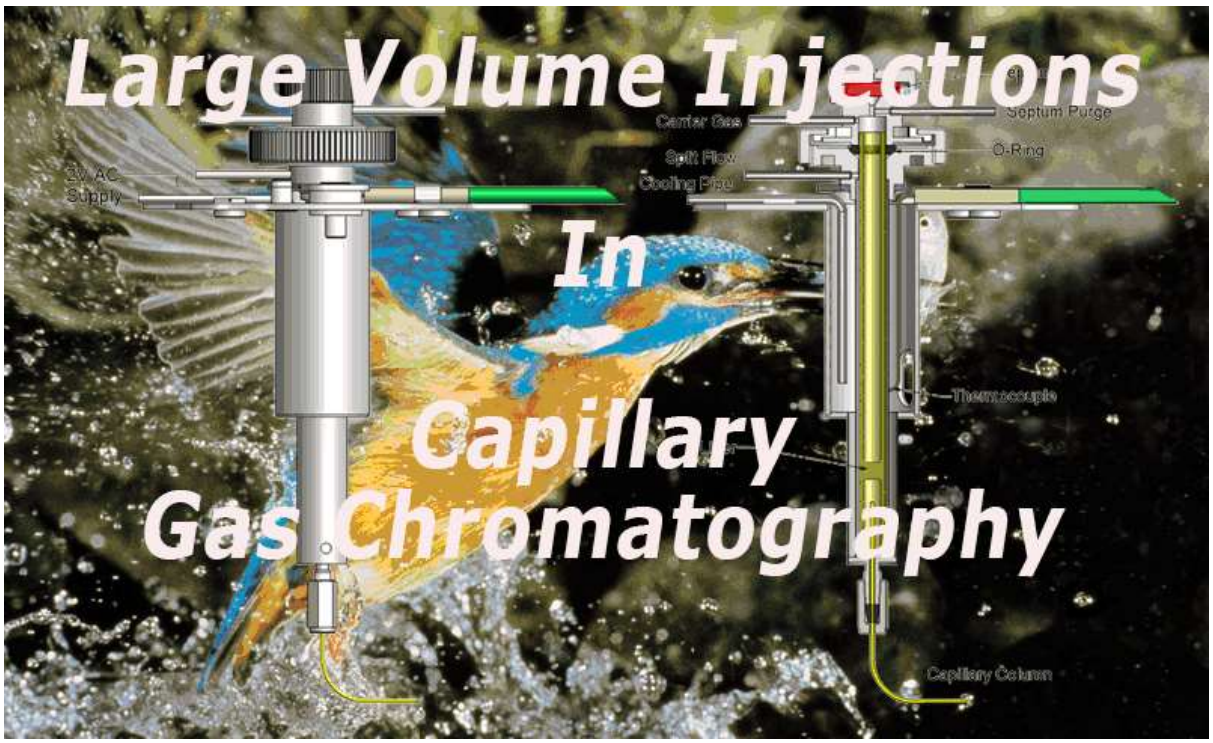
GL Sciences



1.1 Use of large volume injection

Large volume injection can also be used as an alternative for an intermediate evaporative pre-concentration step in an extraction procedure of a complex sample. This could, for example, be of advantage in the analysis of solid samples, such as soil or sediment samples or polymeric samples. The first step in the analysis of these solid samples is usually a liquid extraction. In this step a relatively small amount of the solid sample is extracted with a relatively large volume of a liquid. The extraction can be carried out in different ways, *e.g.* by shaking, sonication or in a Sohxlet extractor. Regardless of which procedure has been used, the result of the extraction is a relatively diluted extract that has to be pre-concentrated prior to injection of a 1 to 3 µL aliquot into the GC instrument. The most widely employed procedure for pre-concentration is evaporation of the solvent. Also this step in the sample preparation can be carried out in various ways. Each of these methods, however, is time-consuming. Moreover, irrespective of which method of evaporation is used, there is a serious risk that volatile components are lost in the evaporation step. Unstable solutes can decompose, especially if the evaporation is carried out at elevated temperatures. Finally, evaporative pre-concentration is difficult to automate. It will be evident from what has been stated above, that two important application areas of large volume injection are the improvement of detection limits in trace analysis and the reduction in the overall analysis time that can be obtained due to the elimination of the time consuming and laborious solvent evaporation step. A third application area of large volume sampling is encountered in the on-line combination of sample pre-treatment and chromatographic analysis. This direct combination is greatly simplified if the gas chromatograph can accept larger sample volumes than the standard microliter volumes. The advantages of on-line coupling of sample pre-treatment and chromatographic analysis are very diverse. On-line systems are easier to automate, more sensitive, more rugged, more reliable and reduce the costs per sample.

Now that the advantages of large volume injection in capillary gas chromatography have been outlined, the various techniques that have been developed for large volume injection in GC will be briefly mentioned. In principle three (groups of) techniques for large volume injection can be distinguished.

- The on-column technique for large volume injection.
- Techniques using the so-called loop-type interface.
- Methods based on the use of the programmable PTV injector.

The PTV large volume sampling method is actually not just one method. PTV large volume sampling is basically a group of large volume injection methods that share the use of a common interface - the PTV injector. The principles of the on-column approach for large volume sampling will be briefly discussed. Loop-type injection is not treated here as this approach of large volume injection offers no advantages in comparison with PTV or on-column large volume injection. The various techniques for PTV large volume injection will be described in detail in section 1.3.

1.2 On-column large volume injection

The basic principle of on-column large volume injection is very straightforward. A retention gap is installed before the analytical column. The large volume sample is than introduced directly into this retention gap using some type of an on-column injector. During introduction of the sample the temperature of the column oven is low, some 15µC below the normal boiling point of the solvent. This means that, as in the on-column injection of a standard volume, the sample is introduced in the liquid state. Discrimination or degradation is hence absent because the sample is directly introduced from the syringe into the column system without an intermediate evaporation step. It is evident that the retention gap, in order to accommodate the entire sample volume, should be sufficiently long. As a rough rule of the thumb: every microliter of sample results in an increase of the zone length of the wetted zone of roughly 20 centimetres. This means that for a 100 µL injection a retention gap of at least 20 meters is required. As the solvent plug may move along the retention gap, a safety margin of some 5 meters has to be added to this length. The use of such a long retention gap not only makes the column system expensive, it also renders evaporation very slow. Due to the long retention gap the column system has a serious flow resistance. This means that the gas flow rate through the system is low and, hence, evaporation of the solvent is slow. A second disadvantage of the on-column method for large volume injection is that in the simple set-up described above the entire volume of solvent has to be discharged from the system via the detector. Many of the more sensitive and/or selective GC detectors such as the nitrogen phosphorous detector, the flame photometric detector, electron capture detector and the mass spectrometer, however, do not tolerate the introduction of large volumes of solvent. To overcome this disadvantage, systems for on-column large volume injection are very often equipped with a so-called 'early solvent vapour exit (ESVE)'. This is an additional gas exit installed between the retention gap and the analytical column. A schematic representation of a system for on-column large volume injection in capillary GC is given in figure 1. The ESVE is opened during solvent evaporation and is closed when solvent evaporation has reached completion. An advantage of the use of an ESVE is that solvent vapours no longer have to be discharged via the detector. In this way the detector is protected from large volumes of solvent vapour. Moreover, if an ESVE is used, the back pressure provided by the column system is significantly reduced, which results in a significantly higher column flow at a given inlet pressure. Evaporation of the sample solvent is hence faster. Apart from these advantages the use of an early solvent vapour exit also has several disadvantages, these include more expensive instrumentation and the greater complexity of the system.

