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Automated determination of s-triazine herbicides using solid-phase microextraction

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Abstract

Solid-phase microextraction (SPME) allows the determination of pollutants in aqueous solution by the adsorption of analytes onto stationary-phase coated fused-silica fibres followed by thermal desorption in the injection system of a capillary column gas chromatograph. This technique has been fully automated using a Varian 8100 autosampler and 3400 gas chromatograph fitted with a nitrogen–phosphorus flame thermionic detector. Fibres coated with 7-μm and 100-μm film thicknesses were used to evaluate the adsorption and desorption of four s-triazines. The resulting gas chromatographic peaks desorbed from the fibres were shown to be comparable to those obtained with direct manual injection. The 7-μm fibre, designed for the analysis of semi-volatile analytes was used to investigate the effect of desorption temperature and on-column focusing temperatures on peak response. The desorption temperature was found to be non-critical and an optimum focusing temperature of 40°C was used throughout the analysis. Evaluation of the 100-μm film fibre demonstrated its potential to adsorb greater quantities of analyte from solution and this study established that an adsorption time of 15 min gave an equilibrium distribution of the solutes between the stationary and liquid phases. With the thicker film fibre it was noted that the effectiveness of the desorption process was reduced at temperatures below 140°C. The linear dynamic range of the technique was evaluated over three orders of magnitude. To enhance method sensitivity, the fibre was used to extract a 0.1 ppb solution of herbicide by repeatedly adsorbing and desorbing from the same solution and focusing the combined solutes at the front of the analytical column prior to elution and analysis.

1. Introduction

Modern methods for the analysis of organic pollutants in water are frequently incompatible with the requirements of laboratories to provide a high turnaround of inexpensive analysis without creating additional environmental problems. Semi-volatile compounds are traditionally analysed by liquid–liquid extraction (LLE) procedures using an organic solvent [1]. These methods can be time-consuming, use large volumes of organic solvent and are difficult to automate. In addition, final preconcentration prior to analysis is frequently necessary. The more recent technique of solid-phase extraction (SPE) is now replacing LLE in many situations [2]. However, SPE can be expensive with the cartridges usually being disposed of after one extraction. Also,

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SPE can suffer from high blank results and the entire analysis can be lengthy with intermediate washing and drying stages slowing the process. In spite of these difficulties SPE has been accepted for use both on- and off-line for preconcentration and clean-up of environmental analytes [3–5]. Supercritical fluid extraction (SFE) has attracted much attention for its rapid, non-organic solvent extractions, but low recoveries and long extraction times make it unsuitable for direct extraction from water samples [6]. However, the combination of SPE and SFE has been shown to be useful for extraction of pesticides [6–8] and surfactants [9,10] from water.

Solid-phase microextraction (SPME) offers solutions to many of the above problems. The main advantages of SPME include, little modification of existing hardware, faster sample preparation followed by solvent-free analysis of both volatile and semi-volatile compounds with full automation possible [11]. SPME is commonly used for the analysis of volatiles, such as substituted benzenes [12–16], which are routinely analysed by purge-and-trap methods [17], since they are easily desorbed at low temperatures. However, some analyses of semi-volatile analytes have been reported including PAHs [18] and PCBs [19]. Recent fibre developments have included a new polyacrylate fibre which is capable of extracting more polar compounds and has been used to extract phenols direct from water and headspace samples [20]. Much of the work reported so far has used "home-made" SPME fibres and holders [12–16,18–20] which require specialist knowledge and which inevitably suffer from fibre-to-fibre inconsistency. This paper discusses the automated analysis of semi-volatile s-triazine herbicides using a commercially available SPME fibre and holder. The s-triazine group of herbicides is one of the most widely used soil-applied herbicides in Europe [21]. Two fibres with the same coating but of different film thickness are compared with the aim of establishing an analytical method for extraction and detection at sub-ppb levels.

