Automated Water Analyser Computer Supported System (AWACSS)
Part II: Intelligent, remote-controlled, cost-effective, on-line,
water-monitoring measurement system

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Abstract

A novel analytical system AWACSS (Automated Water Analyser Computer Supported System) based on immunochemical technology has been evaluated that can measure several organic pollutants at low nanogram per litre level in a single few-minutes analysis without any prior sample pre-concentration or pre-treatment steps.


The first part article gave the reader an overview of the aims and scope of the AWACSS project as well as details about basic technology, immunoassays, software, and networking developed and utilised within the research project. The second part reports on the system performance, first real sample measurements, and an international collaborative trial (inter-laboratory tests) to compare the biosensor with conventional analytical methods. The systems’ capability for analysing a wide range of environmental organic micro-pollutants, such as modern pesticides, endocrine disrupting compounds and pharmaceuticals in surface, ground, drinking and waste water is shown. In addition, a protocol using

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reconstitution of extracts of solid samples, developed and applied for analysis of river sediments and food samples, is presented. Finally, the overall performance of the AWACSS system in comparison to the conventional analytical techniques, which included liquid and gas chromatographic systems with diode-array UV and mass spectrometric detectors, was successfully tested in an inter-laboratory collaborative trial among six project partners.

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Keywords: Automated Water Analyser Computer Supported System (AWACSS); Optical immunosensor; On-line biosensor monitoring; Emerging pollutants; Environmental analysis; Immunosensor network

1. Introduction

Industrial activities, extensive farming, sewage water, and many other human activities are polluting waterways throughout Europe. Rivers, channels, lakes, oceans, ground water, and even drinking water can be contaminated by a variety of organic pollutants that can have adverse effects on aquatic life and pose risks to human health (Barceló and Henttonen, 1997a, 1997b). Examples include herbicides, insecticides, fungicides, antibiotics, drugs, detergents, oils, industrial waste or by-products, endocrine disrupting compounds, and carcinogens. Some man-made substances being released into environment are toxic to humans via food chain. The new trial waste or by-products, endocrine disrupting compounds, and carcinogens. Some man-made substances being released into environment are toxic to humans via food chain. The new generation of emerging pollutants or so-called priority substances (2000/60/EC, 2000) to be monitored in water are rather difficult to analyse European Union expert groups working at the implementation of the Water Framework Directive (WFD) (2000/60/EC, 2000) put a considerable effort to propose a range of techniques and methods for their monitoring. True enforcement demands more frequent monitoring of water catchment areas and also industrial plants need greater control of their waste water to meet the demands of increased regulation.

Currently, to analyse the different environmental pollutants, quantitative water analysis is performed with standard analytical methods such as high-performance liquid chromatography (HPLC), or gas chromatography coupled to mass spectrometry (GC–MS) (Clement et al., 1997). Since many of the so-called priority substances (2000/60/EC, 2000) to be monitored in water are rather difficult to analyse European Union expert groups working at the implementation of the Water Framework Directive (WFD) (2000/60/EC, 2000) put a considerable effort to propose a range of techniques and methods for their monitoring. True enforcement demands more frequent monitoring of water catchment areas and also industrial plants need greater control of their waste water to meet the demands of increased regulation.

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2. Experimental

2.1. Materials

Common chemicals of analytical grade were purchased from Sigma–Aldrich, Deisenhofen, Germany, or Merck KGaA, Darmstadt, Germany. The pesticides atrazine, isoproturon, and propanil were purchased as PESTANAL® analytical standards from Riedl-de Haën Laborchemikalien GmbH & Co. KG, Seelze, Germany. The polymer plasticiser bisphenol A was also ordered from Sigma–Aldrich. The fluorescent dye CyDye™
Cy5.5 was purchased from Amersham Biosciences Europe GmbH, Freibour, Germany. The aminodextrans Aminex™ with 40 and 170kDa molecular weight were purchased from Helix Research Company, Springfield, OR, USA. Labelling and purification of antibody were carried out as described in the product information sheet supplied with the labelling kit from Amersham Biosciences Europe GmbH. UV−vis spectra were recorded using a Specord M500 spectrophotometer from Carl Zeiss Jena GmbH, Jena, Germany. The spatially resolved surface modification was performed using a parallel micro-dispensing system (TopSpot) with high-performance piezostack actuation system and integrated temperature adjustment from HSG-IMIT/IMTEK, Germany.

