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Journal of Chromatography A, 813 (1998) 63–70

JOURNAL OF CHROMATOGRAPHY A

Application of an on-line liquid chromatographic system for the determination of polar herbicides in drinking water within a routine laboratory

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Received 29 April 1997; received in revised form 14 April 1998; accepted 16 April 1998

Abstract

A previously developed on-line automated system for the analysis of polar herbicides in raw and drinking water, was further optimised for use within a routine environmental monitoring laboratory. One-hundred-ml portions of sample were extracted onto interchangeable, PLRP-S packed cartridges, prior to desorption by the mobile phase of an HPLC system. Determination of triazine and phenylurea herbicides was achieved by UV diode array detection, with detection limits ranging between 0.002 and 0.012 $\mu g\ l^{-1}$. Precision, in HPLC grade water, ranged from 3.1 to 9.7% R.S.D. at the 0.090 $\mu g\ l^{-1}$ level, as determined at those wavelengths selected for routine analysis. Confirmation of positive results was achieved by library searching of UV spectra. The system was found to be robust for routine analysis, with a sample throughput of ten samples per day.

Keywords: Water analysis; Pesticides; Triazines; Phenylureas

1. Introduction

On-line systems have been developed, for the determination of polar herbicides in surface waters [1,2], particularly in connection with environmental monitoring of the River Rhine. In the past, the river has suffered from serious pollution incidents, that have compromised drinking water supplies, hence requiring the setting up of such systems. The efficiency and performance of the developed systems, as originally intended for remote location, has resulted in interest in the transfer of such techniques, to the routine potable water monitoring laboratory.

This paper presents the development of a method of analysis of selected triazine and phenylurea herbicides, to supersede the former practice of performing separate (off-line) solid-phase extractions and analyses, with a single, on-line extraction and high-performance liquid chromatography (HPLC) analysis for the entire suite of herbicides. The main aim of the project was to ensure that laboratory based methodologies were compliant with the quality standards required, were robust and efficient enough to cope with a high throughput of samples.

European and UK drinking water regulations [3] require that potable water contains no greater than 0.1 $\mu g\ l^{-1}$ of an individual pesticide and, no greater than 0.5 $\mu g\ l^{-1}$ of total pesticides (the prescribed concentration or value). Methodologies for monitoring at these levels are required to meet detection limits down to 0.01 $\mu g\ l^{-1}$. The total error of the measurements, should not exceed 20% (10% bias

0021-9673/98/$19.00
PII: S0021-9673(98)00329-X

and 5% precision). In practice, many methods in operation today, particularly for organic parameters, do not meet these requirements, especially so in terms of precision rather than bias. There is obviously a need to improve analytical performance wherever practicable, in terms of quality and efficiency.

The existing method for triazine analysis was performed by the solid-phase extraction (SPE) of 1-l of sample, followed by gas chromatographic determination of the extract with thermionic specific detection. Confirmation of samples exceeding the prescribed concentration value was achieved by gas chromatography–mass spectrometry (GC–MS). Phenylurea herbicides analysis was similarly performed following SPE of a 1-l sample and analysis by isocratic reversed-phase HPLC with fixed wavelength UV detection. Confirmation of such ''failed'' samples was determined by re-analysis upon an alternative HPLC column. The majority of samples received, required analysis of both triazines and phenylureas, such that a combined analysis was worthwhile. The objective was to develop a method using on-line sample preparation, requiring only 100 ml of sample and, using diode array detection (DAD) to confirm the identity of positive concentrations of herbicide.

2. Background to the analytical system

The off-line extraction and analysis approach suffers from a number of disadvantages, although in the development of an on-line system, many factors have to be taken into account [4].

Previous methods of on-line sample pre-concentration have involved the use of trace enrichment upon a single pre-column [5,6] which has proved effective in obtaining low $\mu g\ l^{-1}$ levels, from less than 100 ml of sample. The approach suffered from the need to renew the pre-column, following a build up of contamination and, a more robust system was required.

Systems, using Empore type membrane discs were developed in order to alleviate some of the problems noted above and, improve detection limits by the extraction of large volumes of sample of up to 1 l [7,8].

