

Review Article

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TOTAD interface: A review of its application for LVI and LC-GC

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Abstract: Large volume injection (LVI) in gas chromatography (GC) and online liquid chromatography-gas chromatography (LC-GC) are useful techniques for analyzing the compounds present at very low concentrations in complex samples since they substantially increase the sensitivity of the analysis and simplify sample preparation. LVI avoids the need to concentrate the extract and even the extraction step itself by directly injecting the sample. In online LC-GC, the liquid chromatography (LC) step acts as the sample preparation and/or fractionation step. The main problem in both techniques is the selective elimination of the large volume of solvent without losing the analytes. The TOTAD (through oven transfer adsorption–desorption) interface, based on a widely modified PTV (programmed temperature vaporizer) injector, allows large volumes to be injected into the gas chromatograph using both nonpolar and polar solvents, including water. Consequently, online LC-GC can be carried out whether the LC step is in the normal phase or the reversed phase. Various methods for analyzing trace compounds in food and environmental samples have been developed for LVI and online LC-GC using the TOTAD interface. Such analysis methods require the optimization of several variables common to LVI and online LC-GC and specific variables involved in online LC-GC, which must be optimized by taking into account the nature of the analytes and the characteristics of the sample matrix. This article reviews how each of these variables affects the performance of the analysis.

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1 Introduction

When the volatile compounds in a given sample are to be determined, the most sensitive technique and the one with the greatest separation capacity is GC. However, in most cases, the sample cannot be introduced directly into the gas chromatograph but requires a series of previous sample preparation steps, which, in general, involve an extraction process followed by concentration of the extract, of which a fraction is finally injected into the gas chromatograph. One way to simplify the analysis is to directly inject the extract, thus eliminating the concentration step, but without decreasing the sensitivity. This can be done by injecting large volumes of extract, for which an injector that allows LVI is necessary. Different techniques for LVI in GC have been developed that enable volumes ranging from several microliters to a few hundred microliters to be introduced into the GC system [1–3]. Water samples can be injected directly into the GC, but special conditions are necessary [4] because water can cause hydrolysis of the siloxane bonds of the stationary phase of the chromatographic column, and water is not compatible with some detectors that are frequently used in GC, e.g., mass spectrometry (MS) [5]. Unfortunately, most samples, e.g. oil, cannot be injected directly into the gas chromatograph because it would damage the GC column [6]. Some authors have injected diluted oil solutions (10%) directly and prevented the bulk of the matrix from entering the GC column by back-flushing after each analysis [7,8]. However, the injection of nondiluted fat or oil will cause peak broadening, changes in the retention time, and the deterioration of the GC column only after a few analyses, so that the bulk of the oil must be removed, meaning that the sample preparation step cannot be avoided [9]. Such preparation steps are tedious and time consuming and are the main sources of analytical errors. However, the extraction step

can be replaced by an LC step, which provides a much cleaner extract since the separation capacity of LC is much greater than that of conventional sample extraction procedures. When LC and GC are physically connected, providing a multidimensional chromatographic system, the first dimension of the system (the LC) acts as extraction, clean-up, and/or fractionation step, and the second dimension (the GC) acts as the analytical step. In online LC-GC, the selected fraction from the first dimension is transferred to the second dimension (heart-cutting). The entire fraction of interest eluted from the LC is automatically transferred to the GC, making the process much faster and more reliable since it is automated. It is also more sensitive since the volume of sample or extract that can be injected in LC is much greater than that can be injected in GC [10]. By coupling two chromatographic techniques that operate with very different or independent separation mechanisms, the resulting multidimensional system can achieve a high degree of orthogonality [11] although it is not a simple operation because the chromatographic systems operate in different physical states, so vaporization of the LC solvent is necessary before introduction into the GC. Because of the nature of the eluent used in the reversed phase LC (RPLC), online reversed phase liquid chromatography-gas chromatography (RPLC-GC) is more difficult than online normal phase liquid chromatography-gas chromatography (NPLC-GC), which uses organic solvent as eluent in the LC step. Online LC-GC methods have been applied for the analysis of food, environmental, biological, and other complex samples, and several reviews have been published devoted to the different applications [12–16]. To carry out this coupling, it is necessary to use an interface which allows the LC fraction containing the compounds of interest to be isolated and transferred to the GC. It is also necessary to be able to remove the solvent without losing the compounds of interest, and to focus the target analytes in the head of the GC column in order to avoid peak broadening and to obtain good separations in the final analytical step (GC) [13].

LVI injectors can serve as the interface for online LC-GC and several LC-GC interfaces have been developed over the years. The transfer techniques involved have been extensively reported in several reviews [17,18], with on-column [19–21], loop type [22–25] and vaporizer interfaces [26–30] being used more frequently. The on-column interface is based on retention-gap techniques involving partially or fully concurrent eluent evaporation, but, since good wettability of the solvent is required, it is not suitable for online RPLC-GC. In the loop type interface, the LC fraction is collected in a loop, from which the

carrier gas propels it to the GC column. Although this interface does not require good wettability, the large amount of vapor is released and the high temperature needed to evaporate the polar solvents limit its use to online NPLC-GC [13]. Some interfaces using a packed vaporizing chamber do not present these problems, the programmed temperature vaporizer (PTV) interface being the most widely used [14,18]. A brief overview of the PTV interface is given below since TOTAD is a PTV-based interface.

The PTV injector has been used as interface since 1992 [31] by many authors [32–34] and some modifications have been proposed [14,35–38]. LVI using the PTV injector and its operation mode have been widely described in the literature [5,34]. The PTV injector has also been used as the interface in online LC-GC by some authors [38]. LVI with PTV can be achieved in various modes, including splitless injection [39], vapor overflow [40], and solvent split [41]. In solvent split injection, the sample is introduced at a lower temperature than the boiling point of the solvent, and the solvent vapor is eliminated through the split exit, while the analytes are retained in the material packed inside the liner. Once the solvent is eliminated, the split exit is closed, the PTV is heated, and the analytes are transferred to the GC column [17,42]. Señoráns *et al.* [43] transferred the liquid from the LC column to the GC by means of a transfer line directly inserted into the PTV-injector septum. The helium flow rate was established to optimize solvent elimination by evaporative and nonevaporative modes with the GC column disconnected during the elimination step. Once the solvent has been totally eliminated, the GC column is connected again, the PTV is quickly heated, and the analytes are transferred to the GC column. This procedure allows large amounts of aqueous solvent to be eliminated [44,45] but has the drawback that the column has to be manually removed during the online transfer step, and it is impossible to automate the process. To overcome this problem, a PTV injector was greatly modified, thus affecting the pneumatics, sample introduction, and solvent elimination in the design of the TOTAD interface.

2 TOTAD interface

The TOTAD interface, first described by Pérez *et al.* in 1999 [46], allows the injection of large volumes of polar and nonpolar solvents and online LC-GC, with LC in normal or reversed phase. The TOTAD interface allows the injection of much larger volumes than other injection

systems, while maintaining good chromatographic conditions. The TOTAD interface and its operation mode are explained in this section.

Figure 1 shows a scheme of the LVI-GC or online LC-GC system with different steps of the TOTAD interface operational mode. As can be seen, the TOTAD interface physically connects the liquid chromatograph and the gas chromatograph. In LVI, there is no LC column into the HPLC chromatograph, and the sample is injected into the HPLC injector loop, while the LC pump propels the whole sample to the TOTAD interface. Nevertheless, in online LC-GC, a separation process takes place in the LC column and only the selected LC fraction is transferred to the TOTAD interface. A modified PTV injector forms the body of the interface, which contains a liner filled with an adsorbent or adsorbent packing material (1) held by glass wool at both ends (2). The output of the liquid chromatograph is connected to a six-port valve (3), which is connected to waste through two of its ports, one of them containing an electrovalve (EV1). Another port is connected by a silica capillary tube (CT) to the body of the interface through the gas chromatograph oven, at the point in a PTV injector where only the GC column is situated. The GC column is connected at a lower depth than the CT, which is attached to the six-port valve. At the opposite end of the body of the interface, where a PTV injector contains the septum and the sample is introduced, is the waste tube (WT), where another electrovalve (EV2) serves to evacuate gases and solvent vapors. The body of the interface has two gas inlets, the usual carrier gas inlet of a PTV injector (A), and the split exit of a PTV injector (B), which, in this case, is also used as a gas inlet.

