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Large volume injection of water in gas chromatography–mass spectrometry using the Through Oven Transfer Adsorption Desorption interface: Application to multiresidue analysis of pesticides

Rosa M. Toledano[a], Jose M. Cortés[a], Juan C. Andini[b], Jesús Villén[a,*], Ana Vázquez[c]

[a] Escuela Técnica Superior de Ingenieros Agrónomos, Universidad de Castilla-La Mancha, Campus Universitario s/n, 02071 Albacete, Spain
[b] CCT CONICET, Santa Fe, Argentina
[c] Escuela Universitaria de Magisterio de Albacete, Departamento de Química-Física, Universidad de Castilla-La Mancha, Campus Universitario s/n, 02071 Albacete, Spain

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ABSTRACT

In the present work, the potential of the Through Oven Transfer Adsorption Desorption (TOTAD) interface for the large volume injection (LVI) of aqueous samples in gas chromatography (GC) using a mass spectrometry (MS) detector is demonstrated. To this end, a new method for the determination of pesticides in water is presented, being the first developed method in which injection of large amounts of polar solvents using the TOTAD interface and an MS detector are combined, is applied to the determination of pesticides in water. Water samples, as large as 5 ml, were directly injected into a capillary GC. No sample pre-treatment step other than simple filtration was needed. The TOTAD interface allows the introduction of several millilitres of water, while maintaining good chromatographic characteristics. The water is almost entirely eliminated, so that LVI of aqueous samples and an MS detector can be used without problems. Organophosphorus, organochlorine, and triazine pesticides were determined in one run. Calibration curves were linear in the range tested and the sensitivity achieved injecting 5 ml of water sample was sufficient for most of the target pesticides but not for all of them. Sensitivity of the analysis can be improved by increasing the sample volume. No variability was observed in the retention times and relative standard deviations from absolute peak areas were good, considering that they corresponded to the overall analysis. The method was applied to the analysis of pesticide residues in real water samples.

1. Introduction

There is a trend in analytical chemistry to minimize manual sample pre-treatment and to promote the use of fully automated methods from sample preparation to analyte detection. LVI and on-line coupled liquid chromatography–gas chromatography (LC–GC) have become powerful tools in the attempt to achieve these goals. LVI increases sensitivity and/or reduces the need for extract or sample concentration steps [1]. In coupled LC–GC, the specific components of a complex matrix are pre-fractionated by LC and then transferred on-line to the highly efficient and sensitive GC system for analytical separation. Sample preparation, i.e., extraction, clean up and preconcetration can be carried out by LC, and the final separation by the more efficient GC.

Coupling reversed phase liquid chromatography (RPLC) to GC is much more difficult than coupling normal phase liquid chromatography (NPLC) because of the polar nature of the solvent used in RPLC. Nevertheless RPLC–GC is frequently necessary, for example, in the case of an aqueous sample matrix [2]. There is, therefore, a need for reliable interfaces, if possible automatic, which will allow the polar solvent to be eliminated and analytes to be retained without loss or contamination. A Programmed Temperature Vaporizer (PTV) injector has previously been used for LVI and as an interface for on-line coupling LC–GC using different packing materials and adsorbents for the insert [3–7]. In spite of good result obtained, the use of PTV is still not routinely applied in many laboratories due to the lack of automation [8]. Our research group has developed a new interface named TOTAD (Through Oven Transfer Adsorption Desorption) for the LVI of polar and non-polar solvents, which is consequently, suitable for the on-line coupling of LC–GC when LC is carried out in normal or reversed phase. The TOTAD interface was first described in 1999 [9], consists of a heavily modified PTV injector. The changes introduced affect the pneumatics, sample introduction, solvent elimination, and operation mode. TOTAD interface and its operation mode have been described in the literature [9]. Although the liner of the interface is filled with Tenax, solid phase extraction (SPE) is not the only process involved, since partial solvent evaporation takes place in the liner. As far as we know,

* Corresponding author. Tel.: +34 967 599200x2839; fax: +34 967 599238.
E-mail address: jesus.villen@uclm.es (J. Villén).

