essential for high recovery of prometryn and terbutryn)
3. Add 50-mL water sample (adjusted to pH 8) and add ISTDs. Draw through cartridge.
4. Wash with 3-mL 10% MeOH. Dry 15 min ~10 psi.
5. Elute with 2 aliquots of 3-mL ethyl acetate. (60-µL H_2O in tubes as keeper). Dry in Turbo-Vap.
6. Make up in 1 mL 5% MeOH/95% H_2O

Data was collected in SIM mode for the 22 main ions, 23 qualifier ions and ions for the two ISTDs. Quantitation was based on the main ions. Qualifiers were processed on positive samples only.

Using the developed SIM method, several water samples, of varying hardness, were analyzed. A typical set of chromatograms is shown in Figure 3.

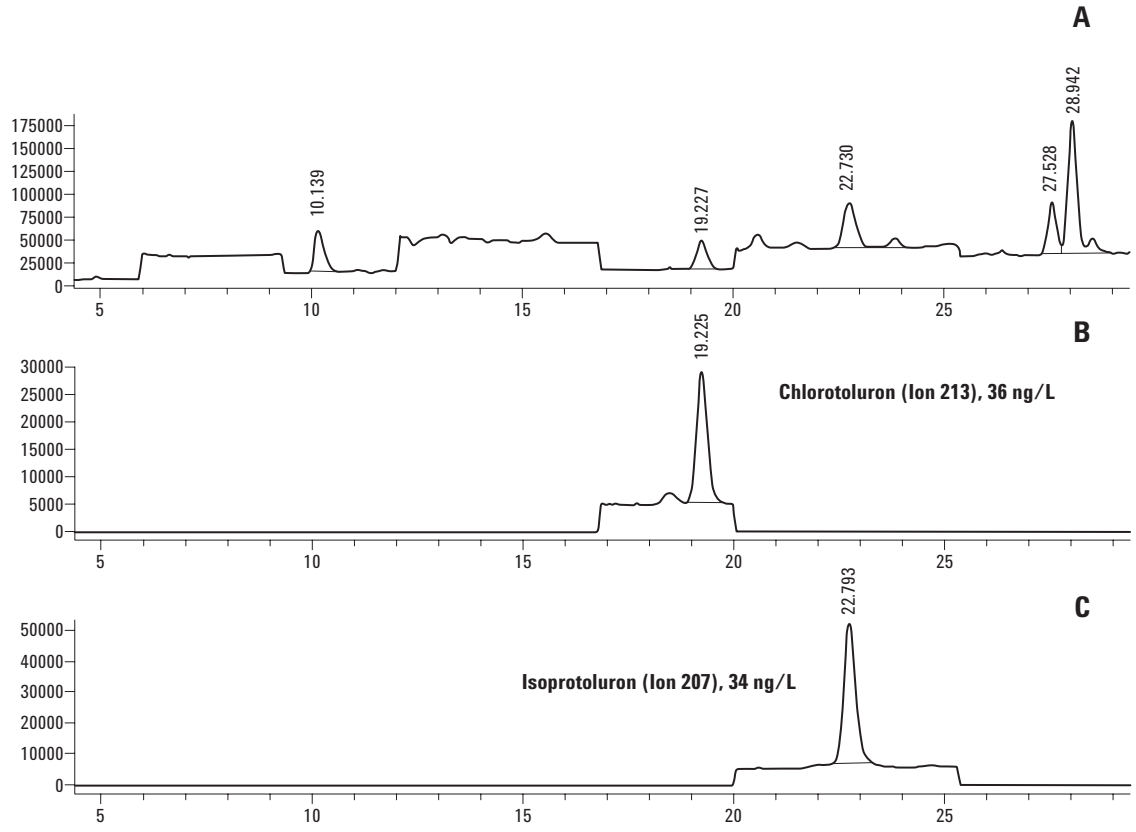

Figure 3. Example chromatograms for a sample of raw surface water. A) TIC of all SIM ions, B) extracted ion chromatogram (EIC) for ion 213, and C) EIC for ion 207.

As part of a systematic validation protocol, 11 batches of duplicates of extracted spiked samples were run and characterized for several key properties, including recovery, detection limits, precision, and accuracy. These data are shown in Tables 5–6.