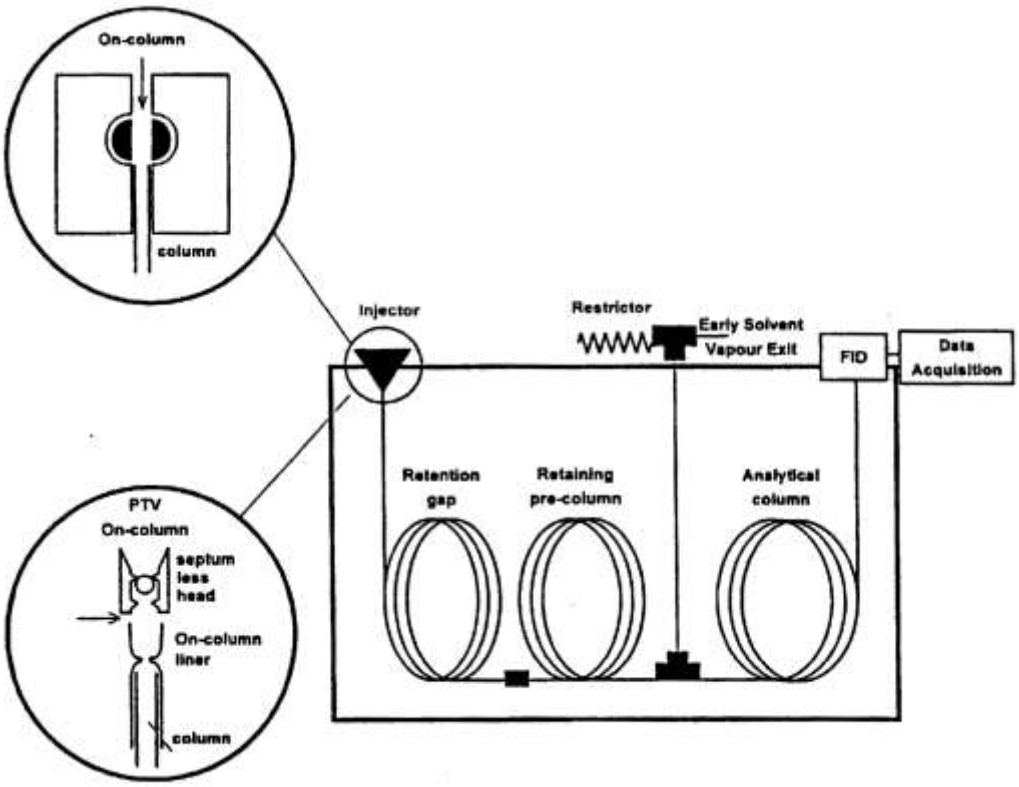


Figure 1. Schematic representation of a system for on-column large volume injection.
A: Standard system, B: System with retaining pre-column.

To avoid losses of volatile sample constituents together with the solvent, the timing of closure of the ESVE is highly critical. This timing is so critical that a system as the one described above consisting of a retention gap, an ESVE and the analytical column can not be used for daily routine analyses. To make the timing of closure of the ESVE less critical and to avoid losses of volatiles, a so-called 'retaining pre-column' has to be installed between the retention gap and the ESVE outlet. This is a short piece (approximately 2 meters) of the analytical column**.** ***This column, in contrast to the retention gap, does contain stationary phase***, it provides some separation between the wide solvent peak and the volatile components. The moment of closing of the ESVE outlet is now less critical and losses of volatile components are reduced. Still a certain minimum difference in boiling point between the solvent and the first components of interest is required. Quantitative recovery of n-C_7 in a large volume injection using hexane as the solvent is impossible. For a really reliable method a boiling point difference of at least some 35°C to 40°C is required. In previous chapters it is already stated that from the fundamental point of view, on-column injection is superior to any of the other sample introduction techniques in capillary GC. This is because with on-column injection the sample is transferred from the syringe directly into the column in the liquid state. No intermediate evaporation step is involved in the transfer of the sample. Sample loss by selective evaporation or by the absence of evaporation, as well as losses by adsorption or thermal degradation can hence be precluded.

Despite the evident advantages of on-column sample introduction one would, for practical reasons, usually try to avoid the use of this technique, even in analyses in which only small volumes are injected. Only if each of the alternative injection techniques fails one would resort to on-column injection. This because on-column injection is less rugged, difficult to automate and because column contamination by high molecular weight impurities occurs more readily than in (hot) split and splitless or PTV injection. In principle the same remarks also hold for on-column large volume injection. From the theoretical point of view, on-column large volume injection is superior to the other methods of large volume injection. For practical reasons, however, one would again often try to avoid the use of the on-column interface in large volume injection. Contamination of the retention gap readily occurs in the on-column injection of large volumes. This results in losses of adsorptive and/or unstable components and makes it necessary to replace the retention gap and to re-optimize the time of closing the ESVE. Moreover, the various column connectors incorporated in a system for on-column large volume injection each form a potential source of leakage. The short description of the on-column method for large volume injection given here is not intended to be a detailed discussion of the principles, advantages and disadvantages and applications of on-column large volume sampling. Its only aim is to give the reader an impression of the on-column large volume injection method. For a more detailed discussion of the technique as well as for further details, the interested reader is referred to literature [1-3].

1.3 Techniques for PTV large volume injection

The PTV injector is a very flexible injector that can be used for a wide range of different sample introduction techniques. Large volume injection using a PTV can be performed in five different ways. These five ways are:

1. Multiple injection
2. Direct or 'at-once' injection
3. Speed controlled sample introduction
4. Multiple 'at-once' injection
5. PTV on-column injection