2. Experimental

2.1. SPME apparatus

The fibres used were coated with poly(dimethylsiloxane) at both 100 μm and 7-μm thickness and designed for automated analysis only. The holder used to protect the delicate fibres during adsorption and desorption was also used specifically for automated analysis (both supplied by Supelco, Poole, Dorset, UK). A Varian 8100 autosampler was used to automate the SPME. The standard syringe assembly was removed from the autosampler and replaced with the SPME holder containing the fibre. The autosampler was used together with a Varian 3400 gas chromatograph (GC) fitted with conventional split/splitless injector and a thermionic specific detector (TSD), both supplied by Varian (Walton on Thames, Surrey, UK). The GC was fitted with a 30 m $\times$ 0.25 mm I.D. 0.25 μm DB-5 capillary column (J&W Scientific, supplied by Phase Separations, Clwyd, Wales, UK). Pre-drilled Thermogreen injection septa (Supelco) were used throughout the analysis to reduce septa coring and bleed. Various injection and initial column temperatures were investigated during the study, however, the detector settings, gas flow-rates, pressures and temperature programme remained constant. The detector temperature of 310°C with a TSD bead current of 3.100 A was kept constant. Also the inlet pressure of 15 psi (helium) and the make-up gas velocity of 175 ml/min nitrogen with 55 ml/min hydrogen, remained constant. The temperature programme used to elute the analytes focused at the front of the GC column after desorption was as follows: variable initial column temperature, hold time 1 min, then 15°C/min to 150°C (hold 0.5 min), finally 4°C/min to 210°C.

The SPME autosampler was controlled by Labview software through a PC which allowed adsorption and desorption times to be set, whether headspace or liquid sampling was required, and the number of vials to be automatically analysed. Data collection and handling was also PC controlled via a Varian GC Star

workstation. Only minor modification of the entire autosampler/GC system was required including exchange of the syringe assembly for the SPME holder, installation of the Labview software to control the autosampler and connection of the PC to the autosampler.

2.2. Reagents

All herbicides were purchased from Promochem (Herts, UK) and used to prepare stock standards in methanol (Rathburns, Walkerburn, UK). The stock standards were then diluted to the required concentration, in volumetric flasks, using methanol to produce standard solutions. HPLC grade water (J.T. Baker, Berks, UK) was used throughout the analysis. Aliquots (1.2 ml) of standard solutions were injected into 2-ml autosampler vials (Chromocol, Herts, UK) using a micropipette to ensure the fibre was fully emersed in the solution and to prevent sample-to-sample variation. The vials were then sealed with septa and placed in the autosampler carousel to await analysis.

2.3. Automated SPME procedure

The main advantage of the commercial SPME assembly over a home-made version is its ability to perform fully automated analysis of multiple samples. This was achieved using a standard GC autosampler which was controlled using specialist software from a PC. Once the adsorption and desorption times, sampling mode (liquid or headspace) and the option for multiple or single vial analysis had been put into the software the autosampler routine was as follows. The carousel containing the samples was moved forward, rotated under an optical sensor to count the number of vials and retreated. The fibre on the SPME assembly was then fully protruded and quickly returned to ensure no faults were present. The carousel was once again moved under the SPME assembly and the sheathed fibre allowed to pierce the sample vial septa. Once inside the vial the fibre was extended to a pre-set amount, depending on whether liquid or headspace sampling was selected, where it remained for the set adsorption time. Upon completion of the adsorption period the fibre was again sheathed inside the protective needle and the whole syringe carriage moved to retract the fibre from the sample vial. The carousel was then retreated to expose the injection port and the needle placed in the hot split/splitless injector where once again the fibre was protruded from its protective sheath where it remained for the set desorption time. At the end of the desorption time the GC temperature programme was automatically started together with the Star workstation integrator. When multiple samples were extracted, a pre-adsorb delay could be included into the SPME software which allowed the fibre to begin adsorption in the next sample before the GC temperature programme had finished from the current sample. This allowed considerable time-savings when long adsorptions were being used.