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there is no similar system, so that we gave a new name, TOTAD, to the interface. It has been used for the analysis of pesticide residues in water by means of on-line RPLC–GC [10] and LVI [11]. In both cases, the interface used was home-made and manually operated,

In recent years, GC–MS has become a useful tool in pesticide residue detection, because it offers simultaneous identification and quantification of a large number of pesticides, avoiding successive analyses with different selective detectors [12]. On the other hand, the low sensitivity of GC–MS, even with selected-ion monitoring (SIM), and the very high sensitivity needed for the analysis of environmental matrices means that the limits of detection reached by GC–MS need to be improved. The use of LVI increases sensitivity, thus allowing the determination of pesticides at much lower concentrations.

The TOTAD interface allows the introduction of polar solvents (even water) in GC, as solvent elimination is almost complete, meaning that it is possible to use water-sensitive detectors. However, as stated above, initially, the TOTAD interface was manually operated and several valves had to be opened or closed at the beginning and the end of the transfer step. A simple error would cause the water to enter the GC and reach the detector, so that it was not possible to use MS as a detector. Consequently, a fully automated TOTAD interface was developed, which avoided operator error. However, the first prototypes occasionally experienced problems of flooding, which damaged the GC system and the possibility that such a problem might arise made it unadvisable to use such a water-sensitive and expensive detector as MS. For this reason, several RPLC–GC analytical methods were developed, using other detectors different to MS for the automated determination of pesticides residue in olive oil [13–16]. Organophosphorus and organochlorine pesticides were analyzed in nuts using two different detectors [17], while the minor components in edible oils [18] and methyl jasmonate in aromatic samples [19] were also analyzed. Díaz-Plaza et al. [20] described the use of two water-sensitive detectors, nitrogen–phosphorus detector (NPD) and electron capture detector (ECD), operating simultaneously, to analyse organophosphorus and organochlorine pesticides in olive oil in a single run. During the development of these and other analytical methods, the interface was improved [21], eliminating the sources of flooding. Once it was clear that the problem of flooding had been solved, the next step was to combine the techniques with an MS detector, which would provide structural information allowing confirmation of the analyte identity and its quantification. Undoubtedly MS is the best choice because unambiguous identification is, of course, a highly desirable option. However, the totally elimination of water is especially important when working with an MS detector because even if a small amount of water reaches the detector it will be seriously damaged because of the reactivity of the water. The objective of the present work was to demonstrate the potential of TOTAD interface to develop analytical methods combining the introduction of very large volumes of water-containing polar solvents, or even pure water, using an MS detector in GC.

To this end, a new method for the trace-level determination of pesticides in water by LVI-GC–MS, using the TOTAD interface and sampling a very large volume of the raw sample, is presented.

2. Experimental

2.1. Materials

Water samples were obtained from a deep channel and from an underground well. All pesticide standards were obtained from Chem. Service Inc. (West Chester, PA, SA). The organophosphorus pesticides (OPs) used were: diazinon, fenitrothion, malathion, parathion, phenthoate and methidathion. The chlorinated pesticide (CP) used was DDT. The triazine pesticides used were atrazine and terbutryne. A stock solution of 100 mg/l of each pesticide was prepared in methanol and stored at 4 °C. The working solutions were obtained by diluting the stock solution in water at concentrations ranging from 25 to 500 μg/l. Both the ethanol and water used to dilute and to propel the aqueous samples into the TOTAD interface were HPLC grade from pestican (LabScan, Dublin, Ireland). Tenax TA, 80–100 mesh (Chrompack, Middelburg, Netherlands) was used as packing material in the liner of the modified PTV (TOTAD interface). The glass-liner was packed with a 1 cm length of Tenax TA between two plugs of glass wool to keep it in place and was then conditioned under a helium stream by heating from 50 to 350 °C at 50 °C/10 min, at which it was maintained for 60 min.