Table 5. Recoveries versus Diluted Standards*

	Maundown Soft water, uncorrrected		Rodbourne Hard water, uncorrrected		Sutton Bingham Raw Intermediate, uncorrrected	
	%Recovery	%RSD	%Recovery	%RSD	% Recovery	%RSD
Desisopropyl	67.9	20.7	63.80	19.15	64.90	12.85
Metamitron	83.0	13.0	76.75	16.37	78.56	13.94
Chloridazon	96.6	11.8	90.62	9.51	95.04	11.11
d_6-desethylatraz 194	92.9	10.8	86.00	13.10	84.63	8.41
Desethyl	89.9	11.0	85.21	12.50	82.92	10.17
Monuron	101.1	6.8	97.18	5.88	97.66	6.60
Metribuzin	93.9	9.0	82.62	14.71	87.94	8.76
Cyanazine	95.2	7.0	94.01	7.74	92.29	7.70
Simazine	99.6	6.1	95.00	6.24	96.69	6.68
Monolinuron	97.9	5.4	95.90	7.17	102.65	7.38
Chlorotoluron	100.6	5.9	97.02	6.14	100.82	6.31
Methabenzthiazuron	100.4	5.6	96.46	5.94	98.51	6.58
Diuron	101.6	7.1	97.10	6.58	99.52	7.58
Atrazine	102.4	4.6	97.35	4.71	99.96	4.54
Isoproturon	100.5	4.7	96.74	5.98	101.96	7.24
Metazochlor	101.9	5.3	98.57	4.95	101.01	4.54
Linuron	101.2	7.3	95.65	5.74	99.51	6.85
Propazine	98.5	5.1	96.58	4.35	96.90	6.14
d_5-terbuthylazine 237	98.7	6.6	95.01	3.93	99.61	6.66
Terbuthylazine	96.3	6.1	94.01	5.35	91.82	7.10
Trietazine	93.6	5.2	92.95	7.21	94.93	7.97
Prometryn	90.9	5.3	89.60	10.75	92.75	6.61
Terbutryn	87.9	5.4	87.64	11.59	89.56	8.87
Napropamide	98.1	6.5	95.79	6.72	94.86	8.71

* This data is from 11 batches extracted and run in duplicate.

Table 6. Validation Data - Limits of Detection (LOD), Precision, and Accuracy for Borehole Water, as an Example†

	Borehole, hard		
	PCV†† = 0.1 µg/L	Spike = 0.1000	
	LOD target = 0.025 µg/L	Target ±0.0125	
	All pass	All pass	
Compound	**LOD calc.*, µg/L**	**Precision****	**Accuracy*****
Desisopropylatrazine	0.008	0.0104	0.0900
Metamitron	0.007	0.0210	0.0951
Chloridazon	0.007	0.0118	0.0988
Desethylatrazine	0.005	0.0046	0.0937
Monuron	0.009	0.0058	0.0933
Metribuzin	0.007	0.0182	0.0973
Cyanazine	0.007	0.0112	0.0949
Simazine	0.007	0.0110	0.0987
Monolinuron	0.009	0.0090	0.0986
Chlorotoluron	0.007	0.0050	0.0969
Methabenzthiazuron	0.007	0.0068	0.0958
Atrazine	0.005	0.0048	0.0975
Diuron	0.005	0.0060	0.0997
Isoproturon	0.007	0.0140	0.0926
Metazochlor	0.008	0.0048	0.0971
Linuron	0.006	0.0066	0.0967
Propazine	0.003	0.0040	0.1003
Terbuthylazine	0.003	0.0045	0.1076
Trietazine	0.003	0.0096	0.0977
Prometryn	0.008	0.0160	0.0976
Terbutryn	0.009	0.0159	0.0952
Napropamide	0.002	0.0056	0.0983
Methabenzthiazuron 165	0.007	0.0054	0.0954

† This data is from 11 batches extracted and run in duplicate.

†† PCV Prescribed Concentration Value, which for pesticides is 0.1 µg/mL.

* LOD calc. determined from 3 x within-batch standard deviation on the 0.040 µg/L spiked sample.

** Precision 2 x standard deviation

*** Accuracy target ±12.5% of the added spike

Qualifier ion 165 included when interference occurs on main ion 222.

Summary

An LC/MS method for pesticides was developed and optimized for selectivity and sensitivity. It was streamlined for multi-analytes and data-handling. It is a robust and fully validated method, meeting UK regulatory requirements, and is in routine operation.

Reference

1. Riezve Soniassy, Pat Sandra, and Claus Schlett, "Water Analysis -Organic Micropollutants", (1994) Hewlett-Packard.
2. (1998) *Acta Hydrochimica Hydrobiol.* **26**, 6, 318-329.

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Printed in the USA
January 18, 2005
5989-1688EN

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