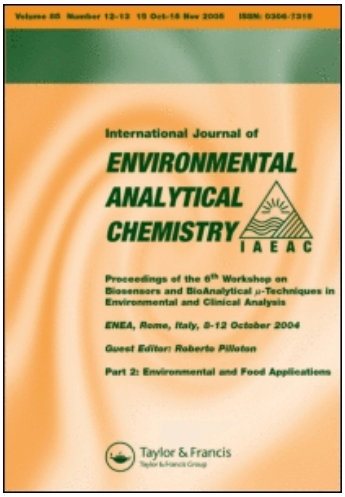

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DETERMINATION AND MONITORING OF POLAR COMPOUNDS AND ACIDIC HERBICIDES USING A MODIFIED SAMOS SYSTEM

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A system for the automated monitoring of organic pollutants in surface waters (SAMOS) has been developed over the last 5 years to monitor for the presence of organic contaminants in surface water. It uses a solid-phase extraction (SPE) trace enrichment step followed by on-line elution and separation by HPLC. Detection and provisional identification is obtained with diode array detection. For pesticide and herbicide analysis, mainly medium volatile, neutral compounds such as carbamates, triazines and phenyl ureas have been reported.

Ionic species such as phenoxy acid herbicides are poorly preconcentrated at normal river pH. These ionic/polar compounds frequently show breakthrough from the SPE cartridge prior to complete loading of the sample. Any retained polar analytes are also often obscured by the presence of co-extracted humic substances in river water samples. The paper presents the required changes to the original SAMOS system to allow ionised and polar pollutants to be successfully analysed. These changes involve allowing the ionic/polar compounds to break through from the loading onto the primary cartridge (PLRP-S), allowing all but the last few ml of sample to go to waste. When breakthrough of the relevant analytes is achieved, the remaining sample is switched automatically on-line to a secondary cartridge (again PLRP-S) with acid being added just prior to this to neutralise the compounds This secondary cartridge effectively preconcentrates the ionic/polar compounds.

The two cartridges are desorbed in two subsequent gradient elution LC-DAD runs. Analysis of several major classes of compounds is achieved, notably members of the triazine, phenyl urea, phenol and acid herbicide groups.

The system has been designed and tested in the laboratory and applied at an installation remote from the laboratory on a river site as part of an intake protection programme. Details of the method performance, experiences of operation and access of the system via telemetry are discussed.

Keywords: Monitoring; polar compounds; acid herbicides; SAMOS

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INTRODUCTION

A System for the Automated Measurement of Organic pollutants in Surface water (SAMOS) has been developed as an on-line river monitor [1]. It is based on automated solid phase extraction (SPE) coupled with liquid chromatography (LC) and was originally designed for the analysis of triazine and substituted urea herbicides and a range of other neutral organic compounds. The current aim was to extend its capability to include the analysis of phenols and acid herbicides from one sample extraction and to install and operate such a developed system unattended as a river intake monitor.

The SAMOS-LC technique enables the trace enrichment of analytes that have to be determined at levels down to 1ug/l or lower. This trace enrichment requires sample volumes in the order of 10–100ml of sample. This volume is loaded onto a precolumn, directing the extracted sample stream to waste.

The precolumn is subsequently eluted directly onto the LC column using switching valves with the total amount of analytes retained from the sample volume being transferred and analysed. This causes a dramatic increase in sensitivity over off-line extraction and elution techniques where conventionally only a portion of the eluate is taken for analysis. The quantitative transfer from the precolumn used in the on-line mode to the separation-cum-detection part of the system enables the analyst to approach or reach the limits of 0.1ug/l or 1ug/l set by the EC (European Community) for the determination of pesticides and herbicides in drinking waters and surface waters respectively.

In the past six years, the fully automated SAMOS-LC technique has been used successfully for analysis of organic micropollutants on the rivers Axios (Greece), Llobregat (Spain), Seine (France) and of course within the Rhine Basin.

Brinkman and co-workers [2, 3] published details of the initial fully automated systems. The groups of compounds that could be analysed in the aqueous environment included triazine and phenyl urea herbicides and additionally a method for organophosphorus pesticides [4] has been published.

Ionic species, however, such as phenoxy acid herbicides are poorly preconcentrated at neutral pH. Ionic and very polar compounds such as phenols frequently show breakthrough from the SPE cartridge prior to complete loading of the sample. Additionally, any retained polar/ionic analytes are often obscured by the presence of co-extracted humic substances in river water applications.

An approach involving the use of two pre-columns was published by Brouwer and Brinkmann [5] in 1994 to counter interference by humic acids with polar analytes. The SAMOS-LC system has also been modified in an elegant and novel way to enable the analysis of a wide range of phenols. Fowlis et al [6] describe an adaptation of the SAMOS-LC which uses a second pre-column being switched in line. This adaptation relies on the breakthrough characteristics of phenol and cresols to again counter the interference caused by humic acids in the subsequent

analysis. Other reported modifications of the technique allow the analysis of rather apolar compounds such as in the laboratory analysis of polycyclic aromatic hydrocarbons [7] and in screening for pyrethroid insecticides [8].

The objective of the current work is the development of a wide-range analysis to include acid herbicides and moderately substituted phenols. This is hindered in that both show the breakthrough described above to varying degrees yet their widespread uses as herbicides (phenoxy acids) and industrial chemicals (phenols) demands that on-line methodology for their analysis is available. In the acid herbicide group, some of the most frequently used herbicides are included, notably MCPA and MCPP (mecoprop) but are all but excluded from conventional SAMOS-LC analysis due to both a low recovery and a rather low response factor.

Three aspects have been addressed in this work, notably the sample treatment, analytical separations and the detection of the analytes.

The approach adopted for sample treatment was to investigate the potential for in-line acidification of the sample stream coming out from the pre-column of the standard SAMOS system (hereafter referred to as precolumn 1).