2.2. Instrumentation

A Konik 4000B gas chromatograph, equipped with a flame ionization detector (FID) and a TOTAD interface, was coupled to an MS (Konik MS Q12). The TOTAD interface (U.S. patent 6,402,947 B1, exclusive rights assigned to KONIK-Tech, Sant Cugat Del Vallés, Barcelona, Spain) was used to inject a very large volume of aqueous samples into the GC. For very large volume sampling a manual injection valve (model 7125 Rheodyne, CA) provide with a 500 μl loop was used. For a 5 ml injection, a quaternary pump (Konik model 550) was used to propel the sample directly from the bottle. A quaternary pump (Konik model 550) was used to push the large volume of aqueous samples into the TOTAD interface. Data acquisition and processing were performed with KoniKrom Plus and MS Control (Konik, Sant Cugat Del Vallés, Barcelona, Spain) software.

2.3. TOTAD operation mode

Initially, the TOTAD interface and GC oven temperature were stabilized at 80 and 50 °C respectively. The carrier gas (helium) stream entered the packed liner through the oven side (B) and through the opposite side (A), both at 500 ml/min. EV_1 was closed and EV_2 was open (Fig. 1). The pump was stabilized at the sampling flow rate. The aqueous sample was introduced in the LC manual injection valve. When this valve was switched, the solvent coming from the pump propelled the sample through the stainless steel tube (ST_1 in Fig. 1) to the six-port valve, which was automatically switched, transferring the large volume of aqueous sample to the GC. The solution reached the glass-liner at 0.1 ml/min. The helium pushed the solution through the sorbent. Analytes were retained on the packed material in the liner and the solvent was vented to waste through the WT tubing. After this, the six-port valve was automatically switched, so that the solvent coming from the pump was sent to waste and the EV_1 was opened. Temperature and helium flow were maintained constant for 2 min to ensure elimination of the remaining solvent in the glass-liner and the SCT tubing. After this time, EV_1 and EV_2 were closed and the flow through B was interrupted while the flow through A was changed to 1 ml/min. Then, the TOTAD interface was quickly heated to 275 °C for 5 min, leading to the thermal desorption of the analytes, which were transferred to the GC column, pushed by the helium. GC–MS analysis was then carried out, after which EV_2 was opened and the interface was cleaned by maintaining the helium stream for 5 min at 300 °C. Finally, it was cooled to 80 °C so that another analysis could be carried out.

2.4. GC-conditions

Gas chromatography separations were carried out on a Quadrex (Weybridge, UK) fused-silica (30 m × 0.25 mm i.d.) column coated with 5% phenylmethylsilicone (film thickness 0.25 μm) with

