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COUPLED REVERSED-PHASE LIQUID CHROMATOGRAPHY–CAPILLARY GAS CHROMATOGRAPHY FOR THE DETERMINATION OF ATRAZINE IN WATER

KONRAD GROB, Jr.*
Kantonales Labor, P.O. Box, CH-8030 Zürich (Switzerland)
and
ZHANGWAN LI
West China University of Medical Sciences, Chengdu, Sichuan (China)

SUMMARY

A fully automatable method for determining atrazine in water is described, based on enrichment of the atrazine from 10 ml of water on a small ODS liquid chromatographic column, desorption with methanol–water (60:40) + 5% 1-propanol and transfer to a gas chromatograph by concurrent eluent evaporation using a loop-type interface. The total analysis time is 35 min with a detection limit of *ca.* 3–5 ppt. Concurrent eluent evaporation of aqueous eluent mixtures suffers from a large temperature difference between transfer and elution of the first sharp peaks. On transfer at 112°C, the atrazine peaks were perfectly shaped only when eluted at about 250°C.

INTRODUCTION

Atrazine is one of the most widely used herbicides and it is frequently found in drinking water. Legal limits are low (0.1 ppb in Switzerland and some other European countries), calling for very sensitive analytical methods. Atrazine is determined in large numbers of samples, rendering a fully automated method desirable. Currently used methods involve sample enrichment by liquid–liquid[1] or solid-phase extraction[2], followed by reconcentration and analysis by liquid chromatography (LC) with UV detection or gas chromatography (GC) with alkali flame ionization detection. Detection limits in the routine application of these fairly labour-intensive methods are between 0.01 and 0.1 ppb.

Solid-phase extraction is often considered to be the state-of-the-art technique for sample enrichment. However, in several respects, LC sample enrichment is clearly superior. The enrichment column is further miniaturized, control of the enrichment step is more accurate (owing to on-line detection) and elution of the material of interest is more selective (owing to higher efficiency and on-line detection).

With GC as a final analytical step, on-line coupled LC is a highly attractive sample preparation method. LC enriches the sample, provides some clean-up by the

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transfer of a small fraction and on-line coupling allows full automation, starting with the raw water from an LC autosampler. Miniaturization of the sample enrichment column is possible, as the whole sample material introduced into the LC system is transferred to the GC column.

The application of coupled LC–GC to the determination of atrazine in water was considered as a first example, testing a technique (concurrent eluent evaporation of water-containing eluents) that had never been used before. There are a large number of other applications that can be carried out along the same lines. Fully automated water analysis outside the laboratory (*e.g.*, in water treatment plants) is of interest to many authorities.

Removal of water from the enrichment column

Sample enrichment necessarily occurs through a reversed-phase system, *e.g.*, an ODS LC column. However, with two exceptions[3,4], only normal-phase LC has been coupled to GC, primarily introducing "easy" solvents such as alkanes and ethers [one of these exceptions involved the use of pure acetonitrile, the other the transfer of a 2-μl volume of methanol–water (80:20)]. To permit the use of *n*-hexane for transferring the sample from the enrichment column to GC, Boo and Krohn[5] and later Noroozian *et al.*[6] proposed displacement and evaporation of the residual water from the LC column in a gas stream and by vacuum (possibly with additional heating). Complete removal of water was essential in order to allow quantitative desorption of the components of interest with the water-immiscible *n*-hexane.

The removal of water from LC columns is demanding. Experimentally, we found that mechanical displacement by gas only removes *ca.* 15–25% of the water. Apparently, a channel is opened through the packing, through which the gas flows without displacing the rest of the water. The remaining water must be evaporated. However, under these conditions the enrichment column becomes a packed GC column. As the GC retention power of ODS LC columns is fairly low, there is a considerable risk of losing volatile components together with the water vapour. On the other hand, Noroozian *et al.*[6] showed that these losses are surprisingly small.