2.2. Instrumentation

The AWACSS instrument employs fluorescence-based detection of the binding of fluorophore-tagged biomolecules to the surface of an optical waveguide chip. The fibre-pigtailed chip, driven by a semiconductor laser, consists of a waveguide circuit which distributes excitation light to 32 separate sensing patches on the chip surface. Bioimmunochemistry is used to sensitise each of the 32 patches to a specific analyte and a micro fluidic system is used to automatically handle the sample injection over the sensor surface, enabling rapid, simultaneous and high-sensitivity fluorescence detection of up to 32 pollutants. A fibre-coupled detection array is used to monitor the 32 separate fluorescence signals, and software has been developed for control of the optics and fluidics and data acquisition and processing for the fluorescence signals, laser power, and ambient and chip temperature.

An HTC PAL autosampler with cycle composer software (CTC Analytics AG, Zwingen, Switzerland) was used for dilutions, sample preparations (transferring 100 μL of the antibody stock solution to 900 μL of the sample followed by one or two mixing strokes) and the sample transfer to the AWACSS instrument. Liquid handling and data acquisition are fully automated and computer controlled. One measurement cycle with washing steps, injection of the sample and regeneration of the surface takes about 15−18 min.

Key features of the instrument, chip fabrication, chip characterisation, hardware requirements, and further details of the instrumentation are described in the first part article published in a previous issue of the same journal.

2.3. Immunochemistry

The immunochemistry utilised in the project takes advantage of a binding inhibition test that requires antibodies directed against specific analytes and analyte derivatives that can be covalently bound to a transducer surface. The previously immobilised aminodextran layer is used to reduce non-specific binding to the surface. Dried (immobilised) aminodextran layers on a glass substrate showed a thickness between one and three nm. The thickness of welled aminodextran layers (aminodextran layers on a glass substrate in contact with buffer solution after a few minutes) were also verified by spectroscopic ellipsometry experiments and these experiments yielded values between 100 and 150 nm.

A huge number of polyclonal antibodies and their corresponding analyte derivatives have been produced for a variety of organic micro-pollutants. After being purified and labelled with a fluorescent marker, they were developed into immunoassays and used in the project. The sample containing the analyte is incubated in solution with the labelled specific antibody. Therefore, 100 μL of the antibody stock solution are mixed with 900 μL of the sample by an autosampler and are incubated for approximately 5 min. The antibody binds the analyte during the incubation step until a well-defined condition of the reaction is reached. When the sample is pumped over the sensor surface, only the antibodies with free binding sites can bind to the surface. For the binding inhibition assay to be quantitative, the binding of the antibody to the surface must be mass transport-limited. This allows the signal to be a function of the diffusion rate to the surface and not of the kinetics of the surface binding. The number of high affinity binding sites on the surface has to be much higher than the number of antibodies used for one measurement. To be sure, that the binding is mass transport-limited, we use small amounts of antibodies and on the sensor surface we immobilise a huge excess of antigen derivatives. This was demonstrated by additional reflectometric interference spectroscopy (RIfS) measurements as already described in literature (Glaser, 1993).

The surface evaluation was performed with covalently immobilised peptide nucleic acid (PNA) for the detection of different endocrine disruptors by the above mentioned label-free detection method. Within these experiments, a hybridisation capacity with DNA oligonucleotides of 180 fmol mm−2 on PNA surfaces has been reported (Kroger et al., 2002). Other experiments to evaluate a covalent strategy for immobilisation of DNA microspots suitable for microarrays with label-free and time-resolved optical detection of hybridisation resulted in hybridisation capacities of approximately 300 fmol/mm−2 (Jung et al., 2001). We achieved linear correlations between the increasing fluorescence signal and the antibody concentration used within TIRF experiments. The linear behavior of the fluorescence signal shows that no saturation effects can be observed even with highest antibody concentrations. Therefore, the immobilised huge excess of antigen derivatives in comparison to the used amounts of antibodies could be verified.