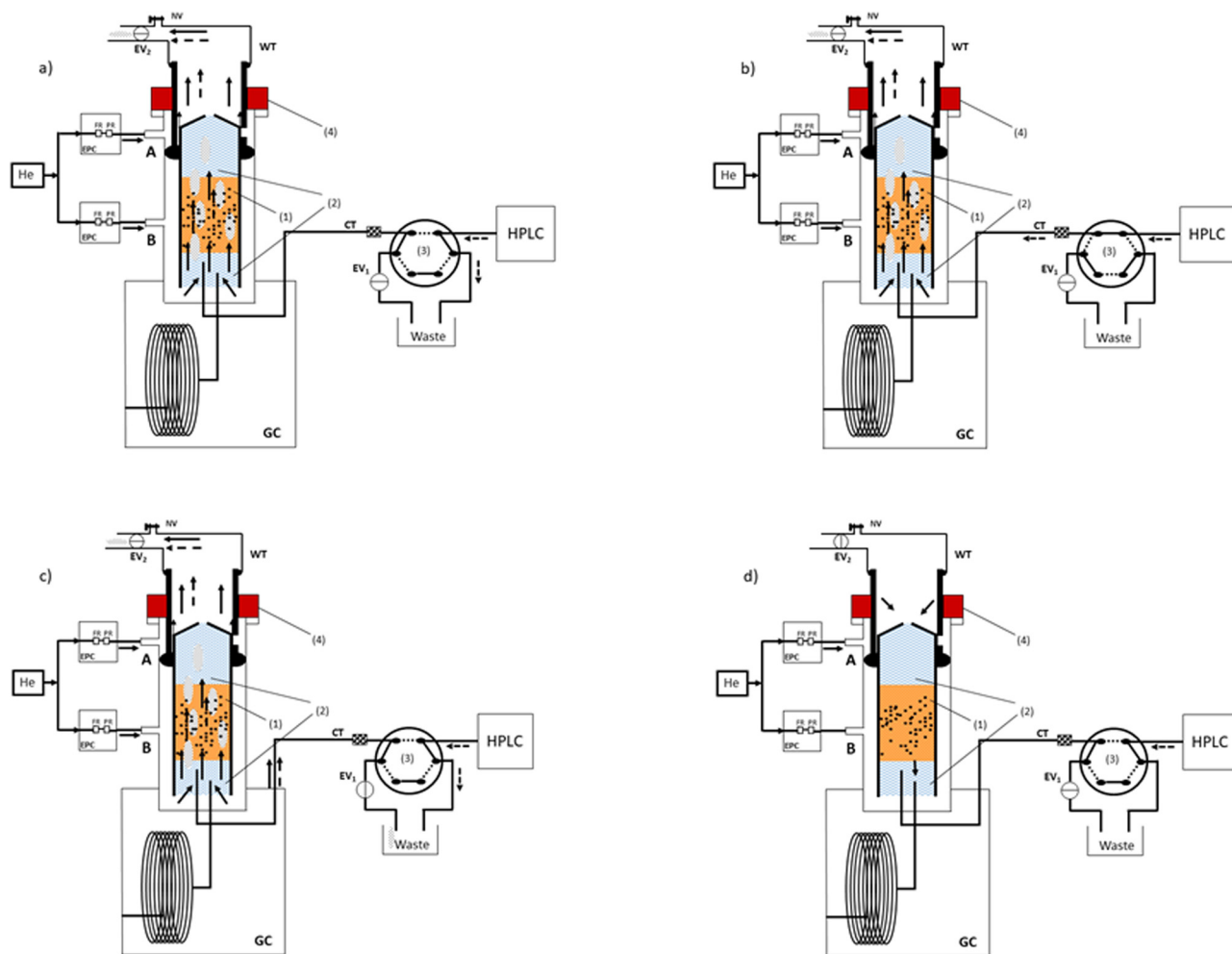


Figure 1: Scheme of the TOTAD interface during different steps: (a) stabilization, (b) injection (in LVI) or transfer (in online LC-GC), (c) solvent elimination, and (d) desorption step. Symbols: 1, sorbent (Tenax TA); 2, glass wool; 3, six-port valve; 4, heated cover; EV1 and EV2, electrovalves 1 and 2; EPC, electronic pressure control; PR, pressure regulator; FR, flow regulator; solid arrows, gas flow; dotted arrows, liquid flow; CT, silica capillary tubing, 0.32 mm i.d.; WT, waste tubing; W, waste; ●, solvent; ••••, analytes; NV, needle valve.

The operation mode of the TOTAD interface includes five steps: stabilization, injection (in LVI) or transfer (in LC-GC coupling), elimination of the remaining solvent, and thermal desorption and cleaning.

The sample is injected into the manual sample injector of the HPLC chromatograph, and the aforementioned mentioned steps occur.

- (a) *Stabilization.* In LVI, the manual sample injector is maintained in the load position. In online LC-GC, the sample injector is changed to inject position and the sample is introduced into the LC column. The eluent from the LC is sent to waste through the six-port valve of the TOTAD interface. The body of the interface and GC oven are stabilized at a fixed temperature, and the electrovalve EV1 is closed and EV2 is opened (Figure 1a). The carrier gas enters the body of the interface through two gas inlets described previously. Part of the gas entering through inlet B goes to the GC column, but most of it passes through the packing material inside the liner and exits to waste through the WT along with the gas coming from inlet A, which circulates outside the liner.
- (b) *Injection (in LVI) or Transfer (in online LC-GC).* In LVI, the manual sample injector is changed to inject position (injection). In online LC-GC, when the fraction of interest reaches the six-port valve (3), it is automatically switched (transfer). The LC pump flow is changed to the optimum transfer flow rate, whereby the eluent is sent to the body of the interface (Figure 1b). The retention material in the liner retains the analytes, while the solvent is propelled by the gas, which enters through B and passes through the liner before being removed through the WT. The gas entering through inlet A prevents condensation of the solvent in the rear part of the body of the interface. It is important that the CT into the liner is placed deeper than the GC column, as this prevents the entry of liquids into the GC column. Solvent venting takes place in both evaporative and nonevaporative modes, while the temperature of the body of the interface and GC oven is kept low.
- (c) *Elimination of the remaining solvent.* Once the analytes contained in the injected sample (in LVI) or in the LC fraction transferred (in online LC-GC) are retained in the liner. To prevent the solvent remaining in the CT capillary from entering the GC column, this remaining solvent must be eliminated before heating the body of the interface. It is very important to completely eliminate the solvent remaining inside the CT because, if some remains in this capillary, the solvent is vaporized when the oven temperature is increased

for the GC analysis and the vapor would enter into the GC column. During this step, the six-port valve is switched and the electrovalve EV1 is opened, so that the gas pressure inside the body of the interface propels the solvent in the CT to waste (Figure 1c). At the same time, on the opposite side of the body of the interface, the carrier gas evaporates and pushes the solvent residue to waste through the WT.

- (d) *Desorption.* Once the remains of the solvent have been removed, the electrovalve (EV2) is closed, and then the gas inlet (B) is closed, so that the gas enters only through inlet A, which is the usual gas inlet of a PTV injector, and exits only through the GC column (Figure 1d). Once the system has stabilized under these conditions, the body of the interface is heated to desorb analytes (thermal desorption), which are propelled by the carrier gas to the GC column, where they are separated and analyzed. It should be mentioned that, during stabilization and injection or the transfer steps, the carrier gas flows in the opposite direction. Desorption is performed by backflushing, which results in maximum trapping of volatiles while simultaneously recoveries of heavy analytes are improved.
- (e) *Cleaning.* When the GC analysis has finished, valves and carrier gas flow return to the initial position (Figure 1a), and the body of the interface is heated to a high temperature (300°C to 350°C) and maintained (from 1 to 5 min) under carrier gas flow (cleaning) to minimize or even prevent memory effects. The body of the interface is then cooled down, and another analysis can be carried out.

