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Large volume injection in gas chromatography using the through oven transfer adsorption desorption interface operating under vacuum

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ABSTRACT

The present work describes a modification of the Through Oven Transfer Adsorption Desorption (TOTAD) interface, consisting of coupling a vacuum system to reduce the consumption of the helium needed to totally remove the eluent for large volume injection (LVI) in gas chromatography (GC).

Two different retention materials in the liner of the TOTAD interface were evaluated: Tenax TA, which was seen to be unsuitable for working under vacuum conditions, and polydimethylsiloxane (PDMS), which provided satisfactory repeatability as well as a good sensitivity. No variability was observed in the retention times in either case. Solutions containing organophosphorous pesticides in two different solvents, a polar (methanol/water) and a non-polar (hexane) solvent, were used to evaluate the modification.

The vacuum system coupled to the TOTAD interface allowed up to 90% helium to be saved without affecting the performance.

1. Introduction

In the field of analytical chemistry, new methods are constantly being sought to reduce the quantity of sample used, simplify preparation of the sample and increase sensitivity, while minimizing the use of toxic solvents that are considered harmful to the environment. Most of the analytical methods that use gas chromatography (GC) involve previous sample preparation, which usually includes extraction and concentration steps. These steps, besides being time-consuming and needing large amounts of solvents, are the principal sources of error in the analytical process [1] hence the need for new methods that minimize these inconveniences.

Large volume injection (LVI) in GC and the direct coupling of liquid chromatography and gas chromatography (LC–GC) permit such sample preparation steps to be substantially reduced (less time and lower solvent consumption), while providing more reliable and sensitive results. LVI increases sensitivity and simplifies sample preparation since it avoids the extract concentration step where analytes loss are prone to occur [2], and even the need for an extraction step if large volumes of sample are injected without prior preparation [3,4]. The direct coupling of LC–GC, besides permitting large volumes of sample or extract to be injected (the volume injected in LC is much higher than is normally injected in GC) leads to effective cleaning due to the great separation power of LC. For that reason, LC–GC coupling is suitable for the analysis of complex samples in which interferences must be eliminated before analysis by GC, while LVI is more suitable when the samples or extracts have a high degree of purity.

Whatever the case, the same difficulty is shared by LVI and the coupling of LC–GC: elimination of the large volumes of solvent – sample or extract solvents in the case of LVI and the LC eluent in the case of LC–GC – while retaining the analytes for transfer to the GC column. Several interfaces have been developed that permit the introduction of large volumes of sample, extract or LC eluent in GC. On-column interface, described by Grob [5] and later replaced by the Y-interface developed by Bierdermann and Grob in 2009 [6], and the loop-type interface are based on retention gap techniques [7]. On-column interface seem unsuitable for the LVI of polar solvents and for RPLC–GC because these eluents show poor wettability of the retention gap. A partial solution was proposed by Grob and Li [8], using an azeotropic mixture [7]. The loop-type interface does not require good wettability of the retention gap but its applicability is limited to high-boiling analytes [9]. Programmed Temperature Vaporizing (PTV) has become the most popular interface (described by Abel [10] and

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developed by Vogt et al. [11,12]), the sample is injected into a liner placed inside a vaporizer. Several parameters must to be optimized and the optimization process is time-consuming and tedious. PTV can also be used for the LVI of polar solvent and for RPLC–GC but only high boiling compounds can be analyzed.

Our research group was responsible for developing the Through Oven Transfer Adsorption Desorption (TOTAD) interface, first described in 1999 by Pérez et al. [13] and used for direct coupling of liquid and gas chromatography working in normal phase (NP) [14–16] and RP [17,18] in LC step and for LVI of sample or extract in the GC [19–21]. The TOTAD interface consists of a PTV injector, which has been substantially modified, with two electrovalves and a six port valve [22]. A retention material, usually Tenax TA, is placed inside the glass liner. Eliminating the solvent (carried out in a partial solvent evaporation mode) implies using more helium than is normally required in a GC operating conventionally, which represents a drawback because of the scarcity of helium reserves and its cost. Hence, the need to reduce the consumption of helium in three of the five steps involved in the TOTAD operation, where its consumption is particularly high: stabilization, transfer (or injection) and the remaining solvent elimination step [23].