2.4. Detection

The AWACSS instruments are based on evanescent field technology. Laser light is coupled into an optical transducer and guided down the integrated optical (IO) chip. The transducer surface is chemically modified in spatially distinct loci with analyte derivatives. Analyte-specific antibodies are labelled with a fluorescent marker (CyDye™ Cy5.5), which upon binding to the transducer surface are
Fig. 1. Schematic set-up of the developed sampling system for on-line monitoring which allows direct intake of river water and its continuous transfer to the autosampler of the AWACSS system.

Active esters were prepared with the derivatives, which are analyte molecules modified with a spacer containing a carboxyl group. Approximately 5.0 mg of the derivative were dissolved in 100 µL of dry N,N-dimethylformamide (DMF). N-Hydroxysuccinimide (NHS) and N,N′-dicyclohexylcarbodiimide (DCC), each in 1.1-fold molar excess (referring to the amount of analyte derivative) were added to the solution. After stirring for several minutes, the solution was kept over night at room temperature. Finally, the solution was centrifuged (12,000 rpm) at approximately 4 °C and the supernatant was stored under refrigeration. 50 mg aminodextran were dissolved in Milli-Q water at a concentration of 1.0–2.0 mg mL⁻¹ and were immobilised by a parallel spotting device TopSpot from HSGIMIT, Villingen-Schwenningen, Germany (http://www.hsgimit.de) and IMTEK (Institute for Microsystem Technology), Fribourg, Germany (http://www.imtek.de).

2.6. Sampling

A sampling system for on-line monitoring has been developed which allows direct intake of river water and its continuous transfer to the autosampler of the AWACSS system. A schematic representation of the set-up is in Fig. 1.

2.7. Measurement

For the measurements, we used a polyclonal IgG antibody from sheep and a suitable analyte derivative. The entire sample volume was 1000 µL. For a calibration routine, 900 µL of spiked Milli-Q water was automatically mixed by the autosampler with 100 µL of an antibody stock solution containing the antibodies and ovalbumin from chicken eggs (OVA) in 10-fold phosphate buffered saline (PBS) (10-fold PBS: pH 6.8, 1500 mM sodium chloride, 100 mM potassium...
phosphate monobasic). After a defined incubation time, this mixture was measured using the biosensor set-up. The experimental design for a calibration routine consists of nine independent blank (Milli-Q water) measurements and nine concentration steps (each measured as three replica), the mean value and the standard deviation (S.D.) for the replica was automatically calculated by the measurement control unit. The measured signal for the mean value of the blanks was set to 100% and all spiked samples could be obtained as a relative signal below this blank value. To fit the data set a logistic fit function (Dudley et al., 1985) with three free parameters (A$_2$, x$_0$, and p) was used. A$_1$, as the upper asymptote was fixed to 100% (relative signal for mean value of the blanks) and A$_2$ is the lower asymptote. The range between A$_1$ and A$_2$ is the dynamic signal range. The inflection point is given by the variable x$_0$ and represents the analyte concentration, which corresponds to a decrease of 50% of the dynamic signal range (IC$_{50}$). The slope of the tangent in this point is given by the parameter p. In compliance with the IUPAC rules (The Orange Book) (Inczedy et al., 1998), the LOD is calculated as 3 times the S.D. of the blank measurements (S.D. blank) and the LOQ is calculated as 10 times the S.D. of the blank measurements (S.D. blank). All statistical procedures and further calculations are included within the AWACSS evaluation software package.

3. Results and discussion

3.1. Selection of AWACSS compounds

A set of new immunochromical reagents was developed for selected organic micro-pollutants to be analysed in environmental water samples. Major criteria for selection of target analytes were: (i) their presence in the environment; (ii) existing environmental legislation (98/83/EC, 1998; 2000/60/EC, 2000); (iii) technical possibilities to prepare sufficiently selective polyclonal antibodies and their corresponding analyte derivatives; and (iv) marketing considerations. More than 20 different polyclonal antibodies were isolated within the project and their corresponding analyte derivatives were synthesised. A priority was given to classes of pesticides, endocrine disrupting compounds, WFD Priority Substances, industrial pollutants and pharmaceuticals (for a list of AWACSS compounds, see Table 1).