Several modifications have been made in the TOTAD interface since the initial model was developed into the one available nowadays on the market (US patent 6402947 B1, exclusive rights assigned to Gibnik Analytical Solutions S.L., Barberá del Valles, Barcelona, Spain). Some of these modifications are strictly technical to improve the performance of the system, but it is worth noting two of these modifications. The first one is the possibility of using a fraction collector. On many occasions, it is necessary to analyze several different LC fractions (multiple heart cutting). In some cases, such as oil or water sample, there is no problem with the quantity of sample available for the analysis, and each of the fractions can be analyzed by injecting the sample as many times as there are fractions to analyze. However, sufficient amounts of samples are not always available, especially in biological samples such as urine or plasma. In these cases, to detect all compounds in one run, it is of

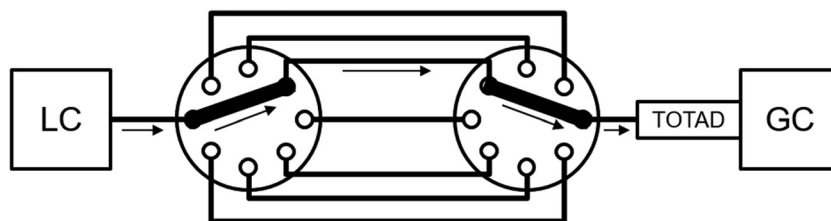


Figure 2: Scheme of the fraction collector used in online LC-GC using the TOTAD interface, when more than one LC fraction is to be analyzed.

great interest to have a system to store the LC fractions for subsequent GC analysis, so that all LC fractions can be analyzed with a single injection of the sample into the LC injector. To isolate these fractions and store them for subsequent GC analysis, a fraction collector was designed; this consists of two multiway valves joined by means of stainless steel tubes, each tube being of a length and diameter that means its internal volume is equal to the volume of the LC fraction to be stored in the tube [47]. The fraction collector is placed between the HPLC and the six-port valve, and it is controlled through the software. Figure 2 shows a scheme of the fraction collector.

The second modification to highlight is that made to allow a derivatization reaction to be carried out online. Hence, an additional LC injection valve is placed between the HPLC chromatograph and the six-port valve to automatically introduce the derivatization reagent. Once the analytes are retained in the packed material inside the liner, the derivatization reagent is propelled to the liner by the HPLC eluent. The derivatization takes place in the packed material inside the liner of the TOTAD interface after the LC separation and before the GC analysis [48].

3 Development of analytical methods

Having explained the operational mode of the TOTAD interface, some aspects and variables that need to be taken into account in the development of analytical methods are detailed in this section. Bearing in mind that the final analysis is a GC analysis, the analytes of interest should be volatile and thermostable; otherwise, a derivatization process is needed either before (pre-analysis) or online [18]. It should be noted that the TOTAD interface allows online derivatization [48]. The first thing we have to consider is whether to use LVI or online LC-GC in the new method. Both techniques have high sensitivity

because the quantity of analyte that reaches the GC detector is much higher than when 1 or 2 μL are injected. However, while LVI allows any quantity to be injected, this is not the case with online LC-GC since the LC step limits the volume that can be injected due to the loading capacity of the LC column. Conversely, in LVI, any possible interferences contained in the sample are also concentrated, while such interferences can be eliminated in the LC step in online LC-GC. Consequently, nonvolatile substances that can alter the system may be introduced in LVI, but in online LC-GC, they are eliminated in the LC step. In short, LVI is suitable for determining analytes that are in very low concentrations in matrices that are not very complex, while online LC-GC is suitable for determining low concentrations of analytes in complex matrices; hence, the nature of the matrix determines the technique to be used. It would be unreasonable to use online LC-GC if LVI can be used, as online LC-GC combines two chromatographic techniques, which is more complicated than LVI. Table 1 summarizes the main aspects to be taken into account when deciding whether to use LVI or online LC-GC.

Figure 3 represents a flowchart that can help the user to make this decision. Bearing in mind that in both techniques the analytes are concentrated in the TOTAD interface, they can be used both when high sensitivity is required and the analytes are presented at a trace level. However, online LC-GC must be used when the sample matrix is complex and an efficient cleanup or fractionation of the components of the sample is required; otherwise, LVI is recommended.

Whether LVI or online LC-GC is chosen, both present the same difficulty: the introduction of large volumes in GC [49]. In the case of online LC-GC, it is the large volume of eluent coming from the LC, and, in the case of LVI, the large volume of extract or sample. The critical problem is the large amount of solvent vapor resulting from the expansion of the large volume of injected solvent.

In the development of analytical methods involving LVI or online LC-GC, several experimental variables must be optimized for each particular analysis, the value of

Table 1: Comparison of LVI and online LC-GC using the TOTAD interface

LVI in GC	Online LC-GC
High sensitivity	High sensitivity
No limitation in the injected volume	The injected volume is limited by the capacity of the LC column
Polar and nonpolar solvents can be used	NPLC and RPLC can be used
Concentration takes place inside the TOTAD interface	Concentration takes place inside the TOTAD interface
Low concentration of the analytes	Low concentration of the analytes
Neither clean-up nor fractionation occurs	Clean-up or fractionation occurs in the LC step
Both interference compounds and analytes concentrate in the interface	Most interference compounds are eliminated in the LC step
Nonvolatile compounds can be introduced	Nonvolatile compounds are eliminated in LC step
No complex matrix	Complex matrix

which will depend on the nature of the analytes and the sample matrix and will allow the sensitivity of the method to be adjusted to that required in each particular case and, at the same time, to obtain good analytical parameters (repeatability, linearity, etc.). It should be pointed out that some of these parameters are interdependent, and often a compromise has to be considered.

Several LVI and online LC-GC methods for analyzing compounds of differing chemical natures (pesticides, minor components, contaminants, steroids, chiral compounds) in different matrices (food, environmental samples, human urine) have been developed using the TOTAD interface. Tables 2 and 3 present the value of

the variables used in some of the methods developed by LVI and online LC-GC, respectively.

4 Common variables to be optimized in LVI and LC-GC

When developing an analytical method, there are a number of experimental variables common to both LVI and online LC-GC, which must be considered. These variables are those that affect the retention of the analytes and the removal of the solvent, regardless of the nature of the solvent in which the analytes are presented (sample, extract, or eluent from LC). Optimization of a large number of experimental variables involved in the PTV performance and their combination have been studied [50]. These variables also determine the performance of the TOTAD interface and include the adsorption temperature (interface temperature during the transfer from LC to GC or during LVI), the flow rate during sampling or transfer, the desorption temperature, the injected volume, the retention material inside the liner, the length of the said material, the flow of the inert gas, usually helium, which crosses the liner, and the flow of inert gas outside the liner that prevents condensation of the solvent.

4.1 Adsorption temperature

Starting with the adsorption temperature, which is the temperature of the interface during the transfer from LC to GC or during LVI, there are two possibilities: to use high temperature or low temperature. With high temperature (60–150°C), solvent evaporation is favored. Most of

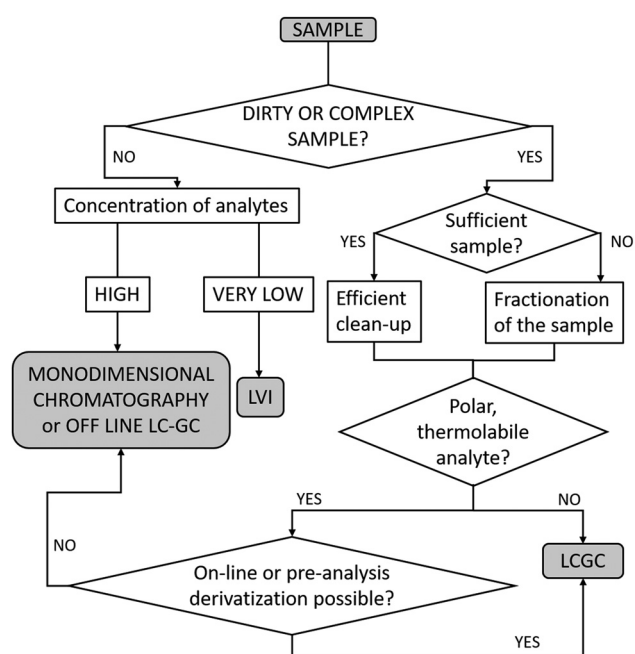


Figure 3: Flowchart to decide when to use LVI or online LC-GC. Modified from Figure 1 of ref. [18].