The first four of the above mentioned techniques for PTV large volume injection are based on the so-called solvent vent or solvent elimination technique. With these techniques the solvent is evaporated in the liner of the injector and the solvent vapour is discharged via the split exit of the injector. Technique number five is slightly different. PTV on-column large volume injection is largely similar to the standard on-column large volume injection technique described in paragraph 1.2. The only difference is that in the PTV on-column injection the on-column injector is replaced by a PTV injector equipped with a special on-column liner. With this liner the sample can be injected directly into the chromatographic column. PTV on-column large volume sampling has the same advantages and disadvantages as the 'standard' on-column large volume injection technique. From the theoretical point of view it is an excellent

technique. For practical reasons, however, one will often try to avoid the use of the method. It is only if none of the other PTV large volume injection methods work, for example because the substances that have to be analysed are extremely volatile or highly unstable, will the PTV on-column technique be the method of choice. For day to day work, this means that the PTV injector is truly a universal interface for large volume injection. With only one injector one can perform various PTV methods for large volume sampling as well as the on-column large volume injection technique. In subsequent paragraphs the various PTV large volume sampling techniques will be discussed in more detail. Particular emphasis will be devoted to the multiple injection technique, 'at-once' injection and speed controlled sampling. As the technique of solvent venting holds a key position in each of the PTV methods for large volume injection, this technique will first be discussed in more detail in a separate paragraph. In all cases it is assumed that the solvent has a lower boiling point than the components of interest. From this it should not be concluded that PTV large volume cannot be used if this is not the case. By carefully exploiting polarity difference or differences in affinity for an adsorbent, also large volumes of samples containing components that are more volatile than the solvent can be successfully analyzed using techniques for PTV large volume sampling. This, however, is outside the scope of this article. More detailed discussions of the PTV methods can be found in literature [4-6].

1.3.1. PTV solvent elimination

In this section the basic principles of PTV solvent elimination will be briefly summarized. In the first instance it is assumed that only a limited volume of *e.g.* 1 µL is injected. With the PTV solvent elimination technique the liquid sample is injected into the 'cold' liner of the injector. The word cold here means that the temperature of the injector should be well below the boiling point of the solvent (*e.g.* at least some 15 to 30 °C below the respective solvent boiling point). At the moment of injection the split exit is open. Hence, a relatively high flow of carrier gas is flowing through the injector. A small portion of this gas flows into the column, whereas the vast majority leaves the system via the split exit. Let us take a closer look to the processes occurring in the liner during injection and shortly thereafter.

Let us assume we have an empty liner. When the plunger of the syringe is depressed, a small liquid droplet forms at the tip of the needle. This starts to evaporate, but evaporation is very slow due to the small surface area of the droplet. The droplet grows until it exceeds some critical size and than falls into the liner. It touches the wall and spreads out as a film of liquid. The droplet will not, as some people expect, fall on to the bottom of the injector. This only occurs if an excessive volume is injected (more than can be accommodated by the wall), if the injection is extremely fast or if a liner with a very large inner diameter is used (» 4 mm). After the injection the sample is present as a thin, small liquid film on the wall of the injector. Because the split line is a open, a high flow of gas is flowing across the liquid film that now will be evaporated by the carrier gas. First the solvent will evaporate, as this has the lowest boiling point. The mixture of carrier gas and solvent vapour now passes along the column inlet. A small fraction will enter the column whereas the bulk of gas will be discharged via the split exit. It is only after all of the solvent has evaporated that the other components also start to

evaporate. This process is of course slower because of the higher boiling points of these components. Just before this starts to occur the split exit should be closed. In that way the only way components can escape from the liner is via the column. If the injector is heated at this time, all of the solutes will be rapidly transported to the column. To obtain good re-focussing here, one would usually work with low initial oven temperatures. It is common practice to choose an oven temperature roughly equal to or slightly above the initial temperature of the PTV injector. The final temperature of the PTV injector should be high enough to yield rapid and quantitative transport of the components of interest within the splitless time selected. If either the final temperature of the PTV is too low or the splitless time is too short, discrimination will be observed for the high boiling sample constituents. It is extremely important that the split exit is closed before the injector is heated. In general it is advisable to wait a few seconds between the moment of closing the split exit and the start of the heating process. In this way flow disturbances that might result from the switching of the split/splitless valve can be damped out.

The PTV solvent injection is schematically depicted in figure 2. As can be seen from this figure a solvent elimination injection consists of two steps. In the first step, the actual solvent elimination, the solvent is selectively removed from the injector. In the second step, the splitless transfer, the components left in the liner are transferred to the column in the splitless mode. It is needless to say that both steps have to be optimized carefully. In practice it is generally easier to start optimization with the second step.

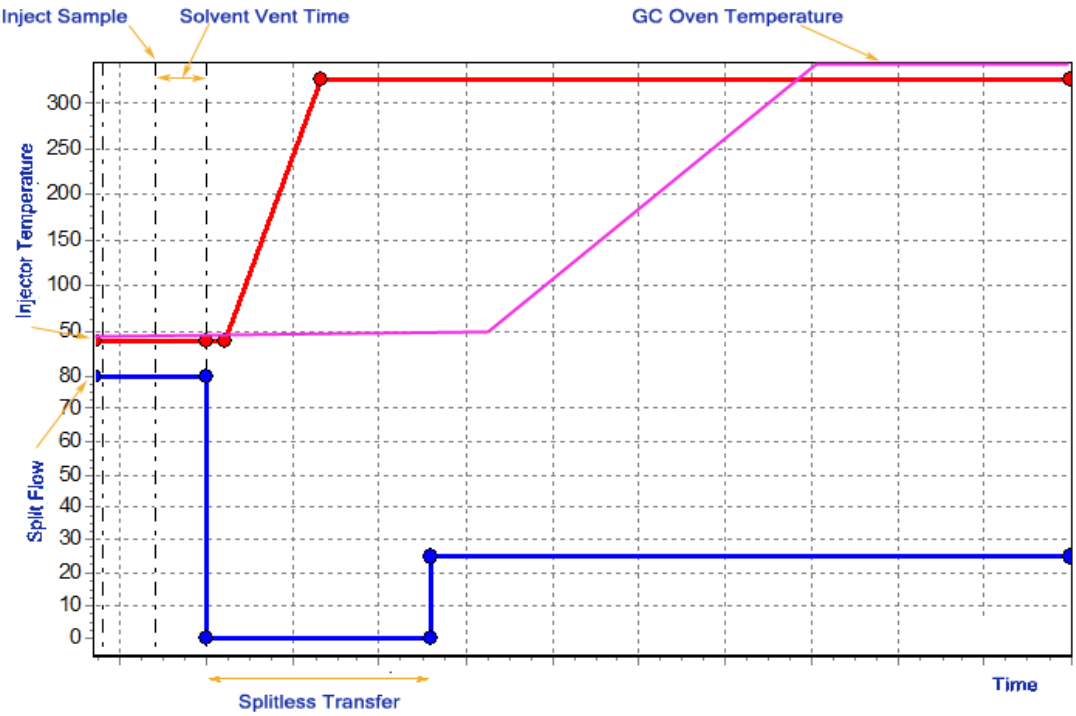


Figure 2. Schematic representation of the temperature and split status during a PTV solvent elimination injection. Dashed line: split exit open. Solid line: split exit closed.

1.3.2. Large volume injection using the multiple injection method

The easiest method for large volume injection using a PTV injector makes use of the so-called multiple injection mode. This method for large volume injection is schematically depicted in figure 3. As the multiple injection mode is seldom used, only a brief description of this technique is given here. An important advantage of the technique is that it is a very easy way to introduce a larger volume (five to ten times the normal injection volume). This gain in sensitivity can be obtained without the need to make any changes to the instrument. It isn't even necessary to replace the injection port liner.