3.2. Regular monitoring of AWACSS compounds in European rivers

Sampling sites: Four project partners (IIQAB, Department of Environmental Chemistry, CED-CSIC, Barcelona, Spain; DVGW-Technologiezentrum Wasser, Karlsruhe, Germany; Environmental Institute, Kos, Slovak Republic; Water Research Institute, Bratislava, Slovak Republic) of the water-monitoring group have systematically investigated various...
Fig. 2. Characteristic pollution patterns by atrazine (a), bisphenol A (b), and sulphamethoxazole (c) at the River Rhine (Germany) by GC-MS. Sampling sites: Mainz, Karlsruhe, and Düsseldorf. Results were obtained in the period 2001–2003.
types of water around Europe in order to select the analyte panel applicable to real-world situations and future monitoring sites for the AWACSS system. Also, water matrix and cross-reactivity effects related to the AWACSS immunosensor chemistry were addressed.

In Spain, sampling sites at the Llobregat River, supplying drinking water to the city of Barcelona, two of its tributaries, the Ebro River and the Mediterranean Sea were regularly monitored by RIANA and LC–MS techniques. The occurrence of target analytes was monitored also in the ground water serving as a source for abstraction of drinking water. A removal efficiency of the detected pollutants was studied at the successive water treatment steps applied in the Sant Joan Despi waterworks.

The AWACSS monitoring network in Germany consisted of three sampling sites alongside the River Rhine. Among the techniques applied for monitoring were GC–MS and LC–DAD UV. Typical examples of pollution patterns by selected analytes over the years 2001–2003 are given in Fig. 2a–c. It is shown on the example of atrazine (Fig. 2a) that the sensitivity and selectivity of individual analytes remained unchanged compared to measurements of individual compounds. A good agreement between the results obtained by biosensor technique and the on-line SPE–LC–MS method were obtained for each analyte (Rodriguez-Mozaz and Reder, 2004).

<table>
<thead>
<tr>
<th>Concentration (µg·L⁻¹)</th>
<th>Propenal</th>
<th>Atrazine</th>
<th>Isoプロトロン</th>
<th>Sulphamethoxazole</th>
<th>Biphenyl A</th>
<th>Estrone</th>
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<tbody>
<tr>
<td>0.009</td>
<td>0.010 ± 0.01</td>
<td>0.010 ± 0.01</td>
<td>0.010 ± 0.01</td>
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<td>0.027</td>
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<tr>
<td>0.09</td>
<td>0.040 ± 0.04</td>
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<tr>
<td>0.27</td>
<td>0.060 ± 0.06</td>
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<td>0.59</td>
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<td>2.7</td>
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<td>9.0</td>
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<tr>
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<td>2.00 ± 2.00</td>
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<td>90</td>
<td>5.00 ± 5.00</td>
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| ai (%)                 | 100       | 100       | 100       | 100       | 100       | 100       |
| p                      | 0.01 ± 0.01 | 0.01 ± 0.01 | 0.01 ± 0.01 | 0.01 ± 0.01 | 0.01 ± 0.01 | 0.01 ± 0.01 |
| LOD (µg·L⁻¹)           | 0.019     | 0.019     | 0.019     | 0.019     | 0.019     | 0.019     |

Resulting parameters (with standard deviations) for the determined logistic fit functions and the validation parameter LOD for all analytes.

3.3. Development and testing of a sampling device

A sampling system was designed and realised that enables the continuous transfer of water from a river (or any other water source) to the autosampler of the AWACSS system (Fig. 1). The system included a pumping system, a valve for controlling the flow rates and the portion of water that is provided to the AWACSS system and a filter unit which allows the filtration of the water sample with a mesh size of one micrometer. By exchanging the (commercially available) filter cartridge, other filters with different pore sizes could be used.