Flores et al. [24] evaluated the use of absorbents as retention material inside the liner for coupling LC–GC. With a PTV as interface and with the column connected and disconnected in each analysis, they compared the performance of the absorbents with that of commonly used adsorbents. The absorbents evaluated were polydimethylsiloxane (PDMS) and poly (50% phenyl–50% methylsiloxane) (OV-17), both on Volaspher A2, and the adsorbents were Tenax TA and Gaschrom. These authors concluded that the use of absorbents, especially PDMS, was a good alternative since it presented advantages over the use of the most commonly used adsorbents. Subsequently, the same authors, Flores et al. [25] compared the performance of Tenax TA with that of the absorbents PDMS and OV-17 as retention material inside the liner for pesticide residues in olive oils by direct coupling LC–GC. The obtained results showed that PDMS provided the best sensitivity and selectivity.

The aim of the present work was to substantially reduce the consumption of helium by carrying out the three above mentioned steps of TOTAD operation at a reduced pressure by connecting a vacuum pump to the interface. The vacuum system would favor solvent evaporation and so reduce the helium needed for its elimination. System performance in vacuum conditions was evaluated by the LVI of standard solutions of pesticides in a polar and an apolar solvent.

2. Materials and methods

2.1. Materials

The organophosphorous pesticides used were diazinon, methylchlorpyrifos, fenitrothion, chlorpyrifos, parathion, phenthoate, chlorfenvinphos and ethion, all of which were supplied by Chem Service Inc. (West Chester, PA, SA). The methanol, water and hexane used as eluents were HPLC grade from Pestican (LabScan, Dublin, Ireland).

The retention materials used inside the glass liner of the TOTAD interface were Tenax TA, 80–100 mesh (Supelco, Madrid, Spain) as adsorbent material, and 50% (w/w) PDMS (Sigma-Aldrich, Madrid, Spain) in Volaspher A2 80-100mesh (Merck, Darmstadt, Germany), as absorbent. These materials were selected because they involve two different retention mechanisms and they have previously been used with this interface. The glass liner of the TOTAD interface was packed with 1 cm of retention material between two plugs of glass of wool to keep it in place. The retention material in the liner was conditioned under a helium stream, increasing the temperature by 50 °C 10 min^{-1} to reach 300 °C, and maintained for 60 min at this final temperature.

Individual solutions of each of the pesticides were prepared in methanol or hexane at 1000 mg L^{-1}, and subsequently used to prepare solutions of 1 mg L^{-1}. Solutions were stored at 4 °C.

2.2. Instrumentation

The analyses were carried out using a 4000B Konik gas chromatograph with flame ionization detector (FID) equipped with a TOTAD interface (US patent 6,402,947 B1, exclusive rights assigned to KONIK-Tech, Sant Cugat del Vallés, Barcelona). A vacuum pump (KNF Neuberger GmbH, Laboport, Freiburg, Germany) was connected to the waste tubing (WT) (Fig. 1). A manual injection valve (model 7125 Rheodyne, CA) with a loop volume of 500 μL was used to inject the solutions. A ternary LC pump (model Konik 560) was used to push the high volume of solutions into the TOTAD interface. For data acquisition and processing the Konikrom 32 program (Konik, Sant Cugat del Vallés, Barcelona) was used.