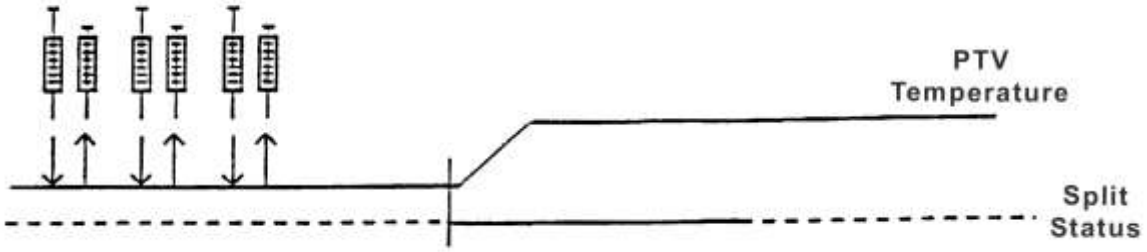


Figure 3. Schematic representation of the multiple injection method.

In the multiple injection technique a small volume of sample is injected into the cold injector. This volume should be well below the maximum volume of sample that the liner wall can accommodate. After injection the liquid sample will form a thin film of liquid on the wall of the liner. Depending on the solvent used and the temperature of the PTV injector a typical maximum that can be accommodated by the liner wall is between 5 and 10 µL. If this volume is exceeded, the liquid can not be retained by the liner wall and will be lost via the split exit. During injection the temperature of the injector is low (at least some 30°C below the boiling point of the solvent). One now exploits the mechanism of solvent venting to selectively remove the solvent from the liner. This means that the injection is performed with the split exit open. A high flow of gas is now flowing through the injector. Due to this high flow of gas the solvent evaporates and solvent vapour will be discharged from the injector via the split exit. In one injection only a limited volume of sample can be introduced into the liner because the capacity of the liner to retain the sample is limited. If one now wants to inject a larger volume than can be accommodated by the injector, the process of injection has to be repeated. At a low temperature multiple aliquots of the sample, each of no more than some 5 µL are injected. Between two injections one has to wait to give the solvent of the previous injection sufficient time to evaporate. During this entire process the split exit is kept open. The injection of small sample volumes can be continued until the desired injection volume is reached. After the last injection one waits for a short time to give the solvent from the last injection sufficient time to evaporate. Immediately after this the split exit is closed. Finally, after a few seconds the injector is heated and the components are transferred to the column in the splitless mode.

An important parameter in the multiple injection is the time between two subsequent injections. This time is called the solvent elimination time. The optimum value for this parameter has to be established experimentally. If the time is too short, build up of liquid in the liner will occur and

the maximum sample capacity of the liner can be exceeded. This will result in losses of sample. The sample will leave the split exit in the liquid state. In this case all components (both volatile and non-volatiles) will be lost to approximately the same extent. When the time between two injections is too long, serious losses of volatiles will occur. The high boiling components will not be lost. These differences in losses (all components or only volatiles) are the key to the optimization of the multiple injection.

The optimization of a multiple injection can be carried out as follows: Let us assume we want to inject 20 µL of sample as 5 injections of 4 µL. First the PTV conditions are selected. A good initial PTV temperature, when using hexane as the solvent is 30 °C (the boiling point of hexane is 69 °C). For other solvents the PTV temperature has to be adjusted in accordance with the difference of the boiling point of the solvent relative to that of hexane. Apart from the initial temperature of the PTV the final temperature and the splitless time also have to be selected. If the final temperature is too low and/or the splitless time is too short, losses of heavy components (discrimination) can occur. To achieve a rapid evaporation of the solvent it is advantageous to use a split flow that is sufficiently high (>100 mL/min). Split flows as high as 75 to 200 mL/min are more or less standard in the various methods for PTV large volume injection. As a rule of the thumb: The evaporation rate doubles when the split flow is increased by a factor of two. Or expressed as evaporation times: the evaporation time is reduced by a factor of two when the split flow is doubled. When all conditions are selected a first reference chromatogram is recorded. This is done by the analysis of 4 µL of a 5 times or, *e.g.*, 1 µL of a 20 times concentrated sample. The peak areas in this reference chromatogram can be used to calculate the recoveries in the multiple injection of the diluted sample. It is a prerequisite for this method of optimization that the components that are to be analyzed are available in the laboratory. If this is not the case, the optimization can also be carried out using a similar substance or any other substance with a similar boiling point as the component of interest. Because the losses that occur from the injector are almost exclusively based on the vapour pressure of the components, the procedure can also be optimized using a standard solution of a series of normal alkanes in the solvent that is to be used. An additional advantage of using an alkane standard for optimization is that losses of components due to adsorption or thermal degradation can be precluded. Once again, good results in a multiple injection are only obtained if the conditions for splitless transfer of the components to the column (PTV final temperature, heating rate, splitless time) are carefully chosen.

Once the reference chromatogram of the concentrated standard is recorded, the experimental optimization of the solvent elimination time (time between two injections or interval time) can be commenced. To avoid contamination of the carrier gas inlet system and the detector, it is advisable to start the optimization with interval times for which one is sure that it is too long. Under the conditions mentioned above (5 injections of 4 µL each, solvent hexane, PTV temperature 30 °C, split flow 250 mL/min) an interval time of 10 seconds between two subsequent injections is amply sufficient. Under these conditions losses of volatiles will occur. In subsequent experiments the time between two injections can be reduced slowly in order to reduce the losses of volatiles and, eventually, reach conditions where losses are negligible. If the time is reduced too far, one will move from a situation in which only volatiles are lost, to a situation in which all components show less than quantitative recoveries. The optimum is

somewhere in between these two times. It is possible that the time between two injections, required to avoid the loss of volatiles, is impractically short. In this case the solvent elimination can be carried out at a lower temperature or at a lower split flow. In both cases the evaporation proceeds at a lower rate.
Another valuable rule of thumb is that the rate of evaporation of a solvent is reduced by a factor of two when the temperature is decreased by 10 to 15 °C.

In the process of optimizing the time interval between two injections, one can use the fact that too short an time interval gives different problems from too long a time. If, in comparison with the reference chromatogram, all components are lost to some extent then the time is too short. In this instance the samples is lost from the liner in the liquid state. If, on the other hand, only volatile components are lost, the time is too short. Even after a careful optimization of the solvent-elimination time (vent time) losses of volatile components are difficult if not impossible to avoid. When using hexane as the solvent, components more volatile that roughly normal C_{14} will be partially lost, despite careful optimization. The best possible situation occurs when the solvent is evaporated at as low a temperature as possible. Also a relatively low split flow (around 100 mL/min) is advantageous for these samples. Losses are minimized if the solvent in the injector is not evaporated to full dryness.