2.4. SPME fibre blanks

Before any extractions can be performed using a new SPME fibre it must first be conditioned in an injector usually at a temperature above that which is to be used for routine desorption, but below the maximum operating temperature of the fibre. For the experiments involving the 100-μm fibre a blank desorption temperature of 220°C was chosen as this was the highest temperature which could be used without possible removal of the poly(dimethylsiloxane) phase. The fibre was conditioned in the injector for a minimum of 3 h at this temperature, with the split vent open, to fully remove any contaminants which might cause very high baseline noise and large ghost peaks. After this the fibre was repeatedly injected into the GC until the resulting chromatogram was clear from any contamination. A blank desorption was also carried out each morning prior to extraction to ensure any airborne interferences adsorbed when the

fibre was left unused overnight in the laboratory atmosphere, were removed.

3. Results and discussion

All experiments were initially done at the 1 ppm level to establish the appropriate protocol before determination of the s-triazines at a more realistic environmental level.

3.1. Comparison between a manual injection and a SPME extraction

Initially a manual injection of 1 ppm of all four s-triazines studied was compared with a 1 ppm aqueous sample containing the same concentration, using the 100-μm fibre. An acetone solution containing simazine, atrazine, propazine and trietazine at 1 ppm was manually injected into the injector at 250°C with the split vent closed. The split vent was re-opened after 0.75 min. This was compared with a SPME extraction using the freshly blanked 100-μm fibre which was absorbed for 5 min in the 1 ppm aqueous solution. After the adsorption was completed the fibre was desorbed for 15 min in the GC injector at 220°C. The injector was operated in split mode throughout all extractions. During the desorption stage the column temperature was maintained at 40°C which allowed the desorbed analytes to be refocused at the front end of the analytical column. No cryogenic cooling was required because of the semi-volatile nature of the herbicides. The two chromatograms resulting from the manual injection and the initial SPME extraction are shown in Fig. 1.

It is obvious from the chromatograms that there is no degradation of peak shape during a SPME extraction and that peak width does remain constant with no tailing observed. This indicates that a column temperature of 40°C during desorption is sufficiently low to successfully focus all of the herbicides. Also the retention times of the four peaks in the extraction exactly match those in the manual injection proving that the peaks shown in the second chromatogram are actually the s-triazine herbicides of interest.

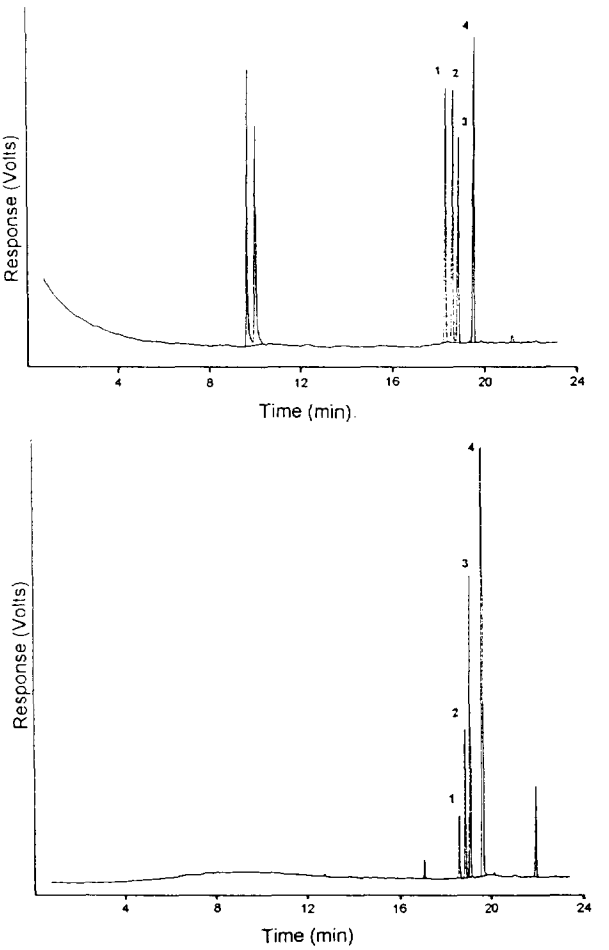

Fig. 1. Comparison between a manual injection (upper) and a SPME extraction using a 100-μm fibre (lower). Peaks: 1 = simazine, 2 = atrazine, 3 = propazine, 4 = trietazine; 1 ppm solution injected in acetone; SPME extraction; 5 min adsorption followed by a 15-min desorption at 220°C.