Table 2: Values of variables used in the analytical methods developed by LVI in gas chromatography using the TOTAD interface

Analyte/sample	Injected volume (μL)	Injection flow (mL min^{-1})	Propelled solvent	Packing material	Adsorption temperature ($^{\circ}\text{C}$)	Desorption temperature ($^{\circ}\text{C}$)	Gas flow (mL min^{-1})	Ref.
OPs*/vegetables	20, 40, 50, 60, 80, and 100	0.1, 0.2	Ethylacetate	Tenax TA	100	275	500 A, 500 B	[64]
OPs, OCs*, triazines/ water	500/not specified	0.1	Water	Tenax TA	80	275	500 A, 500 B	[82]
PAHs*/standard solution	20, 40, 60, 80, and 100	0.1	Methanol	Tenax TA	80	275	He: 500 A He: 150–1,000 B N ₂ : 500 A N ₂ : 100–1,500 B 40–250	[67]
OPs/standard solution	500	0.1	Methanol:water, hexane	Tenax TA, PDMS	80	275		[66]
Diclofenac/water	100	0.1	Water:acetonitrile	Tenax TA	125	325	300 A, 300 B	[54]

* OPs – organophosphorus pesticides; OCs – organochlorines pesticides; PAHs – polycyclic aromatic hydrocarbons.

the solvent evaporates, but a small quantity of solvent is eliminated as liquid, so elimination occurs in evaporative and nonevaporative modes. Although the temperature in the injector is programmed to a given value, the temperature at the point where evaporation occurs is lower, since evaporation of the solvent requires the input of heat (the heat of vaporization), which lowers the temperature at the point where evaporation occurs, that is, in the retention material inside the liner. The process is mainly evaporative, and so it is suitable for analytes of low volatility, since very volatile compounds would be removed along with the solvent. In 1999, a study was published that included, among other parameters, the influence of adsorption temperature on the sensitivity of an analysis of a standard solution of pesticides in acetonitrile by LVI using a PTV [37]. The optimum temperature for most of the pesticides tested was between 60°C and 120°C . These conditions were applied to the determination of pesticide residues in olive oil by online LC-GC using the TOTAD interface [51]. Figure 4 shows the LC and GC chromatograms obtained. The temperature of the interface body during the transfer was 100°C . High adsorption temperatures of 125°C and 150°C were also used to analyze sterols [48] and waxes [52] in edible oils, respectively. For the enantiodifferentiation of a mixture of γ -lactones (compounds of relatively low volatility), an adsorption temperature of 75°C was used [53].

Under high temperatures conditions, it is better to transfer or inject at a low flow rate, and in most applications, a flow rate of 0.1 mL min^{-1} has been used [51,54]. The nature of the retention material inside the liner has little influence on the sensitivity.

However, below 60°C , the process is essentially one of absorption or adsorption depending on the packing material used inside the liner. In this case, the retention material inside the liner has a strong influence. As in the case of high temperatures, it is a partially evaporative process, although the percentage of solvent that evaporates during the transfer is relatively small. The process that takes place inside the liner is similar to a solid-phase extraction (SPE). The temperature inside the liner is lower than the set temperature because of the heat of vaporization of the solvent. These conditions are suitable for highly volatile analytes [18], which, at high temperatures, would evaporate together with the solvent. For instance, for the determination of methyl jasmonate, a compound of medium volatility, in commercial jasmine essence, the temperature of the interface was set at 50°C [55], while for the analysis of chiral volatile compounds in strawberries [56] and polychlorinated biphenyls (PCBs) in transformer oils [57], the temperature was set at 40°C .

Table 3: Values of variables used in the analytical methods developed by online LC-GC using the TOTAD interface

Analyte/sample	Injected/ transferred volume (μL)	Transfer flow (mL min^{-1})	LC eluent	Packing material	Adsorption temperature ($^{\circ}\text{C}$)	Desorption temperature ($^{\circ}\text{C}$)	Gas flow (mL min^{-1})	Ref.
Parathion/river water	50/400	1	Methanol	Tenax TA	80	250	900 A, 1,800 B	[46]
Pesticides/river water	50/1,400	0.1	Methanol:water	Tenax TA	80	250	900 A, 1,800 B	[72]
OPs*, OCs*, triazine/olive oil	20/600	0.1	Methanol:water	Tenax TA	90	250	1,500 A, 1,500 B	[63]
OPs, OCs, triazine, carbamate/olive oil	20/2,200	0.1	Methanol:water	Tenax TA	100	250	1,500 A, 1,500 B	[73]
OPs, triazine/olive oil	20/3,600	0.1	Methanol:water	Tenax TA	100	300	500 A, 500 B	[51]
Unseparable compounds/edible oils	20/1,600	0.1, 1 and 2	Methanol:water	Tenax TA	80	325	500 A, 500 B or 1,600 B	[58]
Pesticides/olive oil (grown plot part I)	20/2,000	0.1	Methanol:water	Tenax TA	100	–	1,500 A, 1,500 B	[76]
Pesticides/olive oil (part II)	20/2,000	0.1	Methanol:water	Tenax TA	100	300	500 A, 500 B	[77]
OPs, OCs and triazine/olive oil	20/1,300	0.1	Methanol:water	Tenax TA	80	275	500 A, 500 B	[78]
(+)-methyl-epijasmone/lemon	20/750	0.3	Methanol:water	Tenax TA	40	200	Not specified	[79]
OPs and OCs/nuts	50/700 and 1,400	0.1	Methanol:water	Tenax TA	125	275	500 A, 500 B	[80]
Methyl jasmonate/aromatic samples	20/450–650	0.1, 0.3; 0.5, 2	Methanol:water	Tenax TA	50	200	100, 300, 400, 500	[55]
Pesticides/lycopene and carotenoids tomato extracts	20/not specified	0.1	Acetonitrile	Tenax TA	100	275	500 A, 500 B	[81]
Ethyl 2-methylbutanoate, linalool and 4- hydroxy-2,5-dimethyl-3(2H)-furanone/ strawberries	20/600	0.2	Methanol:water	Tenax TA	40	200	300	[56]
Irradiated compounds/dry-cured Ham	250/1,500	0.1	Methanol:water	Tenax TA	75, 100	250	200 A, 200 B	[83]
Wax esters/edible oils	20/3,000	0.5	Hexane:Ethylacetate	Tenax TA	150	350	500 A, 500 B	[52,84]
Chiral compounds/essential oils	20/up to 1,900	0.1	Methanol:water	Tenax TA	75	225	200 A, 200 B	[75]
Total sterols content/edible oils	2.5/6,000	0.5	Hexane:Ethylacetate	Tenax TA	125	350	500 A, 500 B	[48]
Steroids/human urine	50/700–2,200	0.1	Acetonitrile:water	Tenax TA	125	325	500 A, 500 B	[47]
Free and esterified sterols/edible oils	20/1,000	0.1	Methanol:water	Tenax TA	125	300	500 A, 500 B	[85]
PCBs*/transformer oils	20/not specified	0.2	Methanol:water	Tenax TA	40	275	500 A, 500 B	[57]
Radioalytic hydrocarbons/low fat containing food	20, 100, 250/2,170	0.1	Methanol:water	Tenax TA	100	250	200 A, 200 B	[60]
Enantiomeric analysis/mentha essential oils	20/300–810	0.1	Methanol:water	Tenax TA	75	225	200 A, 200 B	[86]
Ethyl esters (ethyl butyrate \rightarrow ethyl dodecanate)/standard solution	20/300	0.1	Methanol	Tenax TA	50	250	100–1,000	[87]
Stereochemical analysis of oak lactone/ oak extract	250/720	0.1	Methanol:water	Tenax TA	75	280	100 A, 100 B	[53]
Racemic γ -lactones/standard solution	250/720	0.1	Methanol:water	Tenax TA	75	290	200–1,000	[65]

When different variable values were tested, the underlined value was that chosen.

*OPs – organophosphorus pesticides; OCs – organochlorines pesticides; PCBs – polychlorinated biphenyls.