Coupling reversed-phase LC to GC

Of course, avoidance of the water evaporation step would be desirable. In fact, the sample material trapped on the reversed-phase column can be desorbed directly with an organic solvent as long as this solvent is water miscible. The problem, however, is the water carried into the GC system.

Theoretically, the transfer could be started exactly at the front of the organic desorption medium, although even in this instance a small amount of water is carried into the GC system, as water mixes into the organic solvent. However, as the solute material of interest is likely to move right at the front of the organic eluent, such cuts exactly at the interface between the water and the organic eluent are hardly feasible in practice. To obtain a quantitatively reliable method, the cut must occur slightly earlier, including some water in the fraction transferred. Transfer of water into the GC system is only one problem; another concerns the change in the water concentration from 100% to nearly 0% during transfer, calling for a corresponding adjustment of the GC conditions.

In the method described in this paper, the problem was solved by using an LC

eluent that gives atrazine some retention on the enrichment (LC) column. This moves the atrazine peak away from the breakthrough of the organic eluent system, *i.e.*, from the water. It provides a stable eluent composition and an efficient sample clean-up. On the other hand, retention of atrazine could only be achieved with a fairly high water concentration (40%) in the desorption medium (LC eluent).

Transfer by concurrent eluent evaporation

Recently we investigated several possibilities of transferring water and water-containing reversed-phase eluents to capillary GC. The use of retention gap techniques[7] (with or without partially concurrent eluent evaporation) is restricted to liquids that wet the pre-column wall. This requirement could not be met with water[8], but was possible with many commonly applied aqueous reversed-phase mixtures. However, a second requirement is more difficult to fulfil: water must evaporate at least as rapidly as the organic solvent in order to prevent non-wetting water from being left behind. The eluent mixture in our application, water–methanol (40:60) containing 5% 1-propanol, does not fulfil either of these two requirements[9], ruling out the use of retention gap techniques.

Transfer to GC by the concurrent eluent evaporation technique[10] through a loop-type interface[11] does not require wettability of the pre-column, nor is it important which solvent evaporates more rapidly. The eluent plug is pushed into the oven-thermostated capillary pre-column by the carrier gas. Flooding of the pre-column is prevented by using an oven temperature above the boiling point of the eluent at the inlet pressure, producing an eluent vapour pressure to stop the eluent flow.

On the other hand, concurrent eluent evaporation causes strong broadening of peaks up to elution temperatures considerably above the transfer temperature. This is a particularly severe problem with aqueous eluents because water does not produce any phase soaking (increase of retention power in the coated column by swelling the stationary phase[12]). With polar stationary phases, even a reversed-phase soaking effect may be observed[13], accentuating band broadening. As reconcentration just relies on cold trapping, peak broadening affects components with higher elution temperatures than when using well soaking, less polar eluents.

LC–GC transfer of atrazine occurred by concurrent eluent evaporation. As will be shown, the difference in oven temperature between transfer and elution was just about sufficient for complete reconcentration.

EXPERIMENTAL

Experiments were carried out on an LC–GC prototype instrument with a Phoenix 20 syringe pump, an alkali flame ionization detector (NPD-40) and a fully automated interface system from Carlo Erba (Milan, Italy).

LC enrichment and desorption

LC sample enrichment column. Of the commonly used reversed-phase LC packing materials, ODS phases exhibited the highest retention power (capacity factor) for atrazine. This was important for obtaining a maximum capacity of the LC enrichment column and for keeping the required column size minimal. As atrazine is highly retained, it forms a short band at the top of the LC column, which, in turn, is

a prerequisite for obtaining an efficient LC separation and sharp LC peaks (small fraction volumes). Finally, a high retention power allowed elution with an eluent containing a minimum of water. Among the 5-μm ODS materials tested, Spherisorb, Nucleosil, Hypersil and LiChrosorb had similar retention powers (columns from Stagroma, Wallisellen, Switzerland). We selected a Knauer 100 × 2 mm I.D. column packed with 5-μm Spherisorb ODS.