3.2. Effect of adsorption time

The 100-μm fibre was used to investigate the effect of adsorption time on the peak areas of the four herbicides. A 1 ppm solution of the four analytes was used as a test mixture and the fibre was adsorbed over a range of times from 30 s to 15 min. All extractions were carried out at ambient temperature (~23°C) and were unstir-

red. After each adsorption the fibre was desorbed at 220°C for 15 min. The results are shown graphically in Fig. 2.

Subsequent blank desorption at 220°C after each extraction indicated no carry over of analyte was occurring between extractions. It can be seen from the graph that, as expected, after an initial steep rise as adsorption time is increased the peak area rises less dramatically and is approaching a plateau. The adsorption was not extended beyond 15 min as the peaks obtained, especially for trietazine, were so large that accurate integration became impossible.

3.3. Effect of desorption temperature on the 100-μm fibre

The desorption temperature was determined by maintaining a constant adsorption time of 5 min and using a 0.3 ppm solution so as to reduce the size of the peak areas obtained. This ensured that accurate integration was always possible. After the adsorption period the fibre was inserted into the injector, which was kept at various temperatures ranging from 220°C to 120°C, for 15 min. This is the temperature range recommended to be used with the 100-μm fibre. After each extraction of a 0.3 ppm solution the injector temperature was increased again to 220°C and a blank desorption undertaken to ensure no carry over occurred. Altering the desorption temperature, within the range 220–140°C, has no real effect on peak area. Below 140°C peak areas begin to decrease. Following 140°C desorptions, blank desorptions at 220°C revealed that a residual 5% of analytes were being retained on the fibre coating at the lower desorption temperature. Subsequently the fibre desorptions were run at the maximum temperature of 220°C since this appeared to have no adverse effect on fibre performance.

3.4. Dynamic range of the 100-μm fibre

A short adsorption time of 5 min and desorption time of 15 min at 220°C were then used to study the dynamic range of the 100-μm fibre. A series of aqueous working solutions containing the compounds at various concentrations were made from the stock standards in ranges between 1.0 and 0.1 ppm, 0.1 and 0.01 ppm and 0.01 and 0.001 ppm. Due to operational time constraints the sets of working standards were run on consecutive days. The linear correlation coefficient, for the three separate experiments, was on average 0.978. Thus indicating that the fibre is linear and may be used over three orders of magnitude. Below 0.001 ppm the peaks obtained were very small and it was not possible to integrate them with any certainty. Increasing the bead current to the TSD detector would have increased its sensitivity and may have allowed a lower concentration in solution to be detected but this would have been at the expense of the bead lifetime which is severely reduced when the detector is operated at elevated currents.

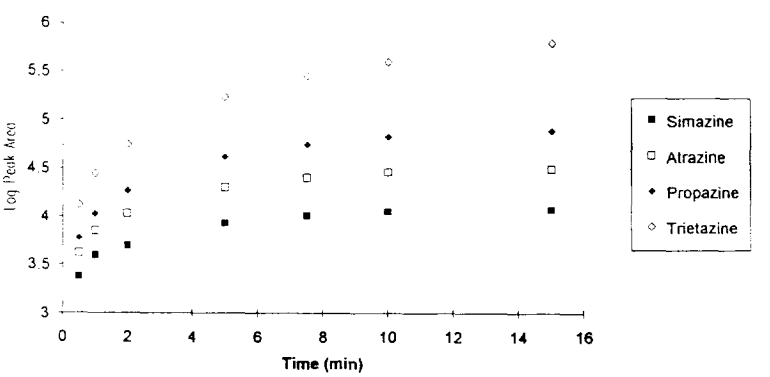

Fig. 2. Effect of adsorption time on peak area using a 100-μm fibre; 1 ppm solution, desorption time 15 min at 220°C.