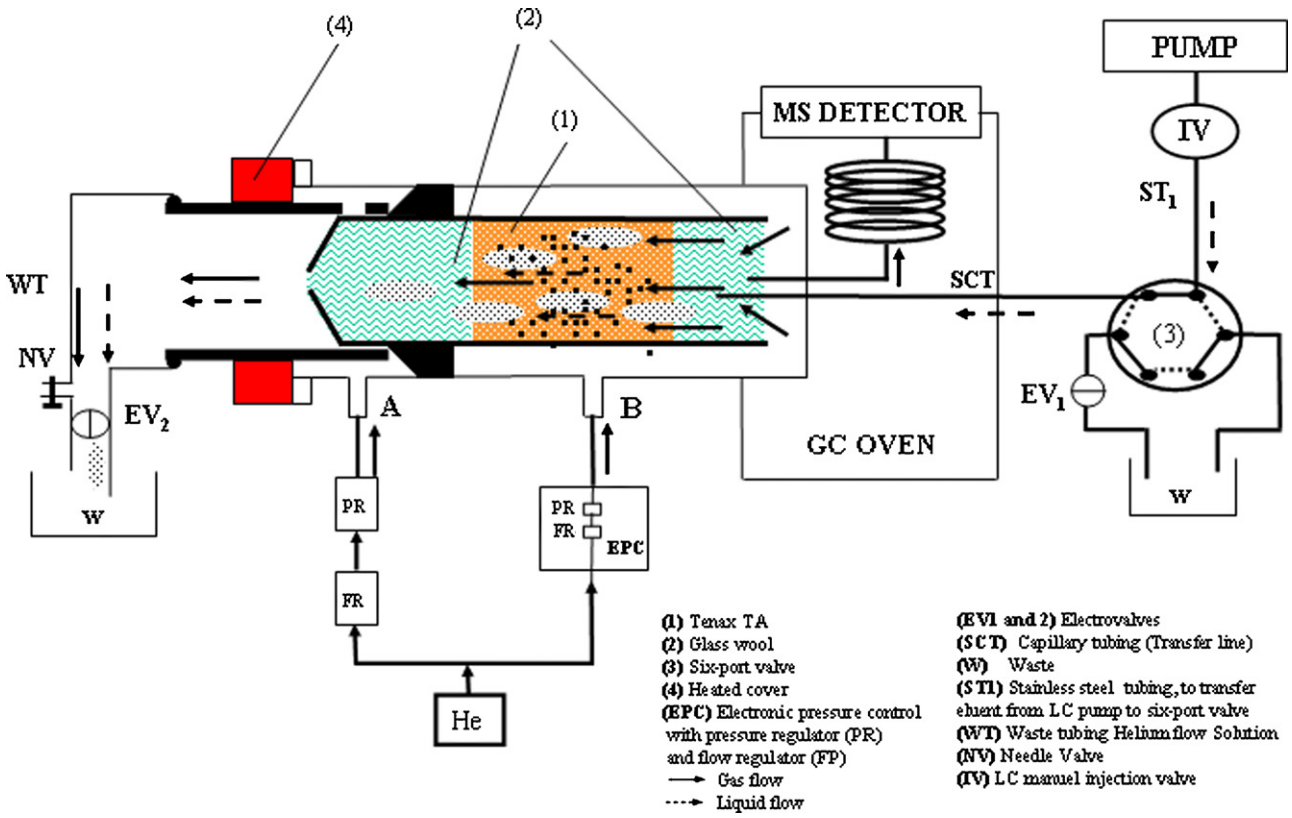

Fig. 1. Scheme of the TOTAD interface during the injection step.

helium as carrier gas at a flow rate of 1 ml/min. The analytical conditions of GC–MS were: interface temperature, 250 °C; ion source temperature, 150 °C; MS operating in full scan mode from 50 to 500 u, to select the characteristic ions corresponding to each compound analyzed (Table 1).

3. Results and discussion

3.1. General considerations

The operation parameters of the TOTAD interface were fixed as indicated in Section 2.3. *TOTAD Operation Mode*, to totally eliminate the water. Before GC was coupled to MS and to verify the complete elimination of solvent, a FID, which is water resistant, was employed. It was observed that there was no solvent peak in the GC chromatogram obtained (results not shown). The capillary used to introduce the sample in the liner and the GC column are both introduced in the liner on the oven side, although the capillary is introduced deeper than the GC column. A helium flow pushes the water to the Tenax, which is on the opposite side of the GC column (see Fig. 1), thus preventing the introduction of water in the GC column. Only the water retained in the Tenax or the glass wool of the liner enters the GC column, which, after the remaining solvent elimination step, is a very low amount. If water is pumped to the GC column and the MS detector, the solvent background would increase but, as can be seen in Fig. 2, this does not happen. A standard mixture of the target pesticide was analyzed by GC–MS in the full scan mode, in which the base peak and qualifier ions were chosen to achieve high sensitivity. The mass spectrum of each compound was characterised in selected-ion monitoring mode. Three product ions were selected among the more abundant and chosen for quantification. For positive identification, both retention time and the presence of at least three characteristic ions (indicated in Table 1) in the correct ratio were necessary. This ion ratio criterion is consistent with that used by other regulatory bodies for GC–MS SIM data [22]. It can be observed from the chromatogram obtained by LVI-GC–MS full scan mode performed with 5 ml injection volume of pesticides in water at 50 μg/l concentration level (Fig. 2) that the pesticides showed good resolution. Other authors who analyzed pyrethroid in water samples by stir-bar-sorptive extraction followed by liquid desorption and LVI-GC–MS indicated that a larger sample volume than 20 μl led to increased solvent background and therefore a lower signal-to-noise ratio [23]. This was not the case using the present method. There is no problem with the volume injected because water is totally eliminated by the TOTAD interface before GC analysis, so that a volume as large as 5 ml could be injected with no problems.