Analytes breaking through from precolumn 1 were combined with a stream of dilute nitric acid in order to neutralise/suppress ionisation of acid herbicides and to some extent phenolic compounds. Passage of the acidified sample stream through a second precolumn (hereafter referred to as precolumn 2) allowed the analytes to be more readily retained in their neutral forms. Desorption of each precolumn was performed separately and sequentially by the LC gradient conditions resulting in two separate analyses, one from the desorption of precolumn 2 followed by restoration of the LC conditions before desorption of precolumn 1.

For the analytical separations, a stationary phase was required which would allow satisfactory separation of acid herbicides with an acidic buffer and also exhibit long-term stability. Ideally, the same stationary phase should allow a good separation and give good peak shapes for compounds of the other major groups of interest.

Finally, with regard to detection, more and more confidence is being sought in identification of analytes that are concerned with legislative requirements. A combination of analyte retention time and its DAD UV spectrum were considered as the best available approach for the study.

EXPERIMENTAL

Chemicals

Herbicides and phenols were obtained from several sources with a high purity being sought. Suppliers used for herbicides were Promochem (Welwyn Garden

City, UK), Greyhound (Birkenhead, UK) and Thames Chromatography (Windsor, UK). Phenols were obtained from Aldrich (Gillingham, UK) and QM_X (Halstead, UK). Stock standards were prepared at 1000mg/l for the phenols and 100mg/l for the herbicides. The solvent was acetonitrile for all types. Storage was in a refrigerator with renewal every 12 months. Intermediates of 10mg/l were prepared from these stocks by dilution with acetonitrile and renewed monthly.

Sodium dihydrogen phosphate monobasic and phosphoric acid (85%) were obtained from Mallinckrodt Baker (Milton Keynes, UK or Deventer, the Netherlands), Baker analysed grade. Acetonitrile and HPLC water were again obtained from Mallinckrodt Baker using the HPLC Ultra Gradient Grade acetonitrile. Nitric acid was obtained from Mallinckrodt Baker. The "70 to 71% for trace metals analysis – Baker Instra-Analysed Reagent" grade was used.

Standards injected from the HPLC autosampler were at nominally 2mg/l concentration with 15ul being injected. Spiked water standards were prepared at 3ug/l of each component using mixed intermediates in acetonitrile by spiking 75ul of 10mg/l solution into 250ml.

Equipment

A Prospekt (Spark Holland, Emmen, the Netherlands) sample preparation module was used in combination with a Hewlett Packard HP 1090 LC system with three pumps and a DAD UV (Hewlett Packard, Waldbronn, FRG) for the initial development. The whole system was under computer control.

A Harvard 22 syringe infusion pump (Harvard Apparatus, Edenbridge, UK) was used for acid addition, being fitted with a Hamilton Model No. 1010W 10ml Gastight™ Priming Syringe rated to 700psi. The equipment was configured as shown in Figure 1.

The Prospekt sample preparation unit consists of a solvent delivery unit (SDU) comprising of three switching valves each with six inlet ports and one outlet port combined with a reciprocating pump and a pulse damper. This unit is linked to a valve switching unit (Prospekt) with three Rheodyne (Berkeley, CA, USA) six-port valves and a clamping system for automated exchange of precolumns. The Prospekt system is supplied with multiple racks each holding ten precolumns which are fed automatically from a magazine arrangement.

For SPE, 10mm × 2mm i.d. precolumns packed with PLRP-S (a styrene-divinylbenzene co-polymer) were used (Hewlett-Packard p/n 5062–8547).

Precolumn 1, mounted on valve 1 of the Prospekt was changed for each extraction. Precolumn 2, mounted in a specially built holder (Mechanical Workshop of the Chemistry Dept., Vrije Universiteit, Amsterdam, the Netherlands) was

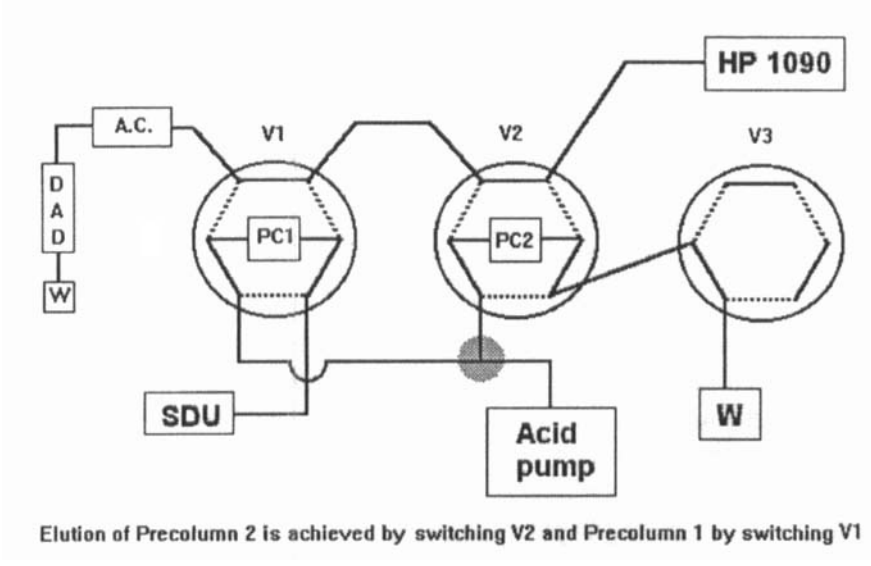

FIGURE 1 Configuration of Prospekt Valves During Loading of Precolumn 2

re-used between maintenance visits, typically between 25 to 40 times. An alternative holder is available from Spark Holland.

The LC separations were performed at 40°C on a 250mm × 4mm i.d. Hypersil BDS C18 column equipped with a 4mm × 4mm i.d. LiChrosphere 60RP select B guard column. Both columns were packed with 5um particles.

Multi-step solvent gradients were employed using phosphate buffer (pH 2.7) and acetonitrile as eluents for each separation. Gradient details are given later. To save solvents, flow rates were set to 0.1ml/min when no separation was being carried out. During separation, UV spectra of all peaks were recorded from 200 to 400nm at the method development stage which took place mainly at the Free University in Amsterdam. The range was extended down to 190nm when the system purchased as the river monitor was installed in the UK.