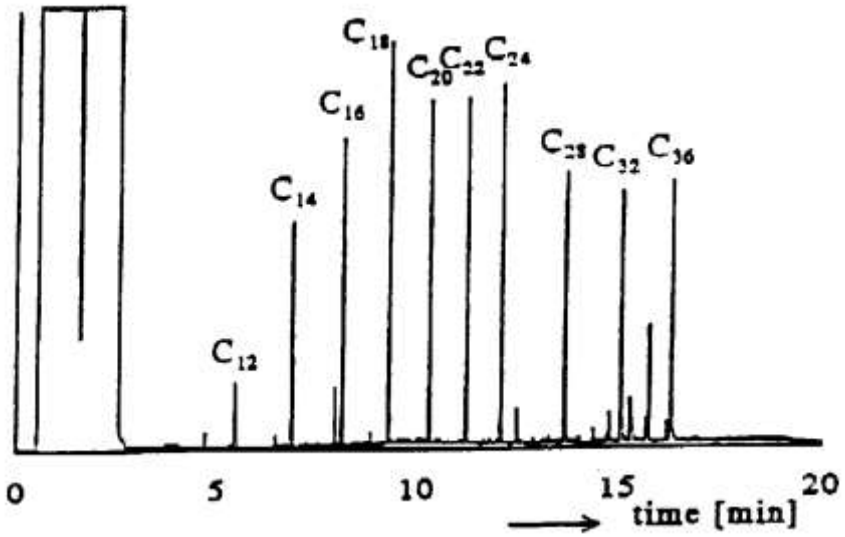


Figure 4. Example of a multiple injection.
Sample: alkane standard (1.5 ppm) in hexane. 10 injections of 1.5 µL each. Interval time = 5 seconds.

Figure 4 shows an example of a chromatogram of a diluted alkane standard recorded using the multiple injection technique. From figure 3 it can be seen that the multiple injection technique, analogous to the solvent vent technique, actually consists of two sucessive steps (solvent elimination and splitless transfer). Each of these two steps has to be carefully optimized. Optimization of the splitless transfer can be performed via an injection of a small volume (e.g. 1 µL). The multiple injection method is not really suited for automated routine applications. It is, however, a simple way to increase the injection volume and sensitivity by a

factor of up to ten. As no instrumental modifications are required, it is a good method when an increased sensitivity is only required once in a while.

1.3.3. *Rapid* or 'at-once' injection

The ***Rapid*** or 'at-once' injection method for PTV large volume injection bears a close resemblance to the standard solvent vent injection. What is different, however, is that the liner is now filled with a packing material. This can, for example, be glass wool, quartz wool or some other type of material. The main purpose of putting a packing material in the liner is to increase the capacity of the liner to retain the liquid sample. In the 'at-once' large volume injection method the sample is injected rapidly (within a few seconds). After the injection the needle is withdrawn from the injector. The temperature during injection is again well below the boiling point of the solvent. The temperature profile of the injector versus time and the status of the split valve are schematically depicted in figure 5.

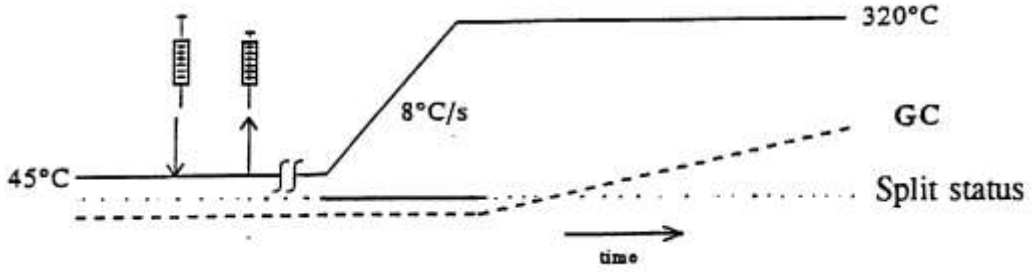


Figure 5. Schematic representation of the temperature/time profile and the split valve status during the direct 'at-once' large volume injection.

Immediately after the injection the liquid sample will spread out as a thin film of sample over the packing material in the liner. During and immediately after the sample introduction, the split exit of the injector is open and a large flow of carrier gas flows through the injector. The largest part of the gas flow leaves the system via the split exit. Only a small fraction enters the column. Due to the high flow of gas through the liner, the solvent film in the liner starts to evaporate. This evaporation consumes a lot of energy. Therefore, a cold spot is created at the location where the evaporation occurs. In this cold spot, the components present in the sample are well retained. This is not only due to the low temperature, the solutes are also retained by the solvent film present in the cold spot. Despite the rapid and strong cooling that occurs, the process of evaporation in the liner continues. Vapours formed upon evaporation are discharged via the split exit (and to a small extent also via the column). Just before all liquid in the injector is evaporated the split exit is closed and the injector is heated. All components are now transferred to the column in the splitless mode. Figure 6 shows the variation of

temperature inside the liner immediately after the injection of a large sample volume. The figure clearly shows the rapid temperature decrease upon injection.

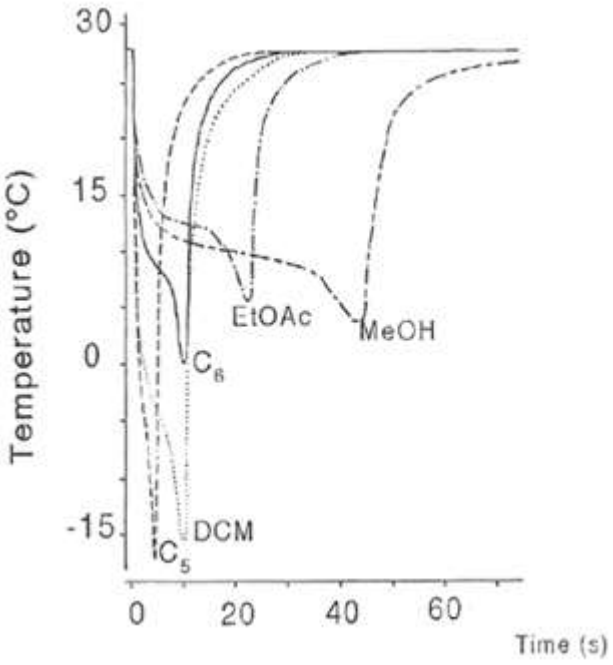


Figure 6.
Creation of a cold spot in the injector during a Rapid injection of a large volume (RLVI) of 100 µL of different solvents in a glass wool packed liner. Initial PTV temperature 30°C. Temperature measured directly below injection point.

Although the principle of the rapid injection method for large volume sampling is extremely simple, a number of parameters have to be optimized before a large volume injection can be performed successfully. The parameters that require some consideration are:

- Choice of the packing material,
- Optimization of the splitless transfer and determination of the inertness of the packing material,
- Determination of the maximum sample volume that can be accommodated by the packed bed (usually provided by supplier),
- Determination of the solvent-elimination time.

In the following paragraphs each of these parameters will be addressed in more detail.