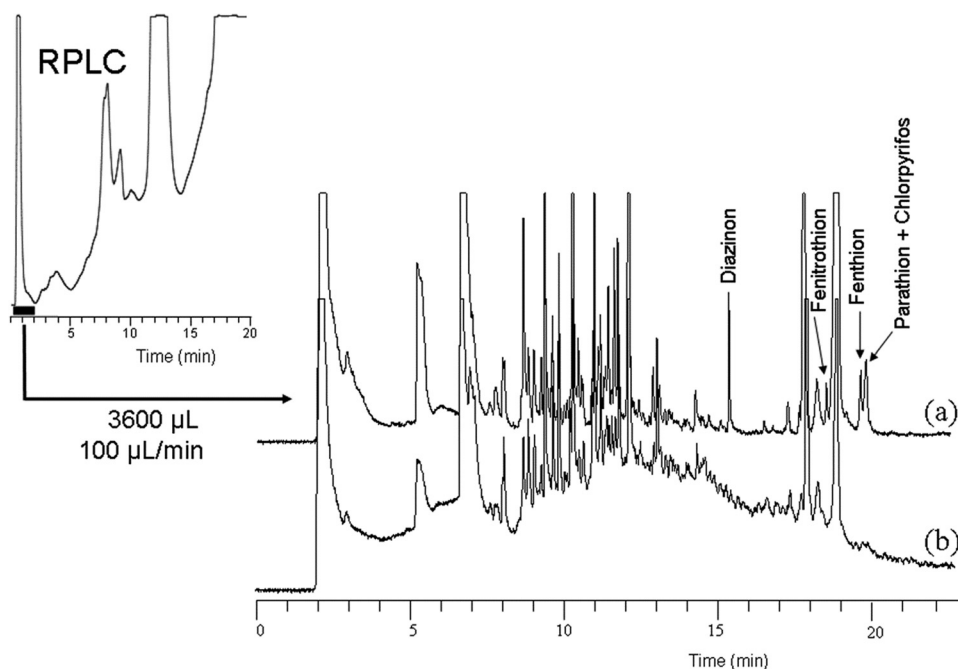


Figure 4: Liquid and gas chromatogram obtained from the online LC-GC-FID analysis of (a) an olive oil sampled spiked with a mixture of pesticides at 1 mg L^{-1} and (b) an unfortified olive oil sample. The thick line in the LC chromatogram indicates the LC fraction transferred from the liquid chromatograph to the gas chromatograph. Reprinted from ref. [51] with permission from Jennifer Diatz (Director of Publications, AOAC International).

Methanol:water was used as a mobile phase in the LC step in all these determinations. Under these conditions, it is better to use higher transfer or injection flow rates. Flores et al. reported that in the analysis of methyl jasmonate, a transfer flow rate of 0.3 mL min^{-1} provided double the peak areas of those obtained at 0.1 mL min^{-1} [55].

4.2 Injection or transfer flow rate

Another variable to take into account is the injection (in LVI) or transfer (in online LC-GC) flow rate, which, in both online LC-GC and LVI, is controlled by means of the HPLC pump. The injection or transfer flow has a great influence on the sensitivity when working at high temperatures: the lower the injection or transfer flow is, the higher the sensitivity [58]. Solvent elimination in the evaporative mode is easier if the injection or transfer flow rate used is low as the requirements for solvent elimination are easily fulfilled if the speed of introduction is close to the solvent elimination rate [37,42,59]. Figure 5 shows the GC chromatograms obtained in the online LC-GC analysis of minor compounds (free sterols, tocopherols, squalene, erythrodiol, and uvaol) in virgin olive oil using different transfer flow rates: 0.1 , 1 , and 2 mL min^{-1} . As can be observed, the lowest transfer flow leads to the highest

sensitivity. Of course, a lower transfer flow rate increases the transfer time and consequently the analysis time. In the LC chromatogram (Figure 5), the elution time of the minor compound fraction can be seen. Bearing in mind that the flow rate in the LC system was 2 mL min^{-1} , the volume of the fraction to be transferred was 1.6 mL , and so, at a transfer flow rate of 0.1 mL min^{-1} , it took 16 min to transfer the total volume. However, at the lower transfer rate, squalene, which occurs at high concentrations in olive oil, could not be quantified because the signal was saturated. However, since sensitivity decreased when the transfer flow was increased, squalene could be quantified by using a transfer flow of 2 mL min^{-1} although the rest of the sterols did not appear in the GC chromatogram (Figure 5c) because of the decrease in sensitivity. The GC chromatograms of Figure 5 were obtained when 20 µL of olive oil diluted 1:50 in 2-propanol was injected. A similar GC chromatogram to that of Figure 5a was obtained when undiluted olive oil was injected and the transfer flow was increased to 2 mL min^{-1} [58], and duration of the transfer flow rate was only 0.8 min . It is clear, then, that a higher transfer flow rate decreases the overall analysis time while maintaining the sensitivity of the analysis if the oil is not diluted. Therefore, the transfer flow rate can be used to adjust the sensitivity when the injection is carried out at high temperatures.

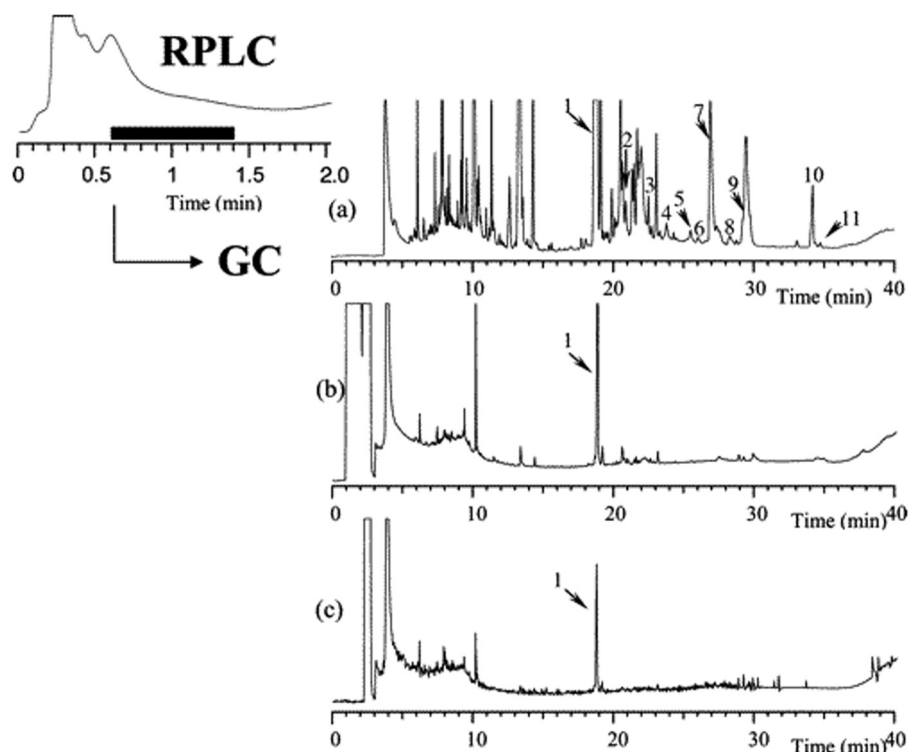


Figure 5: LC and GC chromatograms obtained in the online RPLC-GC-FID analysis of minor compounds (free sterols, tocopherols, squalene, and erythrodiol and uvaol) in a virgin olive oil using different transfer flows: (a) 0.1 mL min^{-1} , (b) 1 mL min^{-1} , and (c) 2 mL min^{-1} . The thick line in the LC chromatogram indicates the LC fraction transferred from the liquid chromatograph to the gas chromatograph. Reprinted with permission from ref. [58]. Copyright 2020 American Chemical Society.

4.3 Time and desorption temperature

The desorption temperature and time must be sufficient to achieve desorption and volatilization of the retained analytes. These values basically depend, therefore, on the volatility of the analytes in question. In most applications described to date, the TOTAD interface was heated rapidly and maintained at the final temperature for 5 min [54,57]. The desorption temperature varied from 200°C to 350°C depending on the volatility of the analytes. Martínez *et al.* applied various temperatures for different durations to accomplish the thermal desorption of the analytes (γ -lactones) retained in the interface and concluded that a temperature of 290°C held for 15 min was best for the experimental conditions [53]. The maximum temperature that the retention material can reach must always be taken into account, and it is preferable not to exceed that temperature. For instance, Tenax TA cannot be heated higher than 350°C .