The UK on-line monitoring system was supplied (Hewlett-Packard, Bracknell, UK) specially tailored for remote monitoring. It included an additional PC workstation for the central laboratory installation. Both central laboratory and site workstations were equipped with modems and Carbon Copy remote access software (Microcom, Woking, UK) to facilitate remote control and viewing of data from the central laboratory.

The Harvard 22 pump was replaced by a Gilson Model 307 HPLC pump with a back pressure regulator (Anachem, Luton, UK) on the outlet side. The replace-

ment was deemed necessary due to a system overpressure causing the Hamilton syringe to burst.

In the development stage, laboratory filtration of raw waters was performed using a glass filter support for 47mm dia. membrane filters (0.45um pores; Millipore, Bedford, MD, USA) mounted onto a vacuum filtration unit with glass collection vessel.

Procedure

Figure 1 shows details of the sample flow paths during the extraction procedure. The SDU multiple inlets were assigned to various streams including conditioning solvents and sample. Elution is effected by switching precolumn 2 in line with the LC flow using V2 and by switching precolumn 1 in line with the LC flow using V1 as required.

Precolumn 1 was exchanged before each run. Next, both columns were switched in line and conditioned using 5ml of acetonitrile followed by 5ml of HPLC-grade water at 1ml/min from the SDU.

During the initial development study in Amsterdam, 50ml of sample was loaded from the SDU onto precolumn 1 with the exiting flow switched initially to waste. The flow rate used was 4ml/min.

During initial sample loading, the acid pump adding the nitric acid after precolumn 1 was switched off, Then after 45ml of the total 50ml sample had passed through precolumn 1, the acid pump was switched on. Precolumn 2 was also switched in series with precolumn 1 at this stage, thus receiving sample that had already passed through precolumn 1 now adjusted to an acid pH.

Finally, each precolumn was eluted in two subsequent LC analyses by switching the precolumn into line with the eluent flow to the analytical column.

A different solvent gradient was employed for each precolumn elution and subsequent HPLC separation. The solvent gradients were achieved using solvent A, a 10mM phosphate buffer solution at pH 2.7 and solvent B, acetonitrile. (Buffer concentrate is prepared with 96.59g of sodium dihydrogen phosphate in about 500ml of water, 17.2ml phosphoric acid is added and then the mixture made up to 1 litre and filtered through a 0.2um nylon filter. The pH should be 2.3. To prepare working buffer, take 25ml of the concentrate into a 51 flask and dilute to volume with water, checking the final pH is 2.7).

Precolumn 2 was eluted first and its retained analytes separated. The gradient used was as follows with all flows at 1ml/min: initially 5% B , increasing to 13% B after 1min, then increasing to 50% B after 34min, finally increasing to 95% B after 35min which was held for 3min.

After restoration of the column solvent start conditions, precolumn 1 was then eluted and its retained analytes separated. The gradient used here was as follows, again with all flows at 1ml/min: initially 5% B for 1min, increasing to 95% B after 31min, holding for 4min. Again the column would then be restored to its start conditions after raising solvent B to 95% for 3min to clean off any residues.

During operation of the SAMOS-LC river monitor, the volumes sampled were increased to aid the limits of detection achievable from the precolumn 2 elution. A total of 55ml of sample was passed through precolumn 1 of which the last 10ml was acidified and passed through precolumn 2. This doubled the volume through the second precolumn.

The LC separations were carried out at 40°C.

Filtration of Samples

The SAMOS-LC requires a highly filtered sample to prevent blockages both during sample extraction and subsequent LC analyses. During development this was performed by the Millipore system utilising 0.45um pore membrane filters.

At the monitoring station, river water was pumped to a primary filtration unit employing a Simplex Easy Autoclean Unit (Cross Manufacturing, Bath, UK) consisting of dual wedge spaced spring filters with automatic backwash facility to remove particles down to 50um. This filtrate was fed to a membrane filter (Stork Friesland, Gorredijk, the Netherlands) removing particles above 0.01um.

The filtrate was fed to a constant head device into which the sampling line to the SDU was placed. In addition, the sampling line had a 10um stainless steel solvent reservoir filter fitted to protect from airborne particles entering the constant head.

RESULTS AND DISCUSSION

Compound selection and the LC/separation

A comprehensive survey of pesticide and herbicide levels in the river where the on-line monitor was to be installed had been commenced 12 months prior to the establishment of any bankside analysers. The results from this ongoing survey indicated a need to have an on-line monitor capable of testing for several herbicide groups, notably the triazine, the phenyl urea and the acid herbicides. Additionally phenolic compounds were requested.

Preselection was made of individual compounds within these groups based primarily on the results from the previous laboratory analyses of spot samples.

For the triazines, simazine and atrazine were selected and for the phenyl ureas, chlortoluron and isoproturon. The acid herbicides selected were MCPA, MCPB, MCPP (mecoprop), 2, 4-D, 2, 4-DB and 2, 4, 5-T and the phenols selected were phenol, p-cresol, 2, 4-dichlorophenol, 2, 4, 6-trichlorophenol and pentachlorophenol (PCP).

The SAMOS-LC has the ability to detect many other compounds provided that they meet with a) the extraction criteria and b) the LC separation and detection conditions. Hence, for the on-line monitoring capability, the observation of peaks outside these standards will be an indicator of the presence of further organic contaminants. It is however considered that the suite of standards used should reflect the likely risk and so this study was performed for the above compounds.

Because the whole set of analytes covers a wide range of polarity, the separations envisaged were carried out using a C_{18}-modified silica analytical column used in the reversed-phase mode. Water (buffer)-acetonitrile gradients were selected to allow use of short wavelengths in the UV region of the detector. Use of these low wavelengths was necessary to achieve realistic detection limits for some of the analytes.

Important parameters are the selection of the LC gradients, the pH of the eluent, the temperature of the column and the choice of appropriate detection wavelengths. With regard to trace enrichment, the selection of precolumn packing type and the in-line adjustment of the sample pH were critical.