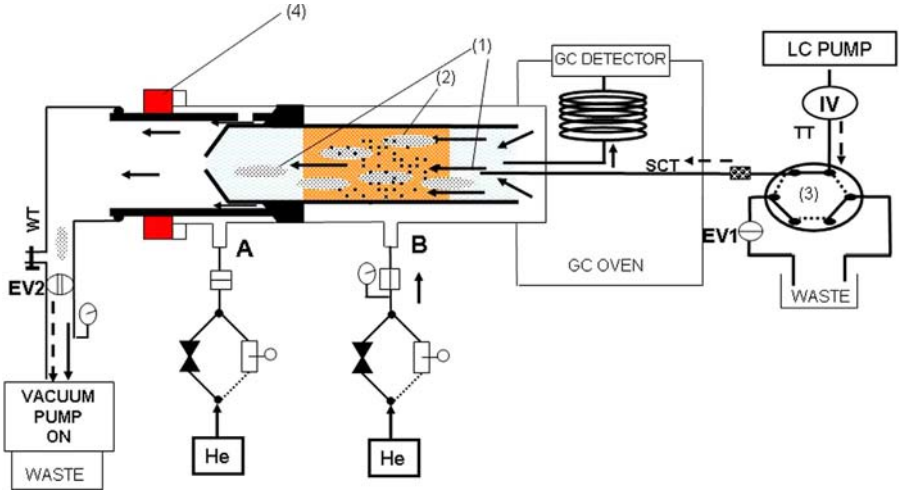

Fig. 1. Scheme of the TOTAD interface with vacuum pump coupled to WT during injection step. Symbols: (1) glass wool; (2) retention material; (3) six-port valve; (4) heated cover; (SCT) silica capillary tubing, 0.32 mm i.d.; (WT) waste tubing; (TT) transfer tubing; (IV) LC manual injection valve; (⊖) electro valve; (⟶) gas flow; (- - -▸) liquid flow; (⊢○) pressure regulator; (▩) filter; (▬) needle valve; (⋈) restrictor; (▭) opening–closing valve; (◔) pressure gauge.

2.3. TOTAD operation mode

The TOTAD operation mode involves the steps detailed below.

2.3.1. Stabilization

The initial temperatures of the TOTAD interface and GC oven were 80 and 50 °C, respectively. These temperatures were used for both retention materials, Tenax TA and PDMS. Different helium flow rates were tested. When the interface was connected to the vacuum pump, the helium flow entered in the liner only through of the oven side (B) (Fig. 1) at flow rates of between 40 and 250 mL min^{-1}. The opposite side (A) was closed. Electrovalve EV1 remained closed while EV2 was opened. The vacuum pump, maintained the pressure at 0.15 bar. The capacity of the pump permits the same pressure to be maintained at any of the helium flow rates. The LC pump was maintained constant at 0.1 mL min^{-1}.

2.3.2. Injection

The solutions are introduced into the LC manual injection valve. When this valve is switched, the solvent coming from the pump pushes the solutions through the transfer tube (TT in Fig. 1) to the six-port valve, which is automatically switched, introducing the solutions (0.5 mL) into the TOTAD interface through the silica capillary tubing (SCT).

The analytes are retained, and the solvent is eliminated, partially evaporated, through the WT.

2.3.3. Remaining solvent elimination

After the injection step is completed, the six-port valve is automatically switched to the initial position so that the eluent from the LC pump is sent to waste. EV1 remains closed for an additional 2 min to eliminate the solvent remaining in the glass liner. Thereafter, the helium flow through side B is shut off, and through the side A is opened. EV2 is closed, the vacuum pump is turned off and EV1 is opened for 55 s to completely eliminate the solvent.

2.3.4. Thermal desorption

After solvent elimination, EV1 is closed and helium flows enters only through side A at 1.8 mL min^{-1}.

The TOTAD interface is quickly heated to 275 °C, which is maintained for 5 min. The retained analytes are thermally desorbed and transferred to the capillary column, propelled by the helium.

2.3.5. Cleaning

After the chromatographic analysis, the valves, vacuum pump and helium flow are changed to the stabilization conditions. The interface is kept at 275 °C for 5 min to clean the retention material. The interface is cooled to 80 °C so that another analysis can be carried out.

2.4. GC conditions

The column used for chromatographic separation was a Quadrex 5% phenylmethylsilicone fused-silica column (30 m × 0.25 mm i.d. × 0.25 μm film thickness) (Weybridge, UK). Helium was used as the carrier gas at 1.8 mL min^{-1} throughout the analysis. During the transfer and solvent elimination steps the oven temperature was maintained at 50 °C. During GC analysis, the oven temperature was programmed as follows: initially 50 °C; 10 °C min^{-1}–160 °C; 2 °C min^{-1}–170 °C; 5 °C min^{-1}–230 °C; and 10 °C min^{-1}–300 °C; hold for 5 min. The FID temperature was kept at 300 °C.

3. Results and discussion

3.1. Helium consumption

To achieve a successful operation mode of the TOTAD interface under vacuum conditions, several modifications were required. The electronic pressure control (EPC) device that allows the entrance of helium flow into the interface is substituted by a pressure regulator, restrictor and open–close valve since the EPC does not work properly when the vacuum system is coupled to the WT (Fig. 1) as it is designed to work at atmospheric pressure. To