3.5. Comparison between the 100-μm and the 7-μm fibre

The 100-μm fibre, designed for analysis of volatile compounds, was then replaced with a 7-μm fibre which was specifically designed for extraction of semi-volatile analytes. The much thinner coating on this fibre allowed the phase to be strongly chemically bound to the silica support thus enabling higher desorption temperatures to be used without risk of phase degradation. The fibre was actually capable of being used over the temperature range 220–320°C which should allow higher boiling point semi-volatile compounds to be successfully desorbed. The obvious disadvantage of the thinner film fibre coating was the poor detection limit. Once again the fibre required several hours blank desorption at an elevated temperature before use.

The difference in the amount adsorbed between the 100-μm and 7-μm fibres is illustrated in Fig. 3 where a 1 ppm solution is extracted for 5 min using a 100-μm fibre and for 15 min using the 7-μm fibre. Both fibres were desorbed for 15 min at 220°C and 250°C, respectively. Although the peak shape in both chromatograms is almost identical, it is obvious that the 7-μm fibre is only capable of adsorbing a fraction of the amount adsorbed by the thicker film of the 100-μm fibre.

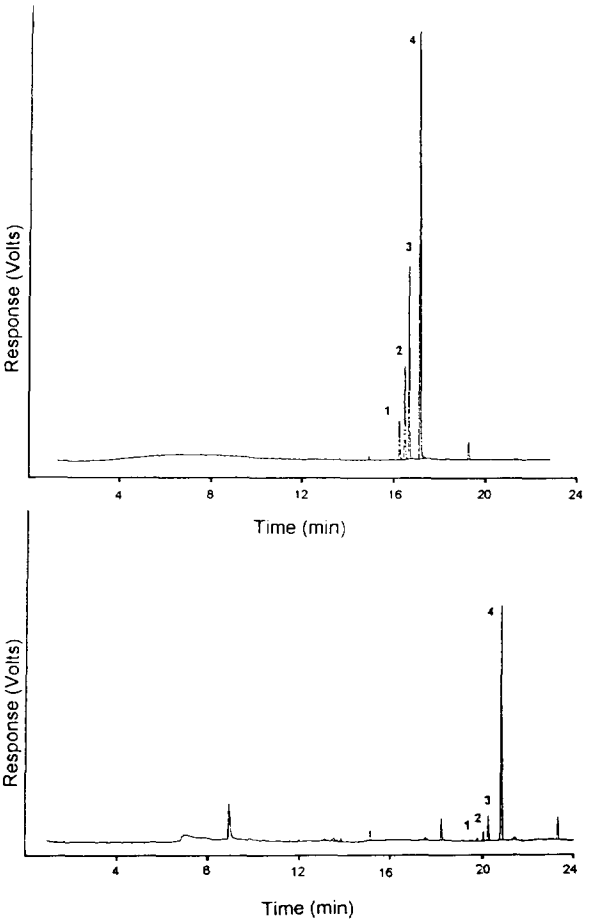

Fig. 3. Comparison between a 1 ppm extraction using a 100-μm fibre (upper) and a 7-μm fibre (lower). Peaks: 1 = simazine, 2 = atrazine, 3 = propazine, 4 = trietazine; 100-μm fibre adsorbed for 5 min, 7-μm fibre adsorbed for 15 min; both desorbed for 15 min at 220°C and 250°C, respectively.

3.6. Effect of desorption temperature on the 7-μm fibre

The effect of desorption temperature on the 7-μm fibre was determined over the working range of the fibre. The 7-μm fibre was used to adsorb a 1 ppm solution for 10 min before a 15 min desorption at the temperature studied. As before, at the end of each extraction a blank desorption was carried out at the maximum operating temperature of 320°C to show any carry over at lower desorption temperatures. No difference in peak area over the temperature range studied was noted. It was observed that regardless of desorption temperature no carry over of any herbicide occurred. Therefore, it was concluded that all analytes could be removed at a desorption temperature of 220°C, irrespective of fibre coating thickness.