Techniques have also been developed to reduce the problems caused by matrix effects when analysing low-molecular-mass, polar compounds, such as by the use of dialysis [9] or advanced data analysis [10]. The use of MS as a detector, enables low level detection, with positive identification of these analytes [11] without the same degree of peak separation required i.e., shorter run times.

The method described in this project, was developed as an extension of the on-line analyses developed previously, utilising an exchangeable, low volume pre-column system [1], linked to a HPLC system with DAD. Detection limits have been improved, compared to previous similar systems and, by exchange of pre-columns, performance is maintained. As a further advantage over other systems [2], the system can be fully software controlled for the concentration and analysis, integration and reporting stages. In this project, sensitivity, reproducibility and resolution of compounds for this application were optimised, prior to validation.

3. Experimental

3.1. Analytical system

The system utilised in this project, is known as SAMOS (System for the Automated Measurement of Organic micropollutants in Surface waters) which was developed as part of the European River Basin Program [12]. It comprises a Prospekt on-line SPE system, coupled to a Hewlett-Packard 1090 HPLC with UV DAD. Samples pumped to the Prospekt via a delivery unit, are extracted and the analytes are directly desorbed by the mobile phase of the HPLC system. Subsequently, the analytes are determined by means of LC–DAD UV. The entire system is software controlled, linked to an HP Vectra XM-2 486 PC and configured such that both extraction and chromatographic analysis occur simultaneously.

Samples were extracted onto packed cartridges, 10×2 mm I.D., containing 15–25 μm PLRP-S styrene–divinylbenzene copolymer, through which samples were pumped, following conditioning and rinsing.

HPLC separation of the analytes was performed upon a Zorbax SB-C_{18} 150×4.6 mm column with 3.5 μm particle size (Rockland Technologies).

HPLC grade methanol and acetonitrile were obtained from Rathburn (Walkerburn, UK), HPLC grade water from a Milli-Q system following pretreatment by reverse osmosis and, ammonium acetate (99.9999% pure) from Aldrich (Gillingham, Dorset, UK).

Herbicide reference materials were obtained from QMx (Gt. Yeldham, Essex, UK), at least 99% pure and comprised the following compounds: atrazine, chlorotoluron, diuron, isoproturon, linuron, methabenzthiazuron, propazine, simazine, terbutryn and trietazine.

Prior to analysis, samples were filtered through a 0.45-μm cellulose acetate filter (Whatman, Maidstone, Kent, UK), with the tap water for spiking experiments being taken from the source at Schwyll water works (Ogmore-by-sea, Bridgend, UK).

3.2. Analytical procedures

Stock solutions of herbicide were prepared by adding 10 mg of each compound to a 100-ml volumetric flask to give 100 mg l^{-1} solutions, made up in acetonitrile, stored in the dark at 4°C and made up freshly every three months.

Working solutions were prepared by adding 100 μl of stock solution into a 50-ml volumetric flask and, making up with purified water. The solution was stored at 4°C and made up weekly.

The aqueous portion of the mobile phase, A, consisted of a 1 m*M* solution of ammonium acetate and, was produced by the addition of 1 ml of a 1 *M* solution to 1000 ml of purified water. HPLC grade acetonitrile was used as the organic component of the mobile phase, B, and, purified water used for flushing of the column at the end of a run, C. All components of the mobile phase were degassed with helium, before and during use. The gradient program shown in Table 1 was used and was programmed such that the final analysis in a sequence, flushed the system with acetonitrile and water.

Standards and samples were enriched on-line by passing 100 ml of solution through a PLRP-S cartridge, at 4 ml min^{-1}, after conditioning of each cartridge with 7.5 ml of methanol and 10 ml of water. The solvent delivery unit (SDU), comprised of a system for delivering solvents and samples to the Prospekt unit, at constant flow-rates and pressures up to 150 bar. The SDU, using three solvent selection valves, was configured for 16 positions, 1 and 2 being used for the conditioning solvents, the remaining 14 positions for standards and samples. Valve 2 on the Prospekt was configured such that desorption of analytes, by the mobile phase, occurred in reverse direction to sample loading i.e., backflush mode (Fig. 1).