The LC gradients chosen were governed mainly by two factors. The first of these was to ensure that an adequate separation was achieved for the compounds selected as standards. The need to prevent ionisation of the acid herbicides and phenols eluted from precolumn 2 dictated that their separation was carried out at acid pH. This was selected at 2.7. As the same column would ideally be used for the separation of the neutral compounds eluted from precolumn 1, it was hoped to effect this separation at the same pH thus removing the need for either extensive reconditioning of the column or introduction of the complexity of column switching. This requirement was fulfilled for both separations where operating conditions were obtained that gave sufficient retention of the most water soluble compounds present and additionally gave an adequate separation of the relevant group of compounds ie. the group of nine compounds that would be determined from precolumn 1 and the group of six compounds that would be determined from precolumn 2. Hence, both precolumn 1 and precolumn 2 are eluted at pH 2.7 and chromatographic gradient conditions were determined for the separations required. The analytes retained on precolumn 2 required a much lesser acetonitrile concentration for their elution and separation. Of the standards selected, MCPA, MCPP, 2, 4-D, 2, 4, 5-T, phenol and p-cresol were quantified using the eluate from precolumn 2.

Stronger gradient conditions were used for the elution and separation of the analytes retained on precolumn 1. Of the standards selected, simazine, atrazine, chlortoluron, isoproturon, 2, 4-DB, MCPB, 2, 4-dichlorophenol, 2, 4, 6-trichlorophenol and pentachlorophenol were quantified using this eluate.

Closely eluting compounds for the precolumn 1 analysis were a) chlortoluron, atrazine and isoproturon and b) 2, 4-DB, MCPB and 2, 4, 6-trichlorophenol. Optimising of the gradients involved carrying out the separation with a short gradient, then a long gradient followed by chemometric calculation.

The second major factor in selection of the LC gradients was to give consideration to keeping the analysis as short as practicable for the given system. This is important in the application of the equipment as an on-line intake protection monitor. This requirement applies to the whole process where the major time periods involved are the sample extraction and the two chromatographic analyses.

The choice of temperature was arbitrary. It was however made considering the equipment would be located outside of a conventional laboratory. The 40°C temperature selected should be easy to control in most bankside environments likely to be encountered by a surface water monitor. It is most important to control this parameter effectively as the methodology is based on reproducibility of an analyte's retention time together with its spectral characteristics.

The selection of appropriate detection wavelengths was governed by sensitivity and selectivity requirements for the analysis.

Extraction and elution

For the trace enrichment, the initial choice was the type of precolumn packing to use. Silica based adsorbents suffer from a much reduced capacity for retention of medium polarity compounds when compared to copolymer materials. Hennion and Coquart [9] report C_{18}-modified silicas being 15–30 fold less retentive than copolymeric adsorbent.

Additionally sample pH is much more critical for the silica-based adsorbents. Compounds which are either partially or fully ionised will have retention seriously reduced whereas adsorption by the copolymer type of adsorbent is much less affected by pH, the ionised form of a compound still often having a significant retention. The performance of the polymeric adsorbent is hence ideal for the more polar compounds.

The adjustment of sample pH after passage through precolumn 1 was achieved by addition of mineral acid. Nitric acid was selected at 0.05M strength. The acid passifies stainless-steel which is advantageous in this application and no recorded problems of it acting as an oxidising agent were apparent.

The pH range of 2 to 2.5 was found experimentally to be the best compromise in obtaining stable and satisfactory recoveries for the compounds extracted by precolumn 2.

One other consideration is the elution of the precolumns which is achieved by switching the appropriate precolumn in line with the LC eluent. Backflush desorption will give the least band broadening. However, operation of SAMOS-LC monitors over the last six years has shown that forward flush desorption, whilst giving some band broadening, creates less blockage problems with real-life samples. Forward flush desorption was hence the chosen option for this study, the precolumn being then used as an additional in line filter to help minimise blockages within the system.

Breakthrough of analytes

The concept of breakthrough of a given analyte is basic to this methodology. For a given compound being loaded onto a precolumn of a defined size, a time will arrive when that compound is no longer retained by the precolumn but appears in the sample stream eluting from it.

All compounds would be expected to be retained to a certain degree on precolumn 1 but breakthrough volumes will vary tremendously compound-to-compound. Not only the breakthrough volumes of the various analytes are of importance. Additionally the presence of interfering compounds are also of significance and it should be appreciated that these compounds are likely to have different breakthrough volumes to any target analytes. This factor may be used to advantage.

Compounds with low breakthrough volumes will be retained to a similar degree on a second precolumn when one is in line with precolumn 1. A significant enhancement is however available where the chemical form of a poorly retained analyte can be altered. For example, the ionisation of an acidic compound can be suppressed to enable its breakthrough volume to be greatly increased, thereby increasing its retention by precolumn 2.

Of interest was the finding that some of the acid herbicides were retained on precolumn 1 with little breakthrough, whilst others broke through from the precolumn very readily and required the acidification stage to be retained on precolumn 2. This is considered to be due to the nature of the analyte-to-SDVB interactions and also relates to pKa values for the various analytes.

Table I includes the pKa values for the compounds and it can be seen that the 4 acid herbicides MCPA, MCPP, 2, 4-D and 2, 4, 5-T would be predicted to be recovered most favourably on precolumn 2 when the pH of the extraction is con-

sidered. The other herbicides in this group, 2, 4-DB and MCPB have significantly higher pKa values and are correspondingly found on precolumn 1.

Quantitative aspects then require that extraction of analytes determined on precolumn 2 should not commence until those analytes are breaking through from precolumn 1. As a result, a requirement of the methodology is that precolumn 2 should be switched into line with precolumn 1 at a certain time period into the sample loading procedure when analytes to be determined on precolumn 2 are breaking through. It additionally follows that analytes that do break through will be present in the chromatograms derived from the elution of both precolumns 1 and 2.