3.7. Effect of column focusing temperature

The effect of column focusing temperature was also investigated to determine whether 40°C was the optimum temperature. A range of temperatures between 40°C and 100°C were chosen, at 10°C intervals, since it was not possible to cool the column, in a short period of time, below 40°C without cryogenic cooling. The effect on both peak shape and area was noted for a 10-min adsorption of a 1 ppm solution using the 7-μm

fibre. The fibre was then desorbed for 15 min at 270°C with the column kept at a constant temperature within the range chosen. The usual temperature ramp was used to elute the compounds from the column regardless of the initial column temperature. The focusing temperature ($n = 7$) has no significant effect on peak area (herbicide: mean peak area counts, %R.S.D.; simazine: 850, 39%; atrazine: 1900, 11%; propazine: 5200, 12%; trietazine: 42100, 6%) or peak shape. The %R.S.D. difference for simazine was attributed to its significantly lower response. It was decided to keep the initial column temperature of 40°C since the majority of work had been done at 40°C and there appeared to be no real benefit in using elevated focusing temperatures.

Increasing the desorption temperature above 220°C did not effect the peak. This indicates that the 100-μm fibre may be of use in extracting not only volatile but also semi-volatile compounds. It was therefore decided, from these results, to use the thicker fibre as it was obvious that the 7-μm coating would be incapable of extracting sufficient analyte in a short space of time to be of any use in the analysis of low concentration solutions.

3.8. *Multiple extractions and analysis of low concentration solutions*

Within Europe the EEC has set limits for individual pesticides and herbicides in drinking water of 0.1 ppb [22]. It is therefore essential for any screening method to be able to detect at this level. From the previous study using the 100-μm fibre it was obvious that it would be impossible to detect a 0.1 ppb solution of s-triazine herbicides in a single extraction. Multiple adsorptions were therefore carried out on the same sample vial and desorbed in the injector. However, instead of following each desorption with a temperature programme to elute the analytes, the herbicides were stacked at the front of the column which was maintained at the focusing temperature. Multiple extractions obviously take considerable time and to reduce this the optimum desorption time was briefly investigated. A 0.5 ppm solution of the four herbicides was placed in the carousel and adsorbed for 3 min using the 100-μm fibre. The fibre was then desorbed at 220°C for the usual 15 min. This was compared with an identical adsorption but with a desorption time of only 5 min. Following the shorter desorption time a blank was run to determine any carry over of analyte. No significant difference in the peak areas after the two different desorptions were noted. The blank carried out after the 5-min desorption indicated a small amount of carry over (<1%) for propazine with the shorter desorption time. Although this is not desirable in single extractions, it is irrelevant when performing multiple adsorptions since all of the analyte is trapped at the front of the column. The 5-min desorption time at 220°C was subsequently used in the multiple adsorption experiments.

The multiple extraction technique was used to extract a 0.1 ppb solution of the herbicides. A 10-min adsorption followed by a 5-min desorption was repeated ten times from a single solution and stacked at the front on the GC column. The whole procedure (15 min per cycle × 10 repeats) in total was repeated three times. The precision of the results ($n = 3$) ranged from 6% R.S.D. for propazine to 20% R.S.D. for atrazine thus indicating the feasibility of the technique to detect low concentration solutions by SPME which would normally be well below the limit of detection of the detector used.

4. Conclusion

SPME has been shown to be a simple and elegant technique which does not require any solvent and only minor modification of existing laboratory hardware. SPME cannot only be used to extract volatile analytes commonly analysed by headspace or purge-and-trap analysis but is also capable of extracting semi-volatile analytes, often extracted using SPE, at a fraction of the cost (the fibres used in this study have been reused approximately 100–150 times). An initial investigation of the principle operating parameters using a 1 ppm mixture of the s-triazines was done. This was followed by the use of a multiple

adsorption technique for the extraction of s-triazine herbicides at the sub-ppb level.

SPME, although still in its infancy as an environmental extraction technique, has indicated sufficient promise to ensure its place for routine analysis of aqueous matrices. At present the amount of data on quantification using SPME is still limited and requires further investigation. Also, the advent of new commercially available fibres with a range of specific analyte coatings will help to promote its use in environmental laboratories of the future.

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