Table 1
HPLC gradient program

Time (min)	% B	Flow (ml min^{-1})
0.00	10.0	1.000
30.00	30.0	1.000
49.00	59.0	1.000
50.00	100.0	1.000
52.00	100.0	1.000
52.50	100.0	2.000
56.00	100.0	2.000
57.00	100.0	1.000
58.00	10.0	1.000

The Prospekt was software-controlled from a link to the HP Chemstation, running the HPLC – refer to preparation program (Table 2).

4. Results and discussion

In order to achieve a rapid separation with baseline-resolved peaks, several parameters that influenced the separation and peak shape were studied. A summary of the optimised conditions is as follows; although several stationary phases (Hypersil BDS-C_{18}, Phenomenex Ultracarb 5 ODS, PLRP-S 100A and Micra NPS RP18) were evaluated, the Zorbax SB-C_{18} 150×4.6 mm, 3.5 μm column was found to give the best results. The best compromise in temperature was found to be 43°C, at which temperature isoproturon and diuron became baseline separated, whilst methabenzthiazuron and chlortoluron, however, still showed some overlap. The pH was maintained at 7 by using ammonium acetate in order to obtain a sharp peak for terbutryn, a compound that occasionally showed retention time drift when no buffer was used. The initial gradient conditions of using 10% acetonitrile rather than 5%, improved the stability of peak resolution (Fig. 2).

Various guard columns were used initially, but as

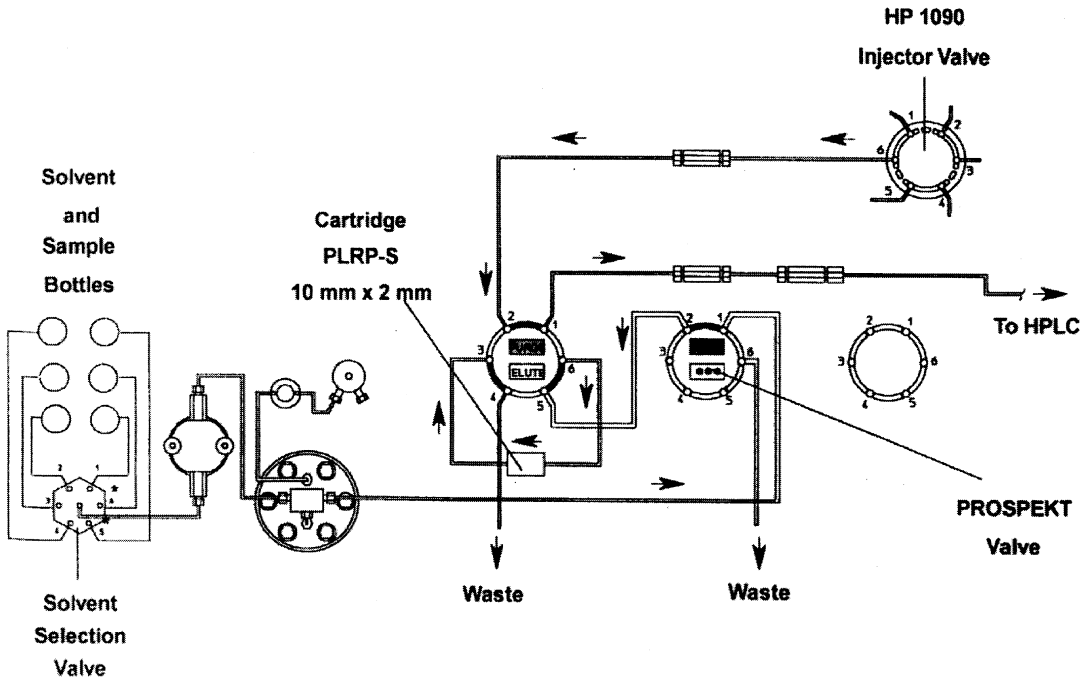

Fig. 1. SAMOS system configured in backflush mode. (Sample enrichment shown).

resolution problems were encountered, it was decided to run the system without a guard column. Analysing potable and raw water samples, column life has been at least three months. However, column lifetime has shown to be reduced following analysis of severely contaminated ground water samples, mainly due to increases in back pressure. The use of a guard column was not found to adequately protect the column from these high pressure problems. Also, the use of an interchangeable 0.5-μm in-line filter was required to remove stray particles from the PLRP-S cartridges and is exchanged on a weekly basis.