LVI is an effective technique that allows the introduction of hundreds of microlitres, while maintaining chromatographic quality [1]. The TOTAD interface is an injection system which allows the injection of volume much larger than other systems with good repeatability and precision [24]. LVI is a simple and efficient way of increase the sensitivity of the method. A 100-fold higher sensitivity can be obtained injecting 100 μl of a sample compared with the injection of 1 μl of sample [25]. Nevertheless, the low levels of pesticides allowed in drinking water, 0.1 μg/l for each pesticide and 0.5 μg/l for their sum, according to EU directives [26], means that authors have to use different extraction systems, such as solid phase extraction (SPE) [25], stir-bar-sorptive extraction (SBSE) [27] or QuEChERS [28]. In a previous work, our research group used the TOTAD interface to directly inject water samples as large as 1 ml into a capillary GC [11]. The technique was applied to the analysis of pesticide residues in standard solutions and in real water samples from the Ebro River (north-east of the Iberian Peninsula). The water sample was injected with no sample preparation other than simple filtration. An NPD was used and its great selectivity

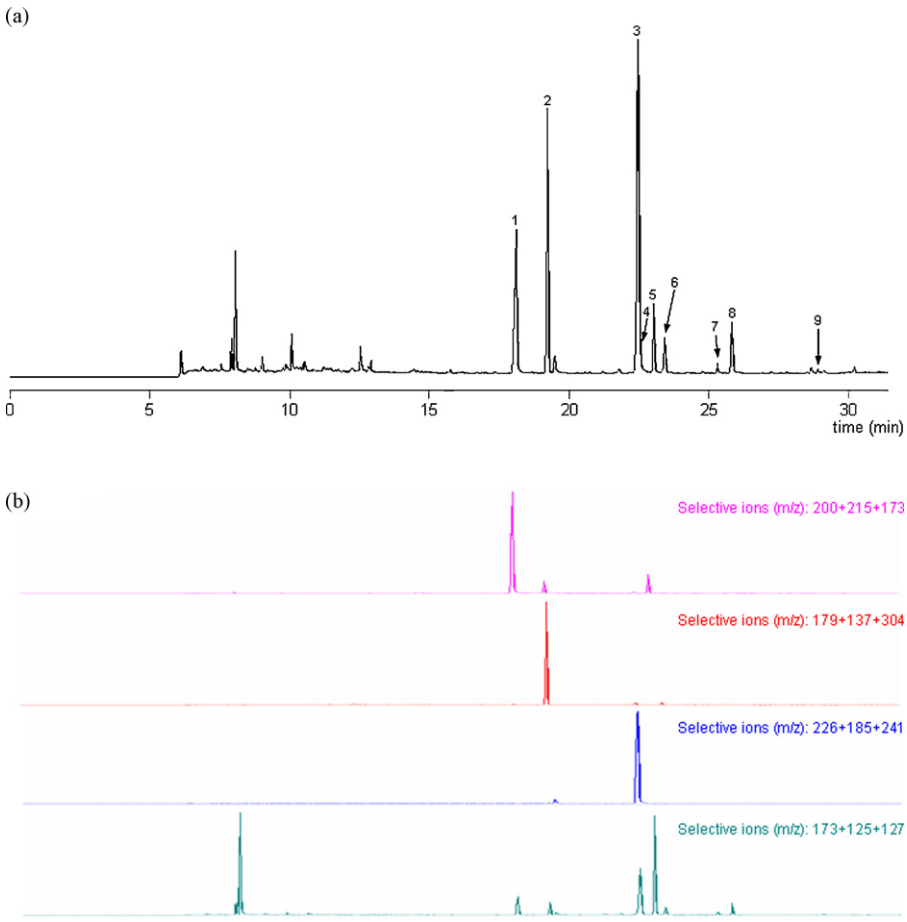

Fig. 2. Chromatograms obtained by LVI-GC–MS a water sample spiked at 50 μg/l of each pesticide: (1) atrazine, (2) diazinon, (3) terbutryne, (4) fenitrothion, (5) malathion, (6) parathion, (7) phenthoate, (8) methidathion and (9) DDT. Volume injected 5 ml. (a) Full scan mode and (b) SIM mode used for pesticide quantification for atrazine; diazinon; terbutryne and malathion.

provided good sensitivity. In the present work, an MS detector has been used because of its ability to identify the analytes. The MS detector has lower sensitivity than an NPD, so that a higher volume of water sample must be injected to achieve the necessary sensitivity. The huge volume injected does not cause any problem because water elimination, which occurs in evaporative and not evaporative mode, is almost complete before GC analysis, as can be observed in Fig. 2.