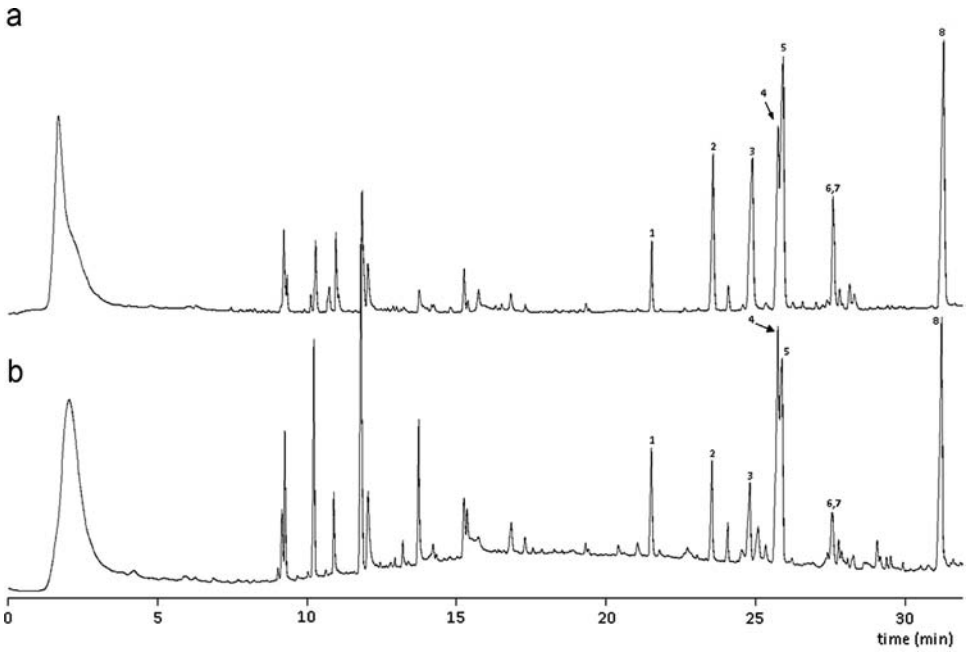

Fig. 2. (a) GC chromatogram obtained by LVI-GC-FID without vacuum conditions. Tenax TA was used as the retaining material. (b) GC chromatogram obtained by LVI-GC-FID under vacuum conditions. PDMS was used as the retaining material. Mobile phase used, MeOH/H_2O in both cases: (1) diazinon, (2) methylchlorpyrifos, (3) fenitrothion, (4) chlorpyrifos, (5) parathion, (6) phenthoate, (7) chlorfenvinphos and (8) ethion.

verify the pressure of the system, a pressure gauge is placed at the entrance and exit of the interface (Fig. 1).

The coupled vacuum system reduces the pressure inside the glass liner, which facilitates solvent elimination since its boiling point is reduced and so a lower helium flow rate can be used.

The analyses were carried out by injecting 500 μL of the solutions prepared as indicated in Section 2.1, both in hexane and methanol, and in the conditions described in Sections 2.3 and 2.4, using Tenax TA as retention material inside the liner. The flow rates commonly used when working at atmospheric pressure were first tested (250 mL min^{-1} for A and B, Fig. 1), providing the chromatogram shown in Fig. 2a. The vacuum system was coupled and the helium flow was gradually reduced, ensuring that the solvent was eliminated at the same time. As a first step in this diminution, the flow of helium through A was shut off. The chromatograms obtained in these conditions (with no flow through A) showed no increase in the solvent peak, indicating that the decrease in pressure was sufficient to prevent condensation. Solvent elimination in these conditions was adequate both in the case of apolar (hexane) and polar (methanol/water) solvents. The elimination of the flow through A implies a saving of 50% of the helium usually used.

The second step consisted of gradually decreasing the helium flow through B (250, 200, 150, 100, 50, and 40 mL min^{-1}). Solvent elimination was almost total, except at the lowest value, when the solvent peak increased, although it was still lower than when a conventional split/splitless injector was used. The conditions in which the solvent was adequately eliminated and the least amount of helium was used involved totally stopping the flow of helium through A, while reducing the flow through B to 50 mL min^{-1}. The pressure inside the liner in these conditions was 0.15 bar.

In this way the helium consumption was substantially reduced from the 500 mL min^{-1} usually used by the TOTAD interface to 50 mL min^{-1} when the vacuum system is coupled to the interface, which represents a saving of 90% helium (Table 1).

3.2. Validation of the modifications using Tenax TA as retention material

To confirm the effectiveness of the modifications made, we evaluated the repeatability of the absolute peak areas and retention times, and compared the limits of detection (LODs) obtained in the different TOTAD operation conditions.