TABLE I Quantitation Wavelengths Used for the Modified SAMOS – LC

Analyte No.	*Quantitation Precolumn*	*pKa*	*LC Retention Time (min)*	*Quantitation Wavelength (nm)*	*Band Width (nm)*
1. Simazine	1	1.62	15.0	230	4
2. Chlortoluron	1	----	17.0	245	4
3. Atrazine	1	1.70	17.5	230	4
4. Isoproturon	1	----	17.9	245	4
5. 2,4-Dichlorophenol	1	7.89	18.9	230	4
6. 2,4-DB	1	4.80	20.6	230	4
7. MCPB	1	4.84	20.7	230	4
8. 2,4,6-TCP	1	6.00	21.4	230	4
9. PCP	1	4.71	25.0	230	4
10. Phenol	2	9.89	11.1	195	4
11. p-Cresol	2	10.17	16.5	195	4
12. 2,4-D	2	2.73	23.8	230	4
13. MCPA	2	3.07	24.8	230	4
14. 2,4,5-T	2	2.83	28.5	230	4
15. MCPP	2	3.78	29.4	230	4

Calibration and limits of detection

The selected wavelengths for quantitation and the precolumn used are summarised in Table I. Reference signal in all cases was 450nm with a bandwidth of 80nm. Wavelengths used are a compromise that is best judged for each analyte for a particular river system. The selection is based on signal-to-noise criteria,

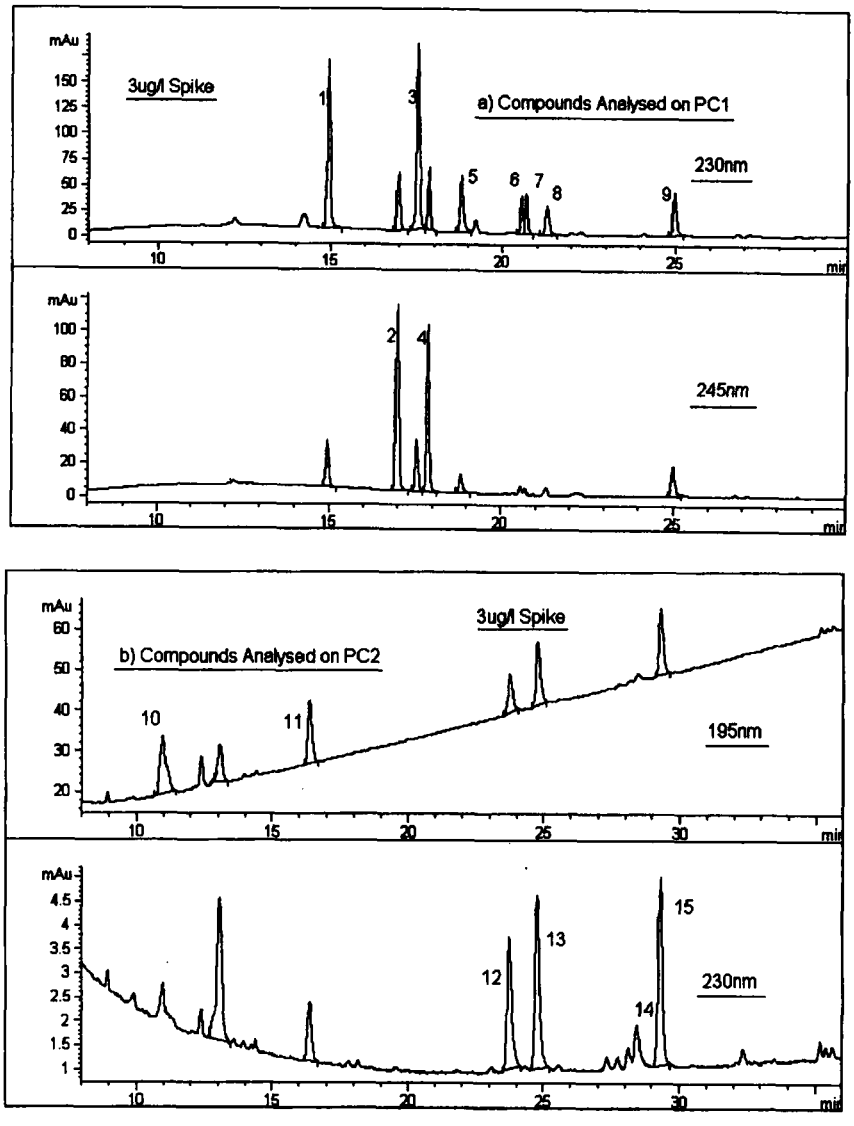

FIGURE 2 Spiked Standards in Spring Water a) Compounds Analysed on PC1 b) Compounds Analysed on PC2

interferences present at the given wavelength and detection limit required. The quantitation wavelength is not necessarily solely optimised for greatest sensitivity. Calibration of the monitor is by individual preference. The method has several options available.

TABLE II Percentage Recovery of Analytes Spiked at 3ug/l

	Analyte Analyte No.	*Measured on PC*	*% Recove from Sprin Water*	*Coefficient of Variation of % Recovery*	*% Recovery from River Trent Water*	*Coefficient of Variation of % Recove*
1	Simazine	1	99.9	2.9	100.5	4.4
2	Chlortoluron	1	101.3	1.7	107.3	4. 1
3	Atrazine	1	99.2	2.7	95.9	1.4
4.	Isoproturon	1	100.6	1.8	104.6	7.2
5.	2,4-Dichlorophenol	1	150.3	3.3	159.0	14.0
6.	2,4-DB	1	98.8	3.7	101.9	5.9
7.	MCPB	1	97.9	5.0	107.9	9.1
8.	2,4,6-TCP	1	112.6	9.4	131.9	11.3
9.	PCP	1	107.0	12.1	94.7	12.0
10.	Phenol	2	81.8	10.3	-	-
11.	p-Cresol	2	54.0	13.7	64.4	20.5
12.	2,4-D	2	108.0	6.2	119.8	13.0
13.	MCPA	2	109.0	2.2	110.2	4.6
14.	2,4,5-T	2	44.4	18.5	85.6	20.8
15.	MCPP	2	106.9	4.5	114.6	4.8

Means calculated from 5 data sets in 6/97.
Coefficient of variation calculated using S for n-1 samples.