4.4 Injected volume

The injected volume refers to the volume injected in the LC injector, which in the case of online LC-GC is

obviously lower than the volume transferred from the LC to the GC, which is usually of hundreds of microliters or even more. The injected volume must be selected according to the required sensitivity because the sensitivity is directly proportional to this variable.

The TOTAD interface accepts a high volume of any solvent, both polar and nonpolar because the solvent is efficiently removed, as indicated by the very low amount of solvent seen in the GC chromatograms. In the case of LVI, either sample or extract can be injected into the GC, but the presence of interfering substances limits the volume that can be injected, since such interfering substances may accumulate just like the analytes at the TOTAD interface. Figure 6 shows the chromatograms obtained during the analysis of pesticide residues in a tomato sample that was spiked with pesticides and a tomato sample collected from an experimental plot. This analysis represents a case of the LVI of an extract. The high sensitivity of the technique can be appreciated. The use of a specific detector, in this case a nitrogen-phosphorus detector (NPD), provides a fairly clean chromatogram. The ability of the TOTAD interface to efficiently eliminate the solvent allows any volume to be injected, although, in the aforementioned case, $50 \mu\text{L}$ were injected because this volume provided sufficient sensitivity. The high sensitivity

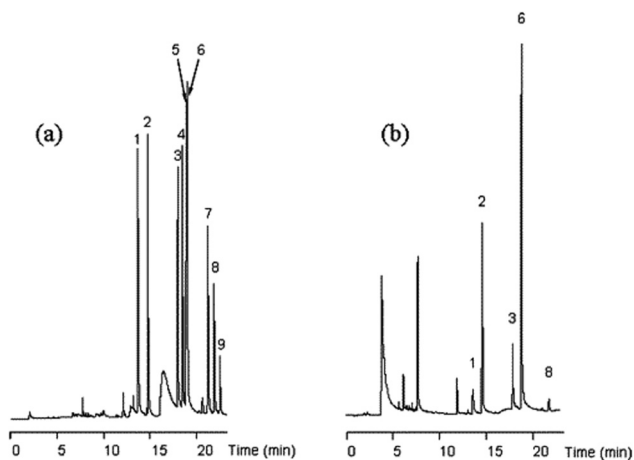


Figure 6: Gas chromatograms obtained in the LVI-GC-NPD analysis of pesticide residues in (a) a tomato sample fortified at 0.05 mg kg^{-1} and (b) a real tomato sample. Reprinted with permission from ref. [64]. Copyright 2020 American Chemical Society.

achieved made it possible to avoid a concentration step and therefore to reduce the sample size required. Thus, only 5 g of sample, 2 g of sodium sulfate, and 5 mL of ethyl acetate were used in the extraction step, and, after shaking, 50 μL of the extract was injected into the GC.

In the case of the online LC-GC, the volume injected in the loop of the LC is limited by the loading capacity of the LC column. Loops of different volumes (20, 100, and 250 μL) have been used [60]. The 100 and 250 μL volume loops enabled the authors to detect more radiolytic markers due to the evident increase in sensitivity resulting from the use of higher volume loops for introducing the sample into the HPLC system. Obviously, the volume of the LC fraction transferred to the GC is much higher and depends on the “window” of the LC fraction in which the analytes elute and the LC flow rate used in the pre-separation step, so the transferred volume is the volume of the LC fraction. For instance, in the analysis of pesticides in olive oil, the volume of oil injected into the liquid chromatograph was 20 μL , and the volume of the LC fraction transferred to the GC was 3.6 mL [51].

4.5 Nature and length of the packing material inside the liner

The packing material placed inside the liner is another variable that must be considered. The correct choice of this material is of great importance because it has to retain the target analytes while allowing the solvent to be eliminated. The packing material should be inert and thermostable. In the case of the LVI of samples

containing water and in online RPLC-GC, a water-resistant material, such as Tenax and Carbofrit, is required [36]. Glass wool has long been used as a packing material inside the PTV liner although it is difficult to deactivate and pack the liner in a reproducible way [32]. Mol et al. evaluated several packing materials to pack liners in PTV injectors and concluded that Tenax is especially suitable for volatile analytes [33]. Any adsorbent or absorbent material can be used with the TOTAD interface. In the case of absorbent materials, the analytes are retained by dissolution, while temporary bonds are formed on the surface of an adsorbent packing material. Whatever the case, the material must be chosen bearing in mind the target analytes and the sample matrix. Tenax and polydimethylsiloxane (PDMS) and poly (50% phenyl and 50% methylsiloxane) (both adsorbents) were evaluated for determining pesticide residues in olive oil [61] and terpenes in orange juice [62]. These authors recommended the use of the PDMS since it is more thermally stable. Especially at low temperatures, the nature of the retention material strongly influences sensitivity and selectivity as, in such conditions, the adsorption or absorption process predominates, while at high temperatures the packing material has little influence, since the process is, as mentioned earlier, fundamentally evaporative.

The length of the retention material inside the liner is another factor to consider. When working at high temperatures, the retention material is best if short ($\leq 1 \text{ cm}$), since the decrease in pressure that occurs when the liquid and the gas flow through it is more pronounced, which favors evaporation of the solvent. Flores et al. evaluated the effect of the amount of adsorbent material (OV-17 and PDMS) for pesticide residue analysis, using 80°C and 110°C during the transfer step, and concluded that better sensitivity was attained with a 1 cm length than with 2 cm of either material [61]. However, at low temperatures, it is better if the retention material is long ($> 1 \text{ cm}$); since the retention in the material is mainly an absorption or an adsorption process similar to solid-phase extraction, the greater the length of the material, the greater breakthrough volume.

4.6 Carrier gas flow rate

Another variable is the flow rate of helium since there are two ways for the helium to enter the interface, one whereby the gas crosses the liner and impels the liquid through the retention material inside the liner (helium enters through B) and the other whereby the helium enters through A (Figure 1), the purpose of which is to

prevent solvent accumulating in the chamber located at the end of the liner. Both helium flow rates have to be considered. However, in this case, the flow rate through B has little influence on the sensitivity and the flow rate through A has no influence.

In the first analytical method developed using the TOTAD interface, a high flow rate of $1,500 \text{ mL min}^{-1}$ was used for both inputs to ensure the total elimination of the solvent so as to avoid flooding of the GC system. This flow rate was maintained for 0.25 min before desorption of the analytes to eliminate the remaining solvent from the glass liner as well as from the CT tube [63]. Later it was confirmed that 500 mL min^{-1} was sufficient for solvent elimination, and the time to eliminate the remaining solvent was increased to 2 min [64]; however, Flores *et al.* indicated that analyte absolute areas were practically the same using 500 or 400 mL min^{-1} [55]. In the latest analytical methods developed, a flow rate of 200 mL min^{-1} has been found to be sufficient for the effective removal of the solvent [60]. One aspect to consider here is that lower flow rates decrease the cost of analysis. Martínez *et al.* applied two helium flow rates (200 and $1,000 \text{ mL min}^{-1}$) to evaluate the effect on the orthogonality of the system [65]. The authors found that, for the enantiodifferentiation of γ -lactones, the mismatch between the plug of liquid transporting the selected fraction and the helium flow rates applied can lead to differences in viscosity great enough to cause “viscous fingering” and strangely distorted peaks. However, by increasing the helium flow rate from 200 to 500 mL min^{-1} , well-shaped peaks were obtained. No other authors have described the occurrence of viscous fingering in online LC-GC. Reducing the helium used when a large number of samples are to be analyzed is important due to the low resources of helium and its high cost. In this respect, a modification of the TOTAD interface has been proposed that consists of coupling a vacuum system to reduce the consumption of the helium needed to remove the eluent [66]. Another alternative is to use nitrogen, which is cheaper and more abundant than helium, to remove the solvent (no differences were observed in the performance of the system using helium or nitrogen) and only use helium in the GC analysis step [67].