First, Tenax TA was used as retention material inside the liner. The repeatability was evaluated by injecting the pesticide solutions (in methanol and hexane) five times in the experimental conditions specified in Section 2. The relative standard deviation (RSD) of the absolute peak areas drastically increased when the TOTAD interface was coupled to the vacuum system. In all cases the RSD (values not shown) were higher than 13%, and even exceeded 20% for four of the pesticides used. These high values indicate that the system is not repetitive since the RSD were unacceptable. This may be due to the fact that Tenax TA becomes degraded at low pressure and so loses its adsorbance properties. Indeed, when the chromatograms obtained were analyzed to determine the RSD, the analysis sensitivity gradually decreased, leading to a high degree of variability and suggesting that Tenax TA is unsuitable for use in vacuum conditions. Another retention material was therefore necessary to use inside the liner.

Table 1
Helium consumption (mL min^{-1}) of TOTAD interface during different steps, without vacuum system, and with vacuum system.

Step	Helium consumption (mL min^{-1})	
	TOTAD without vacuum system	TOTAD with vacuum system
Stabilization	500	50
Injection	500	50
Remaining solvent elimination	500	50
Thermal desorption	1.8	1.8
Analysis	1.8	1.8
Cleaning	500	50

3.3. Validation of the modifications using PDMS as retention material

Bearing in mind these results and the problems obtained when Tenax TA was used, we decided to evaluate the use of PDMS as retention material under vacuum conditions. Chromatogram is shown in Fig. 2b. When this absorbent (PDMS) was used as retention material, repeatability was good whether MeOH/H_2O or hexane were used as solvents. The RSD of the absolute peak areas were lower than 12.5%, which is similar to the values obtained in most analytical methods developed to date using the TOTAD interface without a vacuum system coupled (Table 2). We conclude, therefore, that PDMS presents no problem as far as retention capacity is concerned in successive analyses, unlike Tenax TA.

The RSD of the retention time obtained using PDMS as retention material in vacuum conditions were very low and similar to those obtained using Tenax TA at atmospheric conditions, both when MeOH/H_2O (Table 2) and hexane (Table 3) were used as solvents. The low RSD obtained (lower than 0.3% in all cases) confirms that the retention times during GC are not affected by the used of the TOTAD interface coupled to the vacuum system. However, the RSD of the retention times were considerably lower when the TOTAD interface was not connected to the vacuum system, probably because the vacuum system, which might lead to a slight pressure difference at the head of the column during chromatographic separation (see Section 3.1).

Fig. 3 shows the LODs calculated as the quantity of product that gives a signal equal to five times the noise and the standard deviation of the mean ($n=4$) is given as error bars. As can be seen, the values obtained using methanol/water as mobile phase and Tenax TA without vacuum were lower, except in the case of chlorfenvinphos and phenthoate, than those obtained using PDMS with the vacuum system. However, when hexane was used as mobile phase and PDMS in vacuum conditions, sensitivity was higher for all the pesticides except methylchlorpyrifos and fenitrothion, with LODs lower than 0.024 mg L^{-1} in all cases. It is for these reasons it can be said that PDMS in vacuum conditions

Table 2
Relative standard deviation (RSD) from the absolute peak area and from the retention time (t_R), $n=4$, for each solution pesticide, Tenax TA without vacuum system and PDMS with vacuum system. Retention times were calculated as the average of the different values. MeOH/H_2O, 80/20 was used as mobile phase. The solutions were injected at 1 mg L^{-1}.

Pesticides	RSD (area)		RSD (t_R)		Retention time (min)	
	TENAX without vacuum	PDMS with vacuum	TENAX without vacuum	PDMS with vacuum	TENAX without vacuum	PDMS with vacuum
Diazinon	3.54	10.47	0.01	0.12	21.23	21.63
Methylchlorpyrifos	9.76	11.92	0.01	0.12	23.27	23.62
Fenitrothion	12.48	7.15	0.02	0.11	24.58	24.93
Chlorpyrifos	5.11	4.68	0.02	0.11	25.43	25.87
Parathion	2.07	3.26	0.03	0.11	25.60	26.00
Chlorfenvinphos	11.90	10.57	0.01	0.09	27.26	27.71
Phenthoate	6.63	4.43	0.01	0.10	27.29	27.76
Ethion	3.12	8.21	0.01	0.12	31.94	31.43

Table 3
Relative standard deviation (RSD) from the absolute peak area and from the retention time (t_R), $n=4$, for each solution pesticide, Tenax TA without vacuum system, and PDMS with vacuum system. Retention times were calculated as the average of the different values. Hexane was used as mobile phase. The solutions were injected at 1 mg L^{-1}.