Sample introduction. A 10-ml volume of the water sample was introduced into the enrichment column through an injector loop of slightly larger internal volume. After passage of 10 ml of water through the LC column, shortly before complete discharge of the loop contents, the LC injection valve was returned from inject to load, feeding desorption medium. This ensured a sharp transition from water to eluent (water and eluent are mixed at the rear end of the sample plug in the sample loop), and this rear was discarded in this way. Further, the desorption medium entered at an accurately known time, which is important for an accurate determination of retention times during desorption.

Eluent flow-rate. The pump constantly delivered the eluent for desorption, selected as the strongest eluent mixture safely separating the atrazine peak from the breakthrough peak, namely methanol–water (60:40) with 5% 1-propanol. The flow-rate of 400 μl/min was optimized for desorption. Owing to the lack of an additional pump or a possibility of automatically changing flow-rates delivered by the pump, passage of the water sample through the enrichment column had to occur at the same flow-rate. As this led the sample enrichment to be time consuming, this step was

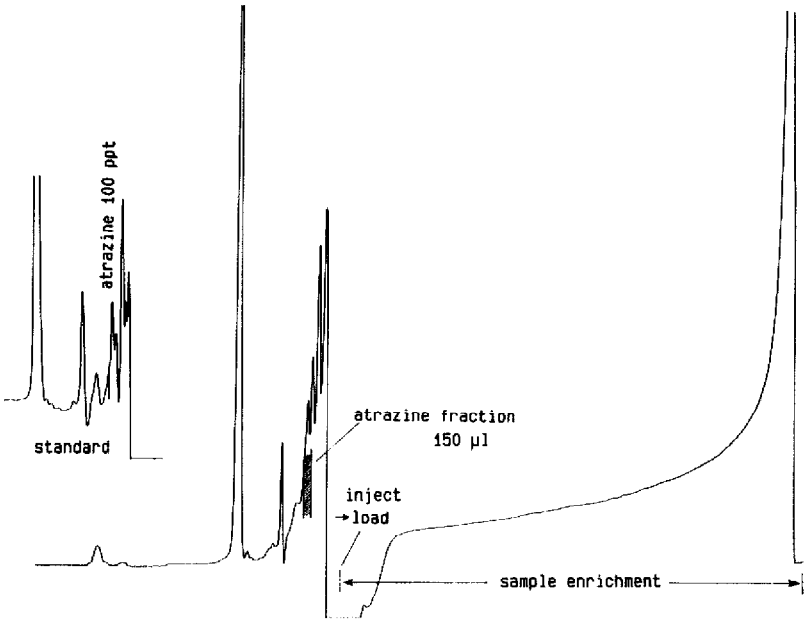

Fig. 1. Liquid chromatogram (UV detection at 220 nm) with initial sample enrichment (10 ml of tap water). At the point indicated, shortly before the content of the sample loop was completely transferred, the injection valve was switched, starting elution. The atrazine fraction is marked. At the left, part of the chromatogram of another tap water sample spiked with 100 ppt of atrazine is shown.

started on the next sample before the preceding GC analysis was completed. From the LC eluate, detected at 220 nm (Fig. 1), an atrazine fraction of 150-μl volume was transferred to the GC system.

Transfer to GC

The GC system involved an early solvent vapour exit in order to accelerate the discharge of the large volume of vapour produced by the aqueous eluent. A 2 m × 0.53 mm I.D. uncoated precolumn (phenyldimethylsilylated fused silica) was used to keep the pressure drop over this precolumn small. It was followed by a 3 m × 0.32 mm I.D. retaining precolumn taken from the separation column. The solvent vapour exit, positioned between the retaining precolumn and the separation column, was constructed of a glass press-fit Y-piece, connected to an electric three-way valve through a 30 cm × 0.32 m I.D. fused-silica capillary. The exit was automatically switched to a high resistance (2 m × 0.10 mm I.D. fused-silica capillary) 2 min after the pressure started to drop from the high transfer level towards the lower analysis level.