5 LC-GC coupling: Specific variables

So far, we have described the influence of the variables that affect both LVI and online LC-GC, and we now turn our attention to the variables that must be taken into

account only in online LC-GC. These specific variables are the nature of the LC eluent, characteristics of the LC column, and the selection of the fraction to be transferred from the liquid chromatograph to the gas chromatograph. Hyöttyläinen and Riekkola described the role of the LC in the LC-GC coupling and provided guidelines for choosing the LC method, but they did not include RPLC among possible methods [18].

5.1 Nature of the LC eluent

With regard to the polarity of the mobile and stationary phases, it must be first decided whether to use NPLC or RPLC.

As indicated previously, NPLC-GC coupling is simpler, since the LC eluents used have both lower boiling points and produce lower volume of vapor per unit of liquid than the LC eluents used in RPLC, which may form a solvent film inside the wall of a capillary and are also unsuitable for GC. Hence, most of the applications that have been developed use the normal phase [12,42,68]. Nevertheless, at least some 70–80% of the LC separations are performed in the reversed phase, and in the case of aqueous samples, for instance, the use of reversed phase is necessary. However, the transfer of polar solvent to GC is difficult because eluents have high boiling points and produce a very high volume of vapor per unit of liquid. RPLC-GC coupling is much more difficult than NPLC-GC coupling. However, the TOTAD interface allows the LC-GC coupling to be carried out in both normal and reversed phases as the solvent is completely eliminated.

Therefore, the choice of RPLC-GC or NPLC-GC coupling will depend on the analytes and the nature of the matrix. For instance, RPLC-GC coupling was used for the analysis of free sterols in edible oils using methanol:water (95:5) as eluent in LC. The free sterols, which are more polar than triglycerides, were eluted first, and the sterol fraction was automatically transferred to the gas chromatograph, thus avoiding the transfer of triglycerides, which would damage the GC systems [58]. Grob *et al.* have previously proposed a method to analyze free and esterified sterols by online NPLC-GC using an on-column interface, which presented this problem. To prevent triglycerides from entering into the GC column, backflush is performed after each analysis [69,70]. However, waxes, compounds of long-chain fatty acids with long-chain alcohols, are less polar than triglycerides, and, when RPLC is used, they eluted after triglycerides and the long tail of the triglyceride peaks overlap the wax

ester fraction and it is difficult to transfer the wax ester fraction without a small quantity of triglycerides. In this case, it is recommended to use the normal phase in LC, so that the wax ester fraction elutes before the triglycerides and does not overlap the triglyceride peak, thus avoiding this particular problem [47]. Grob's group reported an NPLC-GC-MS method using two different interfaces an on-column interface with concurrent solvent evaporation [71] and a loop interface [65]. Nevertheless, these methods present the problem of the variability in the GC retention time, which is not a problem with the TOTAD interface because the analytes are refocused in the packed material inside the liner [72]. Indeed, relative standard deviations of the retention time lower than 0.2 have been calculated [51,64,73].

As mentioned by Hyötyläinen and Riekkola [18], the use of gradient elution in LC is quite complicated, and it has not been used in any method developed using the TOTAD interface. However, it could be used without problem since the TOTAD interface has been demonstrated to totally eliminate any kind of solvent, polar or non-polar, regardless of the composition of the eluent.

5.2 Characteristics of the LC column

Three parameters must be considered as regards the LC column: length, stationary phase, and diameter. The length and the stationary phases are related and basically depend on the application to be developed. It must be borne in mind that in the LC-GC coupling, the LC step is not itself an analytical process but an extraction, clean-up and concentration, and/or fractionation process, so the LC column does not have to be highly efficient. If a group of compounds is to be determined, short columns with stationary phases of low retention power should be used, so that the fraction of interest containing all the compounds of interest elutes in a small volume. For example, a C4 column of 5 cm length was used to analyze unsaponifiable compounds (free sterols, tocopherols, squalene, and erythrodiol and uvaol) in edible oils [58]. The same column was used in the multiresidue analysis of pesticides in olive oil [51], and in the LC conditions used, the volume of the pesticide fraction to be transferred was 3.6 mL.

However, when it is intended to analyze several analytes in very complex matrices, high retention power stationary phases and long columns must be used to provide the necessary high separation capacity. For instance, to determine individual pesticide residues in water, a C18 column 25 cm in length was used [72].

In the analysis of endogenous steroids in urine for the analytical control of doping, it is possible to discriminate between steroids of natural or synthetic origin based on the $^{13}\text{C}/^{12}\text{C}$ ratio by GC-IRMS (gas chromatography-isotope ratio mass spectrometry). Hence, it is essential to obtain very clean peaks in GC with no impurities that would reach the IRMS detector and modify the $\delta^{13}\text{C}$ values. In addition, it is necessary to confirm the nature of the peak by GC-MS (gas chromatography-mass spectrometry). Toledano et al. developed an LC-GC-MS analysis method by using the TOTAD interface, which allowed the unequivocal identification of steroids [47]. The different testosterone metabolites were separated into different LC fractions, which were stored in a fraction collector and subsequently sent to the GC for analysis. The separation in LC of the steroids into different fractions was carried out using a 25 cm C18 column, which provided five fractions containing the nine steroids analyzed according to their concentration in the sample. This same LC column was used to discriminate between the endogenous or exogenous origin of Boldenone (Bo) and its main metabolite (BoM) in urine by LC-GC-IRMS. Bo and BoM were separated into two different fractions in LC and subsequently analyzed by GC-IRMS [74].

Another aspect of the LC column to bear in mind is the diameter. It should be remembered that in online LC-GC, the LC step acts not as an analytical separation process but as a sample preparation step, and any increase in the internal diameter of the LC column will increase sensitivity. The larger the diameter, the greater the load capacity and, therefore, the greater the sensitivity. The choice of the LC column will be a compromise between both aspects. But it must also be borne in mind that the greater the load capacity, the larger the fraction volume that must be transferred from LC to GC. In this case, since the flow rate for transferring analytes of low volatility must be low, the transfer time could be excessively long. For instance, in the analysis of pesticides in olive oil, the transferred volume was 3.6 mL; therefore, 36 min was needed for a transfer flow rate of 0.1 mL min^{-1} [58]. In most of the analytical methods developed to date, a column of 4.6 mm internal diameter has been used for analyzing compounds occurring at trace levels in complex matrices [52,57,58].

5.3 Fraction to be transferred from LC to GC

It is of great importance to establish accurately the "window" of the LC fraction containing the analytes of

interest. Although the TOTAD interface effectively removes the solvent, whether nonpolar or polar, and so there is no problem with the volume of the fraction to be transferred, the unnecessary enlargement of the window may result in introducing undesired material into the GC, thus increasing the risk of peaks overlapping and unreliable determinations [75]. The precise determination of LC fraction to be transferred into the gas chromatograph is important to obtain relatively clear GC chromatograms, which will facilitate the identification and quantification of GC peaks. Conversely, as previously mentioned, the higher the volume of LC fraction, the longer the analysis time.

The LC fraction must be determined previously by injecting high concentrations of a solution of the target analytes into the LC so that analytes can be detected

using an LC detector. For example, for pesticide residue analysis in water, a standard solution of the nine target pesticides, each at a concentration of 100 mg L^{-1} , was used to determine the LC retention time and peak widths. Methanol:water (70:30) at 1 mL min^{-1} was used as mobile phase and an UV detector was used. The beginning and end of the LC peak determined the width of the LC fraction to be transferred to the GC. The volume of the fraction to transfer depends on the composition of the eluent, and the lower the eluent strength is, the larger the volume of the LC fraction. A compromise between a satisfactory degree of separation and the volume of the fraction obtained must be considered. However, the volume to transfer from LC to GC is not a problem using the TOTAD interface, but the lower the fraction transferred,