Pesticides	RSD (area)		RSD (t_R)		Retention time (min)	
	TENAX without vacuum	PDMS with vacuum	TENAX without vacuum	PDMS with vacuum	TENAX without vacuum	PDMS with vacuum
Diazinon	5.79	4.35	0.08	0.28	22.36	22.27
Methylchlorpyrifos	5.14	3.25	0.07	0.28	24.39	24.30
Fenitrothion	4.96	10.78	0.08	0.27	25.66	25.65
Chlorpyrifos	6.14	12.32	0.08	0.29	26.55	26.55
Parathion	4.48	8.39	0.10	0.28	26.75	26.71
Chlorfenvinphos + phenthoate	5.52	7.45	0.07	0.28	28.38	28.39
Ethion	4.87	6.75	0.08	0.23	31.91	31.96

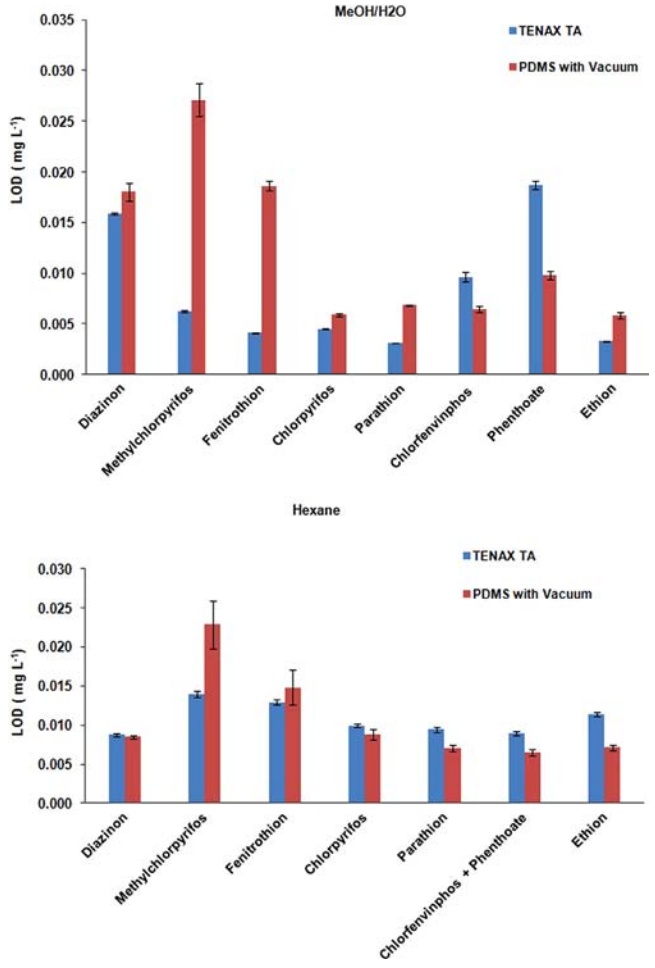

Fig. 3. LOD calculated as the amount of product giving a signal equal to five times the background noise: (a) Mobile phase used, MeOH/H_2O and (b) Mobile phase used, hexane. Error bars denote standard deviations.

provides similar or even slightly greater sensitivity than that obtained using Tenax TA at atmospheric pressure (which are the conditions generally used in the analytical methods developed using the TOTAD interface with LVI in GC or coupling LC–GC).

The fundamentals of analyte retention differ considerably between adsorbents such as Tenax TA and absorbents such as PDMS. Interactions among the analytes and the surface of the porous material are involved when adsorbents are used as retention material. In this case, the affinity of the analytes for the adsorbent, as well as the solubility in the solvent to be eliminated, play an important role. In contrast, a distribution mechanism is involved when absorbents are used, in which the differences between the solubility of the analytes in the absorbent and in the solvent to be eliminated is the main factor to be considered in this case. These differences would explain the apparently random differences in sensitivity found when one retention material or the other is used.

4. Conclusion

Coupling a vacuum pump to the WT of the TOTAD interface permitted the helium flow used in the steps of the analytical process that involve high helium flow rates to be reduced from 500 mL min^{-1} to 50 mL min^{-1} – a saving of 90%. Tenax TA was seen to be a no-suitable retention material when a TOTAD interface is connected to a vacuum system. However, PDMS showed good behavior in terms of sensitivity and repeatability. The ability to evaluate different retention materials under vacuum conditions and its behavior with a variety of analytes, solvents and eluents in LC show the great versatility of the TOTAD interface.

Acknowledgments

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