In order to overcome broadening of the atrazine peak, a GC column with a high retention power was selected, providing a high elution temperature, *viz.*, a 40 m × 0.32 mm I.D. column, coated with Carbowax 20M of 0.4-μm film thickness. In this way, the elution temperature of atrazine could be increased to 260°C.

The carrier gas (hydrogen) pressure delivered to the flow regulator was 1.0 bar. The flow regulator was in position 100, creating an inlet pressure of 0.5 bar at 112°C. During transfer, this pressure increased to 0.8 bar. Higher inlet pressures were avoided in order to keep the transfer temperature low. This change in the inlet pressure during transfer was sufficient for recognizing the end of eluent transfer and causing automatic actuation of the solvent vapour exit valve, as well as starting the GC run.

Under the conditions used, involving a high eluent evaporation rate and a carrier gas inlet pressure of 0.8 bar during transfer, the safe transfer temperature required to rule out excessive flooding was 112°C. This is *ca.* 10°C above the temperature listed[14], which was determined at a lower evaporation rate (strong cooling and a strong tendency towards delayed evaporation of aqueous eluents renders the minimum required column temperature dependent on the eluent evaporation rate).

A problem that occurred with one precolumn, but not with two others, taken from the same piece of deactivated fused-silica, must be examined more carefully in the future. On this one pre-column (2 m × 0.53 mm I.D., phenyldimethylsilylated fused silica), the eluent plug rushed at high speed through the whole uncoated precolumn, too rapidly to be warmed up and to build up the vapour pressure required to stop the liquid. Eluent penetrated into the retaining precolumn and some of it was lost through the solvent vapour exit. Such "shooting" liquid causes peak broadening (spreading of the solutes over the retaining precolumn) and losses in peak area (affecting all components equally). Oven temperatures had to be increased to 150–160°C to stop the liquid in an early part of the uncoated precolumn, *i.e.*, to a temperature far above the actual boiling point of the eluent. This problem could be due to poor wettability of the precolumn wall by the eluent, causing low friction of the moving liquid and poor transfer of heat through the capillary wall–liquid interface. On the other hand, the two other precolumns were not wetted by the eluent either, and even pure water could be concurrently evaporated at 125°C.

RESULTS AND DISCUSSION

Atrazine in water

Fig. 2 shows a full gas chromatogram obtained from 10 ml of tap water spiked with 100 ppt of atrazine. In the upper right corner, part of the gas chromatogram of another tap water sample, containing 15 ppt of atrazine, is shown. The peak that eluted after the atrazine, observed in all samples, was not identified, but is probably an artifact from the interface. The method proved to be simple and the analysis time per sample was about 35 min, with practically no work involved (owing to limitations of the autosampler, full automation was possible only when the sample volume was reduced to 2 ml).

Concurrent evaporation of aqueous eluents

This study also gave some insight into the LC–GC transfer of aqueous eluents by concurrent eluent evaporation, revealing two weak points. First, experiments clearly showed that a large difference in oven temperature between eluent transfer and elution of the solute of interest is required in order to obtain sharp peaks, when water is involved. Fig. 3 illustrates this for atrazine. A 15 m × 0.32 mm I.D. capillary column was used, coated with Carbowax 20M of 0.4-μm film thickness, which eluted atrazine at 238°C. Transfer of a fraction volume of 150 μl at 112°C did not lead to significant peak broadening (full chromatogram). At 120°C, however, broadening is already visible, and at 130°C it is very strong. When the fraction volume was increased to 250 μl, broadening was clearly visible even at the lowest transfer temperature.