In a sampling experiment at River Rhine, it could be shown that with this experimental set-up, a life-time of 3 months could be achieved before an exchange of the filter cartridge becomes necessary because a blocking of the filter occurs, assuming that the average turbidity of the river water is 15 FNU (for River Rhine most often it is lower) and that a flow rate of 1 L·h⁻¹ through the sampling vessel is applied. With this flow rate, the water in a 250 mL vessel is completely exchanged every 15 min.

3.4. Studies on the performance of the biosensor

3.4.1. Multi-analyte measurements

Feasibility studies on multi-analyte measurements were performed with the RIANA system for a mixture of atrazine, isoproturon and estrone. The results showed that sensitivity and selectivity of individual analytes remained unchanged compared to measurements of individual compounds. A good agreement between the results obtained by biosensor technique and the on-line SPE–LC–MS method were obtained for each analyte (Rodriguez-Mozaz and Reder, 2004). Follow-up
Fig. 3. The resulting set of calibration curves for atrazine, bisphenol A, estrone, isoproturon, sulphamethizole, and propanil which were measured in parallel on a multi-analyte transducer. For all compounds, the calculated LOD is below 0.020 µg L⁻¹.

Determinations by HPLC–DAD UV method showed a small positive bias towards higher values obtained by the immunosensor for most of the tested analytes. In the final stage of the project, the multi-analyte determination capability of AWACSS was successfully tested with a mixture of six analytes. The AWACSS IO chip was modified with derivatives of atrazine, bisphenol A, estrone, isoproturon, sulphamethizole, and propanil according to the previously described immobilisation protocol. Then, a simultaneous calibration from 0 to 90 µg L⁻¹ analyte concentration in Milli-Q water with mixed analytes and an antibody stock solution containing the six corresponding polyclonal antibodies (anti-atrazine, anti-bisphenol A, anti-estrone, anti-isoproturon, anti-mixed sulphonamides, and anti-propanil) was performed. The resulting set of calibration curves is shown in Fig. 3. For all compounds, the calculated LOD is below 0.020 µg L⁻¹ and all validation parameters, calculated relative signals, and standard deviations are summarised in Table 2.

This multi-analyte calibration demonstrated the possibility to quantify pesticides from three different classes (triazines, phenylurea herbicides and anilides), endocrine disrupting compounds (bisphenol A), steroid hormones (estrone), and pharmaceuticals (sulphamethizole) within one single measurement cycle, which only takes approximately 18 min. No cross-reactivity effect was observed for any of the tested analytes.

3.4.2. Matrix effects

As expected from the mode of antibody action, matrix effects were observed for different types of samples. The effects were studied in various types of water matrices, including Milli-Q, ground, surface and mixed municipal/industrial waste water. Typical changes in profiles of calibration curves are shown for estrone by Rodriguez-Mozaz and Reder (2004), where the most pronounced effect was observed in the waste water sample. It has been concluded that at analysis of such complex samples a care should be taken to establish calibration curves in similar matrix. Several strategies were proposed and tested to avoid the matrix effects: (i) adjustment of ionic strength and pH of the sample; (ii) dilution of the sample; (iii) sample clean-up; and/or (iv) enrichment of the sample on SPE column and reconstitution of the extract.

One of the major advantages of biosensors compared to conventional techniques is their ability to analyse selectively target analytes in complex matrices. Therefore, a protocol was developed allowing for analysis of solid matrices such as sediments and food samples. In the procedure one gram of solid sample is extracted in ultrasonic bath with 10 mL acetonitrile or methanol, the upper layer of extract (5 mL) is diluted in Milli-Q water in a way to reduce concentration of organic solvent to 2% and further treated/analysed as a liquid sample. Feasibility of the approach was proven in the inter-laboratory study comparing results obtained by on-line SPE–LC–DAD UV system and ELISA for atrazine and alachlor spiked into the Nitra River sediments and baby food (strawberry) samples at 50 and 500 ng g⁻¹ levels. Recoveries rates of both analytes ranged from 89 to 108%.