Choice of the packing material

An important parameter in Rapid Large Volume Injection is the material that is used for packing the liner. This material should meet a number of different criteria. Firstly, the material should be capable of retaining a sufficiently large volume of solvent. This means that good wetting of the material by the solvent should occur. In general, maximum load-ability occurs when the polarities of the packing material and the solvent match. This means that if a non-

polar solvent is used, a non-polar packing material will give the largest capacity. A second important requirement for the packing material is that it is sufficiently inert. If this is not the case, then the components of interest can undergo thermal degradation during splitless transfer to the column. Moreover, the components should not exhibit too strong an adsorption on the packing material as this might hinder their desorption from the material and subsequent transfer to the column. Some interaction between the components and the packing material, however, can be advantageous, in particular for volatile analytes that otherwise might easily be lost together with the solvent during solvent evaporation. Clearly, the packing material should not dissolve in the solvent or swell under influence of the solvent (for example, the structure of the packing material Tenax is irreversibly affected by chlorinated solvents such as dichloromethane). Finally, the packing material should have a sufficient thermal stability and should give good blanks. In table 1 an overview of a number of possible packing materials is given. Apart from the materials given in this table there are numerous other materials that can be used as liner packings for Rapid Large Volume Injection. An important parameter to take into account finally is the particle size of the packing material. Good results can be obtained with particle sizes between 60 and 100 mesh. Larger particles such as glass beads, however, can also be used.

Table 1. Overview of packing materials for the Rapid injection method.

Packing material	V_{max}* (µL)	T_{max} (°C)
Glass wool	115	-
Quartz wool	125	-
Glass beads	40	-
Teflon wool	80	275
Tenax TA	125	310
Dexsil coated support	80	340
Supelcoport	120	330

* Based on a liner with an i.d. of 3.4 mm and a packed bed with a height of 25 mm.

Once the packing material is selected, the liner can be packed with this material. To keep the material in place, it is advisable to use a liner with a frit in its bottom section. The required amount of packing can be packed onto this frit and finally a plug of glass wool can be put on top of it to prevent the packing from being blown out. Prepacked liners with glass frits at either end are nowadays commercially available. When packing the liners, one should realize that in all PTV injectors significant temperature gradients can occur along their length. Due to heat loss at the top and the bottom side of the injector, the temperature in both the top section and the bottom section of the liner will be significantly below the set temperature of the PTV injector. When packing the liners care should be taken that the packed bed does not extend

into the cooler regions of the injector. If required, the needle length of the syringe should be adjusted so that the needle does not protrude into the packed bed in the liner. Figure 7. shows some temperature gradients measured along a PTV liner. Information on which part of the injector is properly heated can be obtained from the manufacturer. Alternatively, the temperature profile inside the injector can be measured using a small thermocouple.

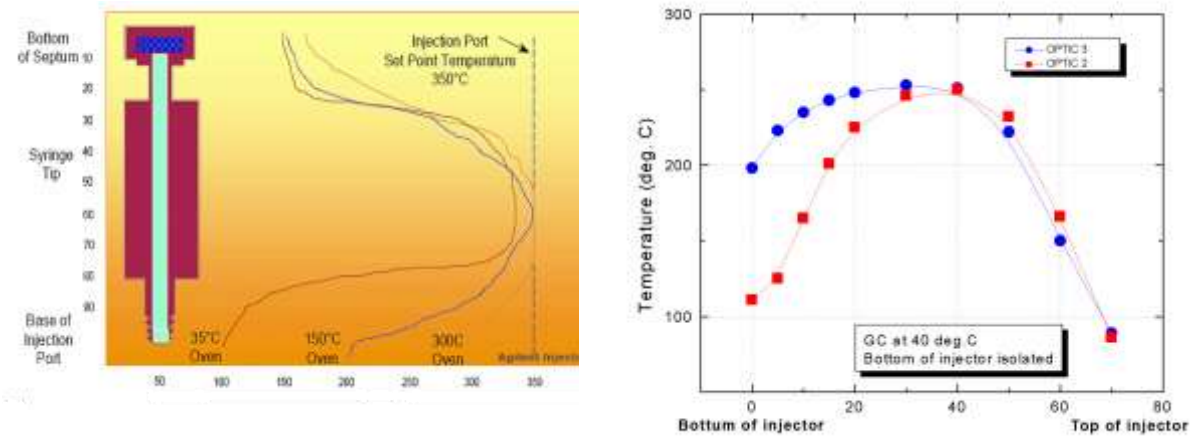


Figure 7. Temperature gradient along a PTV liner (left Agilent, right OPTIC).

Once the liner is filled with packing it should be conditioned. This can be done by temperature cycling the liner several times to a temperature that is roughly 20 °C above the temperature that is going to be used for the splitless transfer of the solutes to the column. Care should be taken not to exceed the maximum allowable operating temperature of the material selected. The time required for conditioning strongly depends on the type of material used. Glass wool, quartz wool and Supelcoport can be conditioned by heating it to 325 °C for one hour. Conditioning of Tenax, on the other hand, requires many hours. In all cases, the conditioning process should be carried out in an inert atmosphere completely free of oxygen and water. Prior to starting the conditioning of the material, all air should first be removed from the liner at low temperature by purging it with carrier gas for a few minutes.

Optimization of the splitless transfer and determination of the inertness of the packing material

Once the liner is conditioned a first series of experiments can be conducted to optimize the splitless transfer of the solutes from the injector to the column. This can be done by injecting a small volume (*e.g.* 1 µL) at different PTV final temperatures and splitless times. It should be emphasized here that the transfer of solutes from a packed liner can be somewhat slower than from an empty liner. This because the packing might show some interaction with the solutes. It is therefore dangerous to transfer a PTV program directly from a method that uses an empty liner to a method in which a packed liner is used. A first guess of the required PTV final temperature can be obtained from the elution temperature of the solutes from a standard GC column. A PTV final temperature some 25 °C above the elution temperature in general gives

quantitative transfer of the solutes to the column in a short time. Standard splitless times are 30 to 45 seconds, calculated from the moment the PTV injector reaches its final temperature.
At this stage, the inertness of the packing material has to be determined. This can be done by comparing the optimum chromatogram from the splitless-transfer optimization experiments with a reference chromatogram recorded using on-column injection or, if this is not available, using a PTV injection with an empty liner. In general, thermal degradation during a PTV injection is reduced if lower heating PTV programming rates are applied. For practical reasons, heating rates below 1 to 2 °C/sec are only rarely used.

Determination of the maximum sample volume that can be accommodated by the packed bed

Once the splitless transfer conditions have been optimized and it is clear that no unacceptable thermal degradation or adsorption of the components occurs inside the PTV liner, a start can be made with the optimization of the actual large volume injection. The first step in this optimization is the determination of the maximum volume of solvent that can be retained by the packing material in the liner. As this volume depends (slightly) on the liner temperature, the gas flow through the injector and the solvent used, first these parameters have to be fixed. For the selection of the optimum liner temperature and the gas flow some guidelines have been given in section 1.3.2. In summary: when using hexane as the solvent, a good initial PTV temperature is 45 °C. For other solvents the initial PTV temperature should be adapted according to the difference of the boiling point of this solvent and that of hexane. To obtain rapid evaporation of the solvent, it is advantageous to use a split flow that is rather high (>75 ml/min). A split flow of 75 to 150 ml/min is, as stated before, more or less standard in all forms of PTV large volume sampling. (Now suppliers of liner will give the maximum volume that can be used for each liner, so these experiments are not needed when packed liner are bought.)