Standard solutions can be injected directly onto the HPLC column via the autosampler facility. These injections serve as a chromatography check and give an absolute value for the injected amount of analyte. They also aid in determining whether significant band broadening is occurring during the on-line elution of precolumns. The one major disadvantage is the shortened retention time of all of the analytes due to the much-shortened pathway between injector and column when compared to the pathway via the Prospekt system.

River water may not be phenol/herbicide free and hence whilst it may be the ideal matrix into which standards can be spiked, then caution must be exercised. Our current preference is to spike standards into high purity spring water and take the spiked water through the analysis procedure as normal. An unspiked water would also be analysed. The spring water is pH adjusted to match the acidification requirements of the river water.

Spiking is performed using a standard mix of compounds in acetonitrile. The resulting chromatograms obtained from the elution of precolumns 1 and 2 would form the basis of the calibration (see Figures 2a and b). The analytes are numbered as given in Table I. Standards are equivalent to a 3ug/l concentration in water assuming 100% recovery.

A spiked river water and blank river water are also analysed as above and serve as a check to establish that recovery of analytes is being achieved for the river being monitored and as an indicator of any likely interferences (see Figure 3). For the precolumn 1 eluate (Figure 3a), the response at 230nm only is shown, peaks 2 and 4 from the phenyl urea herbicides still being clearly visible. The precolumn 2 eluate is shown at 230nm (Figure 3b) to illustrate the acid herbicide responses.

The variety of spiked standards and blanks are introduced into the system via separate lines available on the SDU. The recovery of the various herbicides and phenols is presented in Table II.

The analytes quantified using precolumn 1 are from a 55ml sample (numbers 1 to 9) and the analytes quantified using precolumn 2 are from a 10ml sample (numbers 10 to 15).

From precolumn 1, 2, 4-dichlorophenol shows a high percentage recovery from both waters when compared to the direct standard. Using the spring water as a calibrant does however produce correct values for the river. The other major anomaly for the precolumn 1 group is the enhanced recovery of 2, 4, 6-trichlorophenol for the river water. Using the spring water as a calibrant will give a higher than true value.

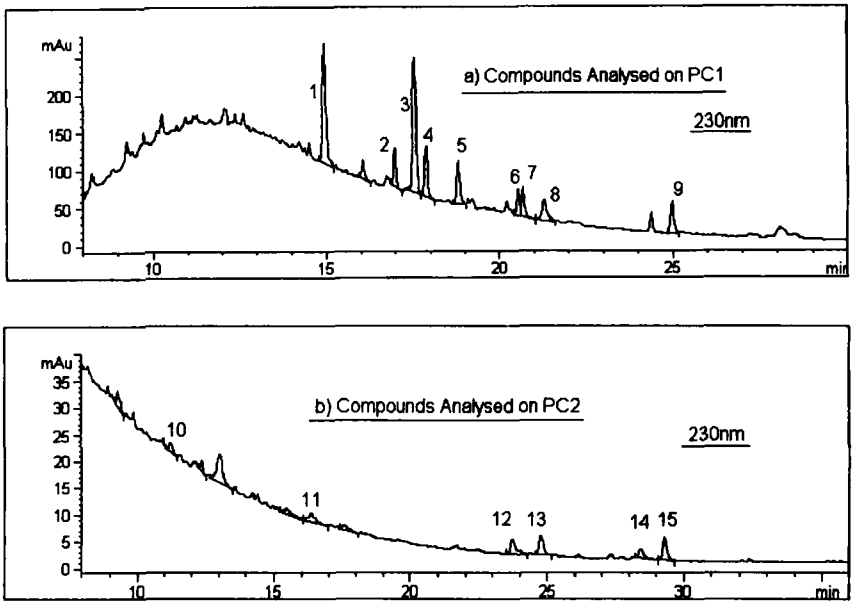

FIGURE 3 Spiked Standards in Trent Water @ 3ug/l a) Compounds Analysed on PC1 b) Compounds Analysed on PC2

For precolumn 2 where a much smaller volume of sample has been processed, the major difficulties involve quantitation of phenol and 2, 4, 5-T. Phenol is apparently totally depleted in the river spike. The explanation is in its rapid metabolism in the river water in which the spike is prepared and then analysed in a sequence some hours after its preparation. The effect is much reduced for the spring water. Trials are being undertaken to stabilise the phenol spikes. Non-the-less, it is considered that phenol present in an on-line river sample would be recorded at its true concentration at the time of the analysis, albeit a concentration that was being metabolised..

The 2, 4, 5-T is recovered poorly from the spring water compared to the river water and calibration using the former will lead to an overestimate of its concentration. Typical chromatograms from the river water are presented in Figures 4a and b.

Limits of quantitation available from the system depend on several factors, the most significant of which is the background signal from the river water study. In the river being monitored, these limits were generally around 0.5ug/l for simazine, atrazine, chlortoluron and isoproturon, and around 0.6ug/l for the remaining compounds. During periods of high rainfall where the river water matrix was diluted, these levels became lower. Conversely, during very low flow conditions where the matrix became more concentrated, then they were raised.

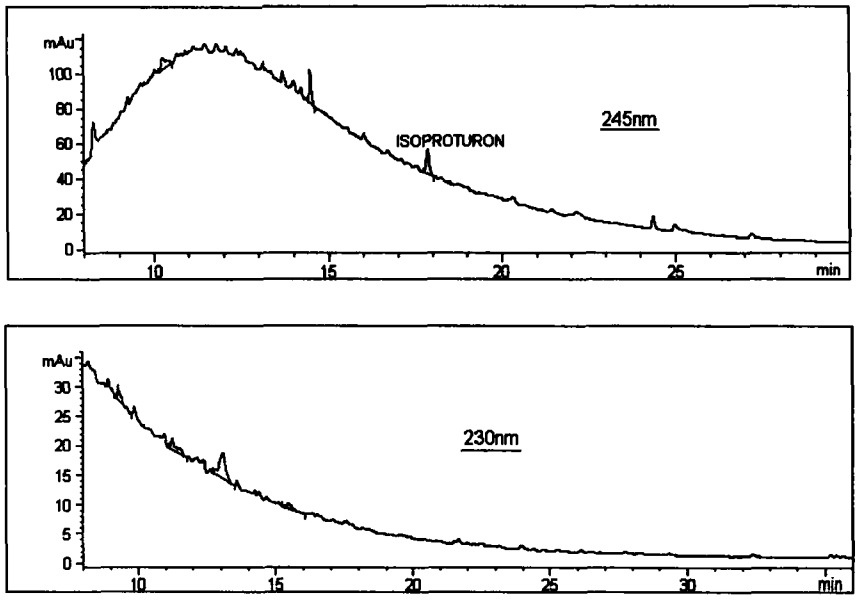

FIGURE 4 – River Trent Analysis a) from PC1 b) from PC2

Interferences / Matrix effects

The effect of interference by humic substances was also found to be a significant factor in the overall performance of the monitor.