Peak resolution is monitored routinely, measuring the separation between the methabenzthiazuron and chlorotoluron peaks. Over a three-month period, the relative standard deviation (R.S.D.) of the resolution between these two compounds has been found to be

Table 2
Prospekt sample preparation program

Time	Function	Set at	Comment
00:00:01	Valve 1	Purge	
00:00:02	Change cartridge		Exchanges cartridge
00:00:03	Valve 2	Port 1>2	Valve 2 connects to valve 1
00:00:04	Solvent channel	1	Methanol
00:00:05	SDU flow-rate	5.0	ml/min
00:01:36	Solvent channel	2	Water
00:03:35	Valve 2	Port 1>6	To waste
00:03:36	Solvent channel	1	Sample selected by Chemstation sequence table
00:03:37	SDU flow-rate	4.0	ml/min
00:06:06	Valve 2	Port 1>2	Enrichment of sample on cartridge
00:31:06	SDU flow-rate	0.0	
00:31:10	End of timetable		

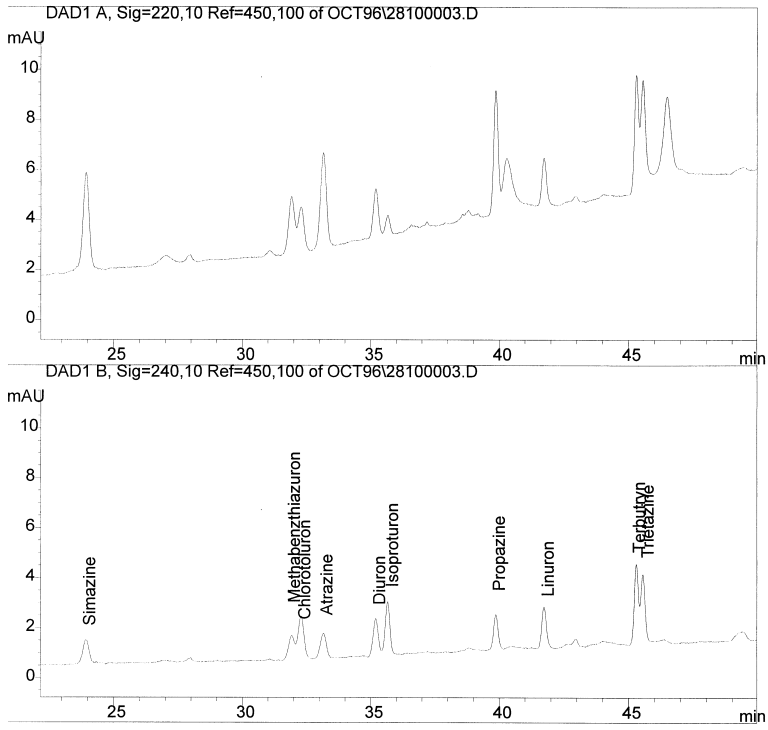

Fig. 2. Separation of analytes on a Zorbax SB-C_{18} 150×4.6 mm, 3.5 μm column. Analyte concentration: 10 ng per compound, on-column. Chromatogram displayed at 220 nm (top) and 240 nm (bottom).

approximately 8%. During this period, analytical columns were exchanged upon the basis of excess back pressure and not due to a loss of analyte resolution. Similarly during this period, the R.S.D. of the daily response factor for chlorotoluron, as an example, in the 200 ng/l standard has been measured as approximately 4.5%.

The importance of being able to positively identify herbicides, particularly at the prescribed concentration level of 0.1 μg l^{-1}, was the main reason for selecting DAD. A user-compiled library of UV spectra was generated from 100 ng on-column amounts of reference materials, and has proved useful in confirming herbicides to levels, below 0.1 μg l^{-1} (Fig. 3), especially for the phenylurea herbicides, which produce more distinctive spectra than triazines.

Table 3 shows the limits of detection, measured as 4.65-times the within batch standard deviation ($4.65S_w$), determined at a spiking level of 10 ng l^{-1} for all herbicides in the suite. Note that the lowest limits of detection were not necessarily achieved at the most sensitive wavelengths. For example, propazine absorbs more strongly at 220 nm than 240 nm, although because of interferences at 220 nm, the limit of detection in practice is higher than at 240 nm. Methabenzthiazuron performed slightly better at 220 nm, than at 240 nm, although a one-off contamination in a blank sample, compromised the detection limit.

Precision and bias are also tabulated and in all cases, are a significant improvement compared to the previous data – in many examples, R.S.D.s were determined as below 5%, at the 0.090 μg l^{-1} level. For example, the former method of analysis of methabenzthiazuron gave a precision of 23.3% at the 0.090 μg l^{-1} level and a bias of 23.3% at the same level. This compares to figures of 5.4% precision and 2.7% bias, at this concentration, using the on-line approach.

Table 4 summarises the recovery of analytes, spiked into tap water, at around the 0.09 μg l^{-1}

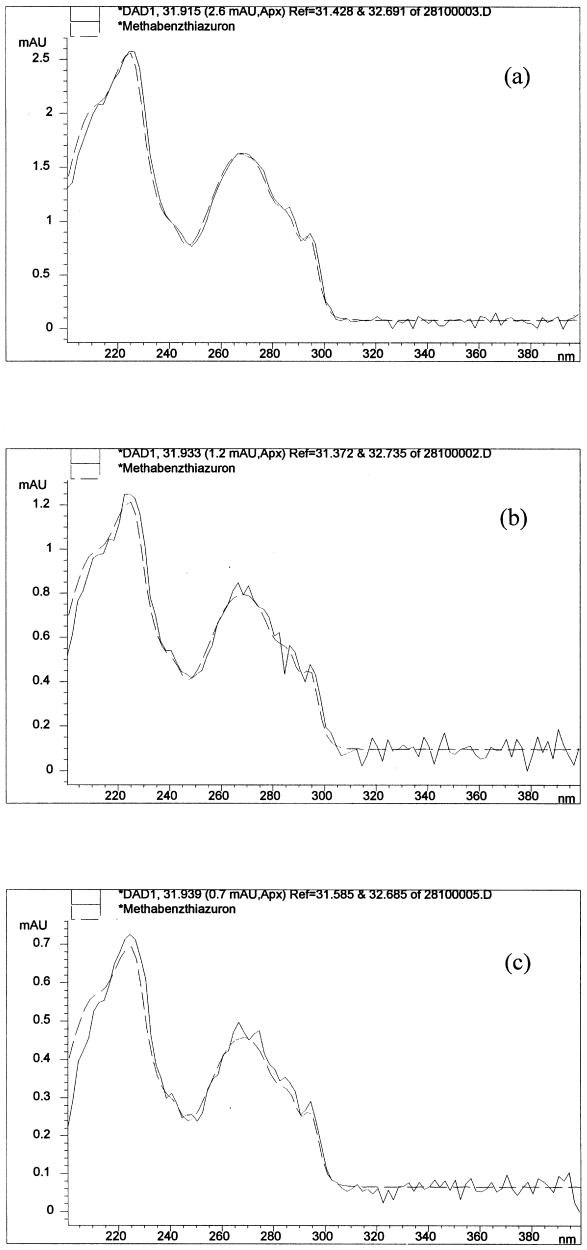

Fig. 3. Library searching of UV spectra (normalised). Spectral composition of methabenzthiazuron at (a) 100 ng/l – match fit 994, (b) 50 ng/l – match fit 982 and (c) 30 ng/l – match fit 973.

level. Most herbicides are recovered within the 95–105% range, although isoproturon gave 112% recovery. The previous off-line method had a similar recovery of 107% for this compound whilst methabenthiazuron had a recovery of 123% in tap water for the former method, compared to 98%, using the SAMOS approach.

In general, former methodologies, gave lower recoveries of analytes in tap water, in addition to poorer precision. The practice followed formerly, was to use unextracted standards for calibration, which partially accounts for recovery loss in samples (and low bias in AQC standards).

The off-line methods used C_{18} SPE cartridges, that have more recently been supplemented by packing materials with a higher affinity for polar compounds. The use of PLRP-S cartridges in the SAMOS system, increases the retention of polar compounds and minimises breakthrough, compared to C_{18}, or other materials [4].

5. Conclusions

Two off-line SPE procedures, with separate GC and HPLC analysis, have been superseded by a fully automated, on-line extraction and HPLC–DAD procedure, described in this paper. Thus the analysis of triazine and phenylurea herbicides in raw and potable water, at a major environmental testing laboratory, is now performed in a single suite, using less resources than previously and producing data of better quality. The latter point is being confirmed by participation in inter-laboratory comparisons in addition to routine quality control. Detection limits range between 0.002 and 0.012 $\mu g\ l^{-1}$, when monitored at those wavelengths, selected for routine operation.

Analytical run times of 65 min are required in order to separate all of the analytes adequately and, with calibration and AQC standards being analysed, the system is able to process approximately ten samples per day. With re-configuration of the SDU and larger mobile phase reservoirs, extended operation over weekends would be possible.

Confirmation of the identity of herbicides, above the prescribed concentration for drinking water, is shown to be successful and, even at levels near to the detection limits, thus alternative confirmatory procedures e.g., GC–MS, are less likely to be required, further reducing costs. The presence of interferences is a possibility, in water samples, particularly in respect to analysis of triazines, such that on occa-

Table 3
Summary of detection limits, bias and precision

	Wavelength (nm)	Detection limit ($\mu g\ l^{-1}$)	% R.S.D. at 0.030 ($\mu g\ l^{-1}$)	Bias (%)
Atrazine	220	0.005	5.98	0.0
Chlorotoluron	240	0.009	10.4	1.5
	215	0.025	8.86	0.5
Diuron	250	0.005	7.40	−2.2
	240	0.014	9.23	−1.7
Isoproturon	240	0.007	8.20	−0.5
Linuron	250	0.005	8.22	−4.0
	240	0.006	14.3	−2.2
Methabenzthiazuron	220	0.049	9.54	1.0
	240	0.009	11.7	1.7
Propazine	220	0.079	38.2	61.3
	240	0.012	17.0	5.0
Simazine	220	0.005	4.69	−0.7
Terbutryn	230	0.002	7.33	0.5
	220	0.019	9.53	5.8
Trietazine	230	0.005	14.0	3.2
	220	0.034	15.2	16.8
			% R.S.D. at 0.090 ($\mu g\ l^{-1}$)	
Atrazine	220		4.14	2.1
Chlorotoluron	240		4.98	0.2
	215		6.42	−0.4
Diuron	250		3.35	2.3
	240		5.61	2.9
Isoproturon	240		8.48	1.8
Linuron	250		4.40	2.8
	240		7.88	2.8
Methabenzthiazuron	220		5.40	2.7
	240		7.43	2.1
Propazine	220		13.4	11.5
	240		9.68	2.9
Simazine	220		3.11	0.5
Terbutryn	230		3.12	4.0
	220		6.13	6.1
Trietazine	230		4.01	6.3
	220		11.9	10.2

Table 4
Recoveries in tap water, at wavelengths selected for routine operation

	Wavelength (nm)	% Recovery of 0.090 μg l^{-1} spiked in tap water
Atrazine	220	101.8
Chlorotoluron	240	97.5
Diuron	250	104.5
Isoproturon	240	111.8
Linuron	250	103.8
Methabenzthiazuron	240	98.0
Propazine	240	100.3
Simazine	220	100.8
Terbutryn	230	102.1
Trietazine	230	108.1

sions, a result below the critical level of 0.1 μg l^{-1} cannot be quoted. Further application of this on-line technique, with MS detection is likely to be a productive means of reducing analytical run-times and increasing selectivity.

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