Other organics in waters

The method described is highly specific for atrazine. The gas chromatograms are correspondingly "empty", just showing the atrazine peak. Such methods are of high

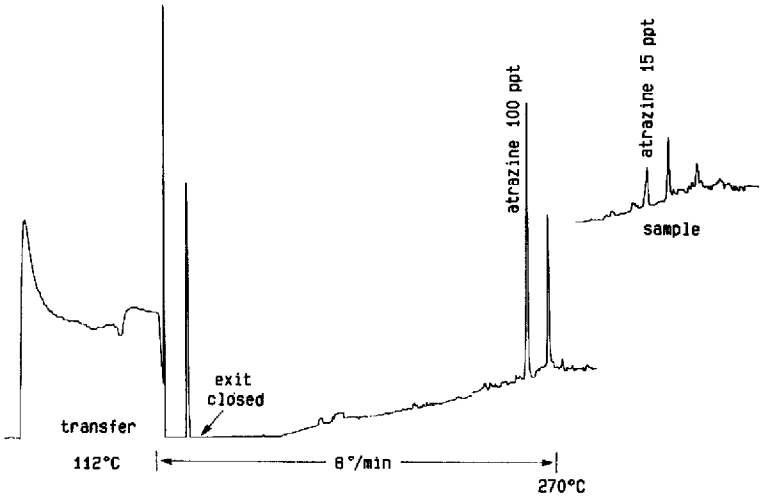

Fig. 2. Gas chromatogram of tap water spiked with 100 ppt of atrazine. Transfer (eluent evaporation) took 7.5 min. The solvent vapour exit was closed where marked. Right, part of the chromatogram of another tap water sample containing 15 ppt of atrazine.

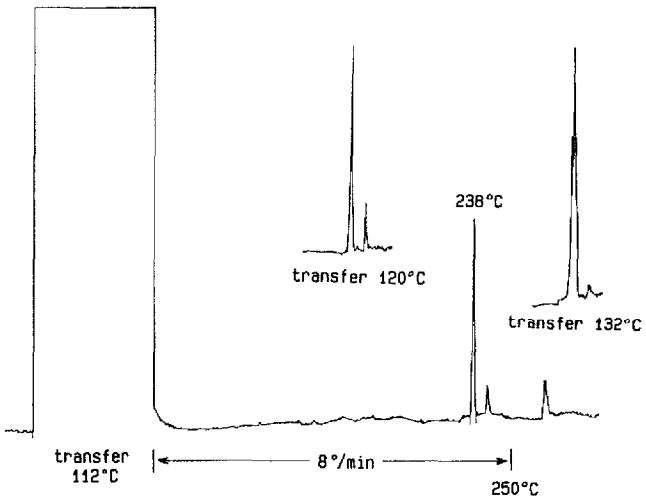

Fig. 3. LC–GC transfer of aqueous eluents requires large temperature differences between transfer and elution of the components of interest. Using a column from which atrazine was eluted at 238°C, transfer at 112°C resulted in negligible, at 120°C in clearly visible and at 132°C in strong peak broadening. Hence, in this instance the required temperature difference was 125°C. Experiments were carried out with 150-μl transfer volumes of methanol–water (60:40) eluent.

value for "dirty" samples, allowing efficient removal of interfering peaks. On the other hand, the method is not suitable for determining atrazine together with its first degradation product, desethylatrazine, or for the analysis of a whole "cocktail", *e.g.*, of triazine herbicides, as the fraction volumes would become very large. Nor is the method suitable for determining components eluted at GC temperatures below *ca.* 240°C owing to the problem concerning peak broadening by concurrent eluent evaporation.

The LC column used was far larger than required for retaining atrazine from 10 ml of water. A smaller LC column would allow elution with a smaller volume of eluent, as would the use of a stronger eluent containing less water. However, both options were discarded in view of the requirement of separating the atrazine peak from breakthrough. As long as conventional concurrent eluent evaporation is used, substantial enlargement of the LC fraction is impossible; with the GC column used in Fig. 2, an increase in the fraction volume above about 300 μl caused visible peak broadening. A further increase in the elution temperature of atrazine, for further improved reconcentration, is difficult.

The method described produced perfect results for atrazine. However, there are a large number of other organic components to be determined in water, and only a limited selection of them could be determined by the same technique. This calls for a complementary method, possibly transferring the LC column in backflushing; problems related to peak broadening, especially of components eluted at lower temperatures, can be solved using concurrent solvent evaporation with co-solvent effects[15].

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