When the initial PTV temperature and the split flow have been selected, the maximum volume of solvent that can be accommodated by the packed liner has to be established. The easiest way to do this is based on visual observation. To this end the packed liner is installed in the injector and the column is disconnected. In doing this the carrier gas flow is kept on. Now a large volume of the solvent that has been selected is injected (*e.g.* 100 µL). During the rapid injection of this large volume one should carefully observed the outlet of the injector, *i.e.* the place where normally the column is installed. If droplets of liquid are observed squirting into the oven, the maximum capacity of the liner has been exceeded. By varying the injected volume, the maximum allowable injection volume can be rapidly determined. To be on the safe side, in a true large volume experiment the injected volume should be only some 60 to 70% of the maximum volume determined using this visual method. The method described here is a relatively crude method, although for most of the situations it is sufficiently reliable. A more sophisticated method is based on the injection of increasing sample volumes of a non-volatile component in separate GC runs starting from a very low value to a very high value (*e.g.* from 1 µL to 200 µL). This should be done with the selected solvent at the pre-selected initial PTV temperature and flow rate. In the sample, only one component has to be present, preferably a high boiling component (boiling point roughly above that of n-C_{20}). A plot of the peak area of this component versus the injected volume should increase linearly until a plateau value is

reached. The volume where the plateau occurs is the maximum allowable injection volume. If in a Rapid Large Volume Injection experiment a volume in excess of the maximum liner capacity is injected, liquid sample will be lost via the split exit. It is also possible that liquid sample can enter the column. This latter phenomenon manifests its self as peak splitting. All peaks in the chromatogram will show a 'pre-peak'. An example of these pre-peaks is shown in figure 8.

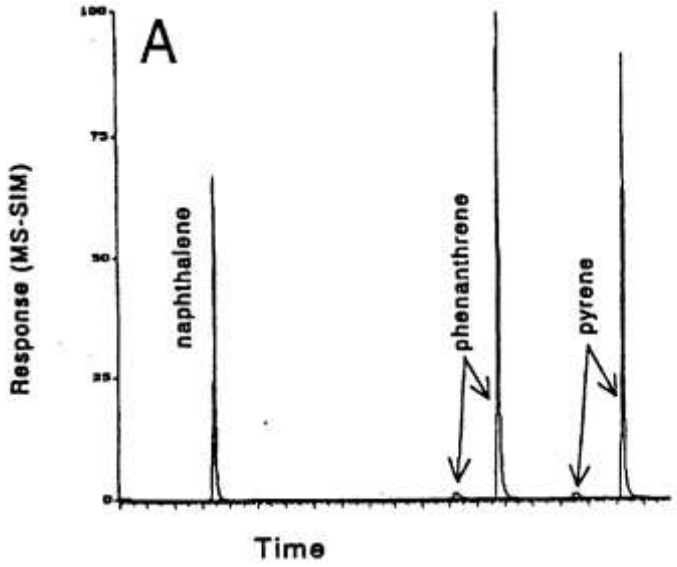


Figure 8. Peak splitting caused by too large an injection volume in the Rapid injection technique.

It is apparent that the maximum volume of liquid that can be retained by a given liner depends on the temperature, the gas flow, liner diameter and type and amount of packing material in the liner. Liners with a larger inner diameter clearly have a larger sample capacity. Therefore, these liners are to be preferred for the Rapid Large Volume Injection method. Unfortunately, however, the use of wide-bore liners also has a few disadvantages. The most important one being the longer time required for splitless transfer. This increases the risk of sample degradation during splitless transfer. Also in this case, bleeding of the packing material can not be completely avoided, the fact that for the Rapid injection technique a large i.d. liner with a larger amount of packing material has to be used is a disadvantage.

Determination of the solvent-elimination time.

In the Rapid or 'at-once' injection method a large volume of sample is injected into a packed liner. After injection the injected sample spreads out as a thin film of liquid over the packing material. Due to the large flow of gas through the injector the solvent film starts to evaporate. The components present in the sample will be trapped in the cold-spot that is created due to the large heat consumption of the evaporation process. The solvent vapour created in the evaporation process is discharged via the split exit. A small fraction of this, approximately 0.5 to 1% enters the GC column. Once the solvent film is evaporated, the split exit can be closed

and the components remaining in the liner can be transferred to the column in the splitless mode.

An important parameter in Rapid large volume injection is the time of solvent evaporation (Vent Time). If this time too short, there will still be a significant volume of solvent left in the liner at the moment the split exit is closed. When the injector is heated, with the split exit closed, a very rapid, uncontrolled evaporation can occur in the liner, creating a pressure pulse. In the worst case this pressure pulse could adversely affect the integrity of the packed bed in the liner and packing material particles, solvent vapour and components could be forced back up in to the carrier gas inlet of the GC, resulting in contamination of the system. The worst case scenario described above only occurs if a very large volume of solvent (some 10 µL or more) is still present in the liner at the moment heating starts. If on the other hand, the time for solvent elimination is excessively long, the liner is evaporated to dryness. When all of the solvent is evaporated, the cold spot, that helps to retain the volatile sample constituents in the liner, disappears and volatile components are lost. From this it will be clear that a careful optimization is required for successful and reliable large volume injections using the at-once method. Under optimized conditions, the split exit is closed at the moment that a few microliters of solvent are left inside the liner. As long as there is some solvent left, volatile components will not be lost.

After careful optimization, even very volatile components can be analyzed successfully using this method. A boiling point difference of only some 10 to 40 °C between the solvent and the most volatile components is sufficient for obtaining quantitative recovery. For components with a higher boiling point the optimization of the solvent vent time is less critical. If hexane is used as the solvent and solvent elimination is carried out at a temperature of 45 °C, solutes with a boiling point above that of roughly n-C_{16} will not be lost, even if an already dry liner is purged with a high flow of carrier gas for some time.

In principle there are four methods for determining the evaporation time of a given volume of solvent.

Method 1: In the split exit line of the GC a monitor detector, as for example a thermal conductivity detector, can be installed. This detector detects the presence of solvent vapour in the carrier gas leaving the split exit. Once solvent elimination has reached completion, the signal of the detector returns back to its original value. At this point the split exit can be closed. Superficially, this method is very attractive, because it allows automation of the split exit control. Unfortunately, however, it is a relatively expensive method as it requires an additional TCD and control software.