A separate study on the effects of residual amounts of organic solvents acetonitrile and methanol on the immunoassay performance was conducted with ELISA for several antibodies by Abuknesha and Griffith (submitted for publication), Abuknesha et al. (submitted for publication) and Abuknesha and Luk (submitted for publication).
Estrone is present in outlets of municipal waste water treatment plants. Estrone — hormone with endocrine disrupting effects, often a contaminant known as an endocrine disrupting compound; and (3) WFD Priority Substances; (2) bisphenol A — industrial polycarbonate plasticizer, being also on the list of hazardous substances (EPPO, 1993); and (1) atrazine as a representative of pesticide class, being also on the list of priority pollutants (EEC, 1997). Each of them was spiked with three analytes: (1) atrazine as a representative of pesticides, (2) bisphenol A — polycarbonate plasticizer, and (3) estrone — hormone with endocrine disrupting effects. Conventional methods used in the collaborative trial among the project partners. In total, eleven different analytical set-ups were used in six laboratories, among them four automated on-line SPE–LC–DAD UV systems (Slobodník et al., 2004a, 2004b, 2004c, 2004d, 2004e, 2004f), ELISA and immunochemistry techniques. An evaluation of the collaborative trial using the Z-score methodology as an expression of deviation of the measured from assigned value showed that none of the results obtained by AW ACSS would be excluded from the evaluation. Here, it should be mentioned that analysis of reconstituted sediment extracts by on-line SPE–LC–DAD UV techniques was possible only by using time-consuming mathematical deconvolution and/or subtraction of the blank signals. As regards the reproducibility, all results by AW ACSS had a coefficient of variation lower than 17% (Table 3), the only exception being bisphenol A at the lower spiked level in sediment (27.8%). The results were well within the range obtained by both chromatography-based and other conventional analytical techniques, ELISA, the immunosensor RIANA, and the AW ACSS instrument.

### 3.5. Comparison of AWACSS performance to conventional analytical techniques

The overall performance of the AWACSS system in comparison to the conventional analytical and immunosensor techniques was tested in the inter-laboratory collaborative trial among the project partners. In total, eleven different analytical set-ups were used in six laboratories, among them four automated on-line SPE–LC–DAD UV systems (Slobodník et al., 1993), on-line SPE–LC–FLD, on-line SPE–LC–MS (Slobodník and Brinkman, 2000), off-line SPE/LVI–GC–MS (Korenková et al., 2001), two RIANA prototypes (Tschmelak et al., 1993), on-line SPE–LC–DAD UV, on-line SPE–LC–MS (Korenková et al., 2001), two RIANA prototypes (Tschmelak et al., 2004a, 2004b, 2004c, 2004d, 2004e, 2004f), ELISA and AWACSS. The tested matrices were Milli-Q water and freeze-dried 63 μm fractions of river sediments from the Nitra River. Each of them was spiked with three analytes: (1) atrazine as a representative of pesticide class, being also on the list of WFD Priority Substances; (2) bisphenol A — industrial pollutant known as an endocrine disrupting compound; and (3) estrone — hormone with endocrine disrupting effects, often present in outlets of municipal waste water treatment plants. Spiking levels were 0.1 and 1.0 μg L<sup>−1</sup> in water matrices and 50 and 500 ng g<sup>−1</sup> in sediment. In order to prevent decomposition of analytes during transport, water samples were directly loaded onto small 10 mm × 2.0 mm i.d. SPE cartridges packed with polymeric sorbent, which fitted into the automated sample preparation device PROSPEKT available in all partner’s laboratories. Sediment samples were first extracted by ultrasonic extraction into acetonitrile, extract diluted in Milli-Q water and concentrated on the same cartridges. Each sample has been prepared in triplicate and sets of cartridges, including blanks, were distributed among the partners. Cartridges were measured either directly by techniques using on-line SPE–LC set-ups (Brinkman et al., 1994) or eluted by 1 mL acetonitrile, which was then reconstituted into 20 mL volume by Milli-Q water (resulting in an acetonitrile concentration of 2%) for analysis by RIANA, ELISA and off-line SPE/GC–MS. For more details on the procedures, the reader is addressed to a reference (Slobodník et al., 2004).