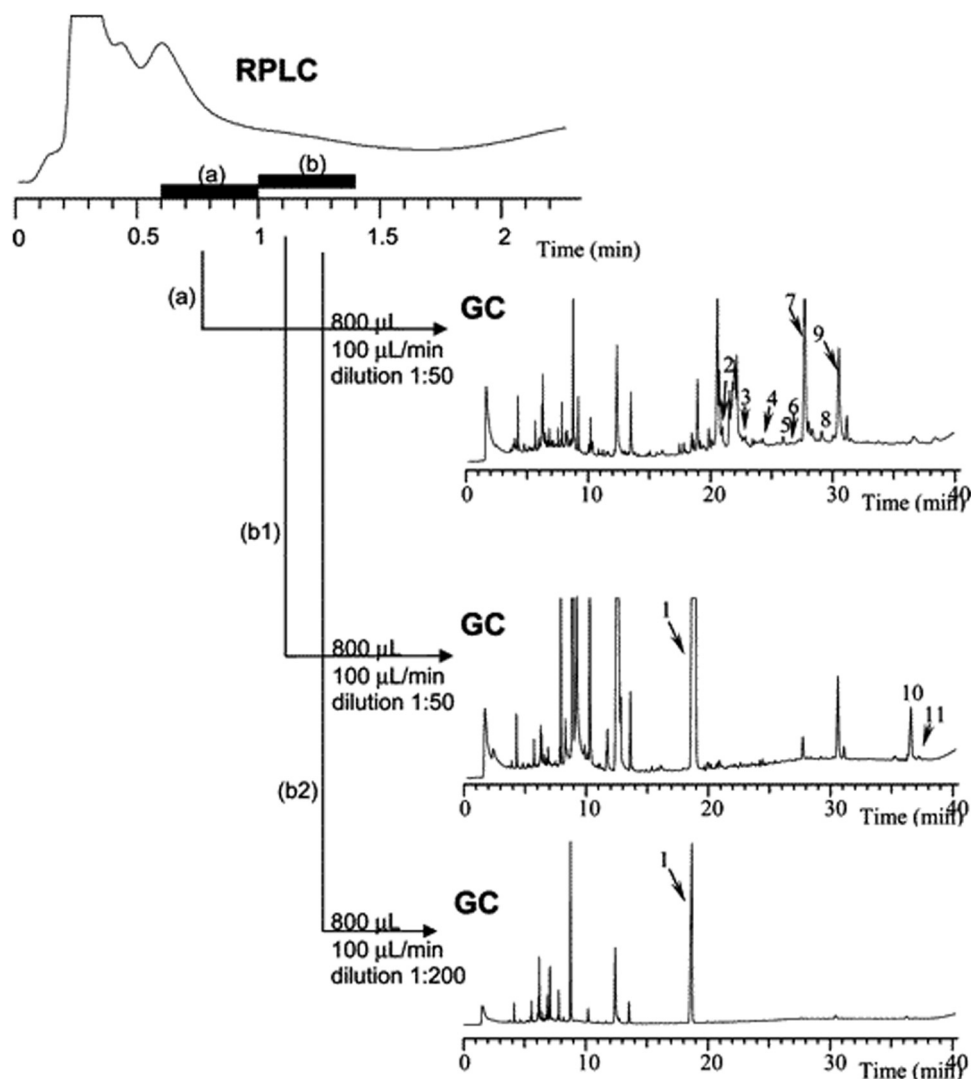


Figure 7: Gas chromatograms obtained from the direct RPLC-GC-FID analysis of the two fractions indicated in the LC chromatogram. Fraction (a) corresponds to sterols and tocopherols, and fraction (b) corresponds to squalene and triterpene dialcohols. Fraction (b) was analyzed twice: (b1) olive oil diluted 1:50 in 2-propanol and (b2) olive oil diluted 1:200 in 2-propanol. Reprinted with permission from ref. [58]. Copyright 2020 American Chemical Society.

the shorter the analysis time. For the analysis of PCBs in transformer oil by online RPLC-GC, methanol:water was used as an eluent and different proportions were tested (70:30, 80:20, 90:10, and 95:5). The ratio chosen was 90:10 since it provided a satisfactory degree of separation and a low LC fraction volume. The PCBs eluted between 0.85 and 2.35 min, resulting in an LC fraction volume of 1.5 mL when the flow rate was 1 mL min^{-1} . The flow rate during the transfer step was changed to 0.2 mL min^{-1} , and so the transfer time needed was 7.5 min [57].

In an analysis of unsaponifiable compounds in edible oils, using methanol:water (70:30) at 2 mL min^{-1} as an eluent, the volume of the LC fraction to be transferred was 18 mL, which would take at least 1.5 h to transfer into the GC at a flow rate of 0.2 mL min^{-1} during the transfer step. To decrease the LC fraction volume, the eluent composition was changed to 95:5 and the LC flow rate to 2 mL min^{-1} , and in these conditions, the LC fraction was fixed between 0.6 and 1.4 min, which resulted in a volume of 1.6 mL. Free sterols, tocopherols, squalene, and triterpene dialcohols can be determined in only one run (Figure 5). As can be seen, the squalene peaks overloaded in the experimental conditions were used. As the LC fraction can be modified to analyze compounds covering a wide range of concentrations [47,72], the LC fraction was split into two: one fraction from 0.6 to 1.0 min, corresponding to sterols and tocopherols, and the other from 1 to 1.4 min, corresponding to squalene and triterpene dialcohols (Figure 7). The two fractions were analyzed in two separate runs. To analyze both fractions, olive oil was diluted 1:50 in propanol, but under these conditions, the squalene peaks could not be quantified correctly, so the olive oil was then diluted 1:200 and the second fraction was analyzed [58].

6 Conclusion

When compounds at very low concentrations are to be determined, LVI provides high sensitivity because of the large amount of analytes that reach the detector. It can also greatly simplify the sample preparation step by avoiding the need for extract concentration, and even extraction in cases where the sample can be injected directly. Online LC-GC provides a system that simplifies the sample preparation step, thus saving time, while increasing the sensitivity and the reproducibility of the analysis. Online LC-GC is a multidimensional system that integrates sample preparation in the first dimension of the system (LC) and analysis in the second (GC). The LC

dimension presents high sample capacity, and the GC dimension presents high separation efficiency and the possibility of using a wide variety of detectors, including MS. Both techniques, LVI and online LC-GC, require an injector or an interface capable of injecting a much higher volume into the gas chromatograph than is usually injected into a GC (1 or $2 \mu\text{L}$).

The operation mode of the TOTAD interface is completely automated, so manipulation errors are avoided. However, although the TOTAD interface offers different operation modes that reinforce the analytical performance (e.g., the possibility of injecting or transferring large volume fractions regardless of the eluent used), the implementation of multidimensional systems may be difficult. One common drawback with other interfaces is that as the number of instruments increases, so does the complexity of operating the system, meaning that the analyst or user must be well trained [12]. The user of this type of system needs to thoroughly understand the mechanisms of both chromatographic instruments (LC and GC) and the interface selected.

The key factor when developing an analytical method is to optimize the multiple variables involved in the process. Some of the variables affect the performance of the TOTAD interface and need to be considered in the case of both LVI and online LC-GC. Other variables only need to be optimized when using online LC-GC and are related to the first dimension of the system. Hence, many variables may need to be optimized before each analysis, always bearing in mind the target analytes and the nature of the sample matrix. Moreover, the optimum values of some variables are not independent of the others, so sometimes optimization is a matter of compromise, which is another drawback shared with other interfaces. In the case of the TOTAD interface, the users have to consider even more parameters that must be optimized, and compromises in order, for example, to achieve full solvent elimination of polar solvents such as aqueous eluents or to avoid peak distortion as a consequence of the different viscosities of the mobile phases used in LC and GC.

Another drawback of the TOTAD interface, again shared with other interfaces that use a packing material, is that it can cause analyte losses, degradation, or even the irreversible adsorption of the analytes after several injections. Therefore, the packing material must be well conditioned before being used and the liner should be replaced regularly. Also, contamination of the packing material may cause a memory effect. In the case of the TOTAD interface, this drawback is minimized or even avoided as a result of the cleaning step, during which the body of the interface is heated under a carrier gas

flow once the GC analysis is finished. Although some analytes (mainly volatile compounds) have presented poor recoveries (less than 50%) with the TOTAD interface, this is not a problem for validating the analytical methods developed because of the good repeatability, linearity, and sensitivity that are possible. In addition, it is expected that the optimization of variables, such as adsorption and desorption temperatures and the carrier gas flow rate, will greatly improve the recovery of volatile compounds.

Two of the main advantages of the TOTAD interface over other interfaces is that it enables the injection of both nonpolar and polar solvents, even water, and consequently the online coupling of NPLC-GC, and, what is much more difficult, RPLC-GC. The other advantage is that the GC retention time does not vary.

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