Method 2: A very simple approach for the determination of the solvent elimination time is based on visual observation of the evaporation process by igniting the vapour leaving the split exit. When evaporation is complete the flame extinguishes. Now the split can be closed and the injector can be heated. Alternatively, the evaporation time can be measured a few times with large volume injections of a pure solvent. In a first real experiment an evaporation time some 5% shorter can this value can than be used. As the time allowed for evaporation is slightly shorter than the required time some solvent is still left in the liner. This solvent

helps in retaining the volatile sample constituents. This approach works well for hexane and pentane (yellow flame) and ethylacetate (weak blue flame). Other solvents such as dichloromethane or chloroform do not give self-sustaining flames. The vapours of these solvents, however, can be monitored by allow the split gas to flow through, for example, a candel flame.

Method 3: Another method for determining the solvent elimination time uses the GC detector itself as a monitor detector. Solvent entering the column during solvent elimination will reach the GC detector after the column dead-time. When solvent elimination is complete the detector signal returns to the base-line. The width of the solvent peak (at half height) now equals the duration of solvent elimination. An example of this is given below. If this method is used, the oven temperature during solvent elimination should be some 10°C or more above the initial PTV temperature. This is to ensure rapid transport of the vapours formed through the column. This method can not be used if the solvent is eliminated at zero injector pressure. When a mass spectrometer is used the vacuum level of the system can also be used to determine the width of the solvent plug entering the detector. It is advisable to measure the width of the plug using a few injections of the pure solvent at the desired volume. This method can be used for most GC detectors including the FID, NPD, FPD or SCD. For the ECD it is slightly more complicated. To allow accurate measurement of the peak width at half height it will often be necessary to perform the experiments at a reduced detector sensitivity setting. In a first real experiment one could again use a solvent elimination time that is some 5% shorter than the time required for complete evaporation.

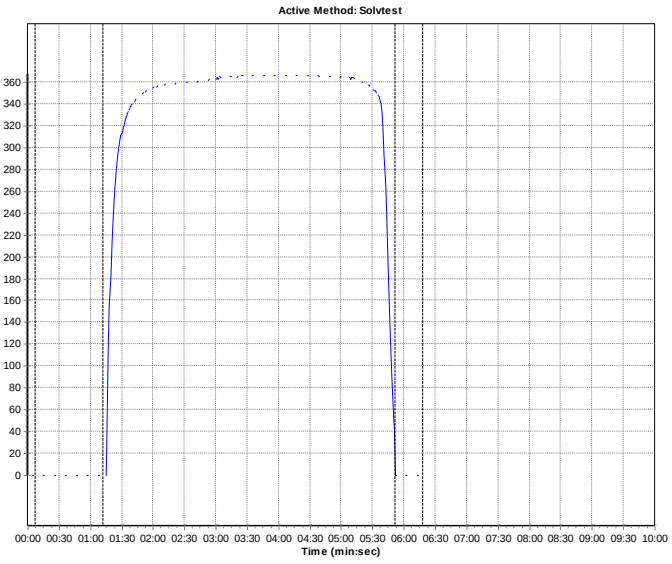


Figure 9. Determination of the solvent elimination time (method 3) in a Rapid large volume injection. Further details: see text.

Method 4: The last method for the determination of the optimum solvent elimination time is based on trial and error optimization. In this approach a large sample volume of a sample containing various components covering a wide range of boiling points in the solvent of interest is injected. The identity of the components is not important. A homologous series of normal alkanes is therefore very well suited for this purpose. The selection of the solvent is of couse important. In the first experiment a very long solvent elimination time is used. At this very low evaporation time the more volatile components present in the sample will be lost. In a series of experiments the solvent elimination time is now reduced in small steps to a value where good recoveries are obtained for all solutes. Figure 3.10 shows the chromatograms obtained for a 100 µL 'at-once' injection of a hexane solution of normal alkanes obtained at solvent elimination times that are too short, good and too long, respectively. As a rule of the thumb, the evaporation rate of a solvent increases by a factor of 2 if the PTV temperature is increased by 10 °C to 15 °C or if the split-flow is doubled.

If the solvent elimination time is far too short, a large volume of liquid is still present in the liner at the moment the split exit is closed. Upon heating the injector this volume of solvent rapidly evaporates ('explodes') and creates a huge volume of gas inside the liner of the injector. Eventually the packed bed of packing material in the liner might be disrupted by the rapid evaporation of the solvent. Moreover, solvent vapours might back-up into the carrier gas lines resulting in severe contamination of the inlet. This situation should always be avoided. During optimization of the solvent elimination time one should therefore always start the optimization procedure with elimination times that are too long and successively reduce the time for solvent elimination in small steps. A very helpful figure is figure 10. The top figure shows the chromatogram of a large volume of an alkane standard at too long a solvent elimination time. Losses of volatile components show that the liner is vented to dryness. The middle chromatogram shows the analysis at an optimized solvent vent time while the bottom chromatogram shows a chromatogram obtained at too short a solvent elimination time. In the latter case a very characteristic peak distortion is observed in the middle part of the chromatogram. The early eluting peaks have good peak shapes and so have the peaks more towards the end of the chromatogram. The middle part is distorted.

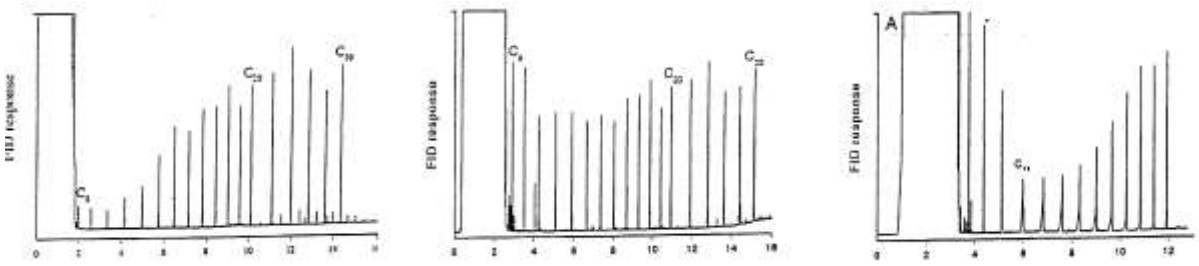


Figure 10. Peak distortion and sample loss during 'at-once' large volume injections of an alkane standard in hexane at a long (left), optimum (middle) and short (right) solvent elimination time.

1.3.4. Large volume injection using the speed controlled sampling technique

An important prerequisite of the Rapid Large Volume Injection technique described in the previous paragraphs is that the sample volume to be injected 'fits' in the liner. Expressed more accurately, the packing material in the liner should have sufficient capacity to retain the volume to be injected. If the sample volume exceeds the volume capacity of the liner, the Rapid 'at-once' technique can no longer be used. In this case one has to resort to the multiple Rapid method or the speed-controlled large volume injection method. As the multiple Rapid method is simply a combination of the multiple injection technique and the at-once method it is not discussed here. In the PTV speed controlled large volume injection technique the sample is introduced into the liner at a constant, and controlled rate. During sample introduction the liner is