### Table 3

<table>
<thead>
<tr>
<th>Compound</th>
<th>Assigned value (μg L&lt;sup&gt;−1&lt;/sup&gt;)</th>
<th>Milli-Q water matrix Assigned value (μg L&lt;sup&gt;−1&lt;/sup&gt;)</th>
<th>Assigned value (ng g&lt;sup&gt;−1&lt;/sup&gt;) Sediment matrix</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (μg L&lt;sup&gt;−1&lt;/sup&gt;)</td>
<td>Mean (%)</td>
<td>CV (%)</td>
</tr>
<tr>
<td>Atrazine</td>
<td>Level 1: 0.095</td>
<td>0.11</td>
<td>116</td>
</tr>
<tr>
<td></td>
<td>Level 2: 0.950</td>
<td>1.12</td>
<td>118</td>
</tr>
<tr>
<td>Bisphenol A</td>
<td>Level 1: 0.097</td>
<td>0.08</td>
<td>82</td>
</tr>
<tr>
<td></td>
<td>Level 2: 0.974</td>
<td>1.25</td>
<td>128</td>
</tr>
<tr>
<td>Estrone</td>
<td>Level 1: 0.076</td>
<td>0.08</td>
<td>105</td>
</tr>
<tr>
<td></td>
<td>Level 2: 0.765</td>
<td>1.04</td>
<td>136</td>
</tr>
</tbody>
</table>

*Milli-Q water and sediment samples were spiked at 0.1 and 1.0 μg L<sup>−1</sup> (water samples) and 50 and 500 ng g<sup>−1</sup> (sediment samples), respectively. For more details on the procedures, the reader is addressed to a reference (Slobodník et al., 2004). Corrected for extraction recoveries from sediments. Calculated from three measurements.

### Table 4

Comparison of atrazine determinations in Milli-Q water obtained by conventional liquid chromatography-based analytical techniques, ELISA, the immunosensor RIANA, and the AWACSS instrument.

<table>
<thead>
<tr>
<th>Method</th>
<th>Mean (μg L&lt;sup&gt;−1&lt;/sup&gt;)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPE–HPLC–DAD UV (Lab 1)</td>
<td>0.11</td>
<td>14.3</td>
</tr>
<tr>
<td>SPE–HPLC–DAD UV (Lab 2)</td>
<td>0.14</td>
<td>3.5</td>
</tr>
<tr>
<td>SPE–HPLC–DAD UV (Lab 3)</td>
<td>0.07</td>
<td>20.8</td>
</tr>
<tr>
<td>RIANA</td>
<td>0.16</td>
<td>24.1</td>
</tr>
<tr>
<td>ELISA</td>
<td>0.14</td>
<td>3.6</td>
</tr>
</tbody>
</table>

Spiked concentration: 0.1 μg L<sup>−1</sup>; number of measurements = 3.
imunosensor techniques (for an example of Milli-Q water, see Table 4).

4. Conclusions

Two prototypes of the AWACSS system were designed and constructed within the project and over 20 antibodies and their analyte derivatives were developed. Analytical performance of AWACSS systems was tested in comparison to a wide range of conventional chromatography-based analytical techniques, such as on-line SPE–LC–DAD UV, on-line SPE-LC–MS and GC-MS systems. Feasibility studies on the multi-analyte analysis and/or matrix effects were conducted with imunosensor techniques RIANA and ELISA. Among the tested matrices were surface, ground, drinking and waste water and, using special sample preparation protocols, sediment samples. The results showed that AWACSS is fully comparable to conventional analytical techniques in terms of accuracy, repeatability and reproducibility. Detection limits of all tested analytes were in the low nanogram per litre range, while selectivity allowed for trace analysis even in complex matrices such as sediment extracts. Time of a single analytical run was less than 18 min and during the system validation more than 70 analyses were performed within a day in a fully automated regime. Four water-monitoring groups from the project team were extensively assessing various fields of potential application of the system and measured sites that could accommodate the final instruments for testing. The fact that monitoring of many of the AWACSS compounds is fully comparable to conventional analytical techniques, such as on-line SPE–LC–DAD UV or on-line LC–MS, opens up a wide range of conventional chromatography-based analytical applications and Quality Assurance. Elsevier.

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