3.2. Validation of the method

Validation of the developed method was evaluated by estimating its the sensitivity, linearity and precision.

Table 1 presents the validation parameters of the developed method. The limits of detection (LODs) for an injection volume of 5 ml were calculated as the amount of product giving a signal equal to five times the background noise. The LODs ranged from 0.02 to 0.77 μg/l. The LODs fulfill the international regulatory directives on water quality for most target pesticides but not for parathion, phenthoate and DDT. Further experimentation is necessary to reach the necessary sensitivity for these pesticides by optimizing the transfer parameter, adjusting the MS conditions or injecting a larger volume of sample. The repeatability, calculated as the relative standard deviation (RSD) of the absolute areas of five replicate analysis of spiked water sample at 50 μg/l of each pesticide, was satisfactory, with values ranging from 4.7 to 19.0. It should be emphasised that

Table 1
Retention time (t_R) and selected ions (m/z) for the analytes investigated under the experimental conditions described in this study. Ions were selected by the inspection of the fragment of each compound. Relative standard deviation (RSD) from the absolute peak area and from the retention time ($n = 5$) when 500 μl of water spiked at 50 μg/l was injected. Correlation coefficient (R^2) from 25 to 500 μg/l of each pesticide. Detection limits (LOD) when 5 ml of spiked water at 50 μg/l was injected.

Pesticide	t_R (min)	m/z	RSD (area)	RSD (t_R)	R^2	LOD (μg/l)
Atrazine	17.95	200; 215; 173	8.6	0.04	0.99	0.05
Diazinon	19.14	179; 137; 304	5.7	0.04	0.99	0.02
Terbutryne	22.31	226; 185; 241	4.7	0.05	0.99	0.02
Fenitrothion	22.4	277; 260; 125	10.0	0.05	0.99	0.08
Malathion	22.91	173; 125; 127	16.3	0.05	0.96	0.07
Parathion	23.32	291; 97; 109	9.3	0.05	0.99	0.12
Phenthoate	25.19	274; 121; 125	19.0	0.06	0.96	0.73
Methidathion	25.75	145; 85; 93	18.3	0.07	0.97	0.04
DDT	30.09	235; 237; 165	10.8	0.05	0.99	0.77

values correspond to the overall method because no pre-treatment steps are necessary. Five point calibration plots using a least-square linear regression were constructed, with the concentration of each analyte ranging from 25 to 500 μg/l. Good linearity was observed.

3.3. Analysis of water samples

The developed analytical method was successfully applied to the analysis of pesticide residues in water samples from a deep channel and from an underground well. No pesticides were found in any case.

4. Conclusion

The TOTAD interface allows a volume up to 5 ml of water to be injected in GC, using an MS detector. Water is fully eliminated, while pesticides are retained and analyzed, with good chromatographic performance. Given that it is possible to inject such a large amount of water in GC–MS, it is clear that the TOTAD interface will make it possible to use an MS detector for the LVI of polar solvents and for the on-line coupling of RPLC–GC.

LVI-GC coupled to an MS detector proved to be an efficient and easy method for the determination of pesticide residues in clean water samples since the water only has to be filtered and injected into the GC–MS. The method presents good linearity, repeatability and sensitivity. However, further optimization is needed to ensure reliable determination at the MRLs established by European legislation. The method is suitable for the determination of pesticide in regulatory laboratories. The analysis of dirty water samples would require the use of an on-line LC–GC–MS system. Further research is being carried out in our laboratory in order to develop an analytical method suitable for such dirty water samples.

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