Humic substances are a major problem in that they are present in the LC chromatogram as a diverse hump with peaks of interest often present on quite significant slopes. This hinders accurate integration and spectral comparisons are degraded even with background subtraction. These effects are shown in Figures 3 and 4.

Humics are generally slower to break through from precolumn 1 than the analytes quantitated by precolumn 2 and hence interference is reduced. This reduction is not however total as some very polar material does break through from precolumn 1 and the acidification enhances its recovery along with the precolumn 2 analytes. This effect can be observed in Figures 3b and 4b when compared to the spiked springwater trace in Figure 2b.

Any other compound extracted by the method and then eluting next to or with one of the standards is an interference. The outcome of such is the generation of a false positive which, in the context of on-line monitoring, is an acceptable occurrence. Such false positives can generally be rapidly resolved by reference to the UV spectral data.

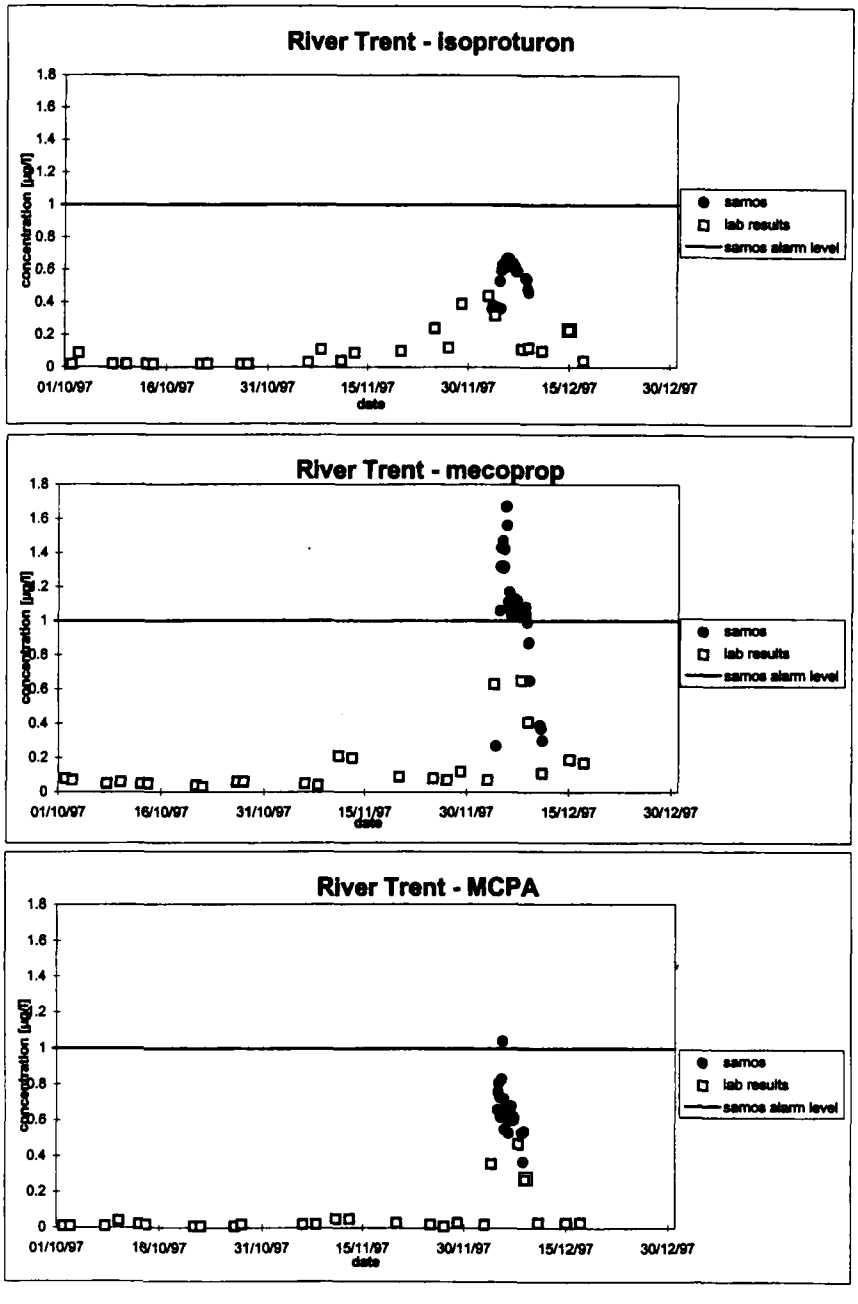

FIGURE 5A Mixed Herbicides Pollution Event

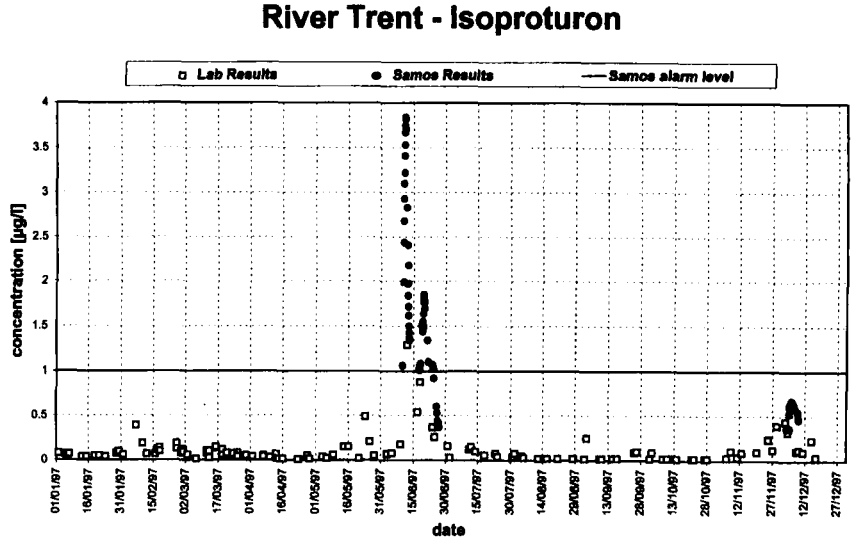

FIGURE 5B Isoproturon Pollution Event

Applications to on-site monitoring

Preparation before commissioning of the system as a river monitor is necessary to determine the amount of acid that is required to achieve the pH range for sample entering precolumn 2.

Regular adjustment of discrete river water samples to within this pH range is made for 10ml spot samples adding acid (with mixing) from a pipette (eg. in 50ul volumes) and monitoring with a pH probe. An average volume of acid required is calculated and for the river of interest was found to be quite consistent even extending into the times of spate.

Depending on the amount of acid required, its concentration can be adjusted so a reasonable volume for accurate delivery can be obtained. In the instance of the Trent on-line monitor, this was set to 0.05M acid with 1.5ml being required per 10ml of river water. The acid is added at a fixed rate by means of a low volume T-piece. As a precautionary measure, a coil of PEEK tubing (green) was installed after the acid/sample mixing point such as to introduce a delay volume of about 1ml between acid addition and application of the sample to precolumn 2. This was to ensure adequate mixing and stabilisation of the sample pH.

The River Trent is very high in humic substances which limit the detection levels to those given in the calibration and limits of detection section. Significant improvements are likely for other rivers with a lower humic acid content.

Indeed, tests were conducted increasing the sample volume taken through precolumn 2 both twofold and threefold and these showed promising results with little detected breakthrough of the neutralised analytes. Background did however increase in proportion to sample volume taken and for our river, the selected volume of 10ml was taken as a satisfactory compromise between sensitivity and too high a background.

The on-line monitor is installed in an air-conditioned environment. Maintenance visits are made each Monday, Wednesday and Friday. These visits allow recharging the precolumn magazine and removal of used precolumns, recharging of HPLC solvent reservoirs and removal of waste solvents (large reservoirs have been fitted), replacement of precolumn 2 by a new precolumn and renewal of the calibration and river water spikes.

Alarming software has been written (TLC, Amsterdam, the Netherlands) which operates contact closures available on the HPLC module and writes details to "Notepad". Alarming is available for both calibrated compounds and unknowns, the latter being assessed by responses over a set area count. A malfunction alarm is also available.

Alarms output via radiotelemetry to a 24-hour manned response centre. The staff contact a standby analyst familiar with the data produced by SAMOS-LC. The analyst then contacts the SAMOS-LC data system using telephone/modem connections and Carbon Copy 32 software installed on either a laboratory based PC (office hours) or a laptop PC (outside office hours). This contact will allow verification of the data. Over and above this response, data is transferred by telephone/modem contact on a daily basis and scrutinised for changes to the river "fingerprint" and any trends taking place below alarm limits.

The system has been performing with a high level of reliability, having been commissioned in February 1997. It has recorded several pollution incidents, examples of which are shown in Figures 5a and 5b. Figure 5a depicts a mixed herbicides pollution event involving detection of herbicides on the precolumn 1 eluate (isoproturon) and the precolumn 2 eluate (mecoprop and MCPA). Figure 5b shows two instances of river pollution by isoproturon.

CONCLUSIONS

The modified SAMOS-LC system described has been performing as an on-line monitor remote from the central laboratory facility since February 1997. It has proven to be a robust and reliable monitor and has been gathering information during construction of the river intake and excavation of bankside storage. The

limits of detection achievable by the system and its ability to raise alarms where these levels become elevated when coupled to the advanced water treatments available at the water treatment plant will contribute to safeguarding the supply of drinking water in the region when abstraction commences.

The SAMOS-LC monitor provides 24-hour, 7 days per week cover and is an integral part of an intake protection strategy involving a series of pollution monitors installed at the site.

References

[1] P.J.M. Van Hout, K.P. Hupe and G.Vallet. *Anal. Methods and Instrument.*, **2**, 208–220 (1995).
[2] J. Slobodnik, E.R. Brouwer, R.B. Geerdink, W.H. Mulder, H. Lingeman and U.A.Th. Brinkman. *Anal. Chim. Acta*, **268**, 55–65, (1992).
[3] J. Slobodnik, M.G.M. Groenewegen, E.R. Brouwer, H. Lingeman and U.A.Th. Brinkman. *J. Chromatogr.*, **642**, 359–370, (1993).
[4] J. Norberg, J. Slobodnik, J.J. Vreuls and U.A.Th. Brinkman. *Anal. Methods and Instrument.*, **2**, 266–276, (1995).
[5] E.R. Brouwer and U.A.Th. Brinkman. *J. Chromatogr. A*, **678**, 223–231, (1994).
[6] I.A. Fowlis, I. Jepson and J.J. Vreuls. Proceedings of "Brighton Crop Protection Conference-Pests and Diseases – 1996", pp999–1006.
[7] J. Slobodnik, S.J.F. Hoekstra-Dussoren and U.A.Th. Brinkman. *Anal. Methods and Instrument*, **2**, 227–235, (1995).
[8] E.R. Brouwer, E.A. Struys, J.J. Vreuls and U.A.Th. Brinkman. *Fresenius J. Anal Chem.*, **350**, 487–495, (1994).
[9] M.-C. Hennion and V. Coquart. *J. Chromatogr.*, **642**, 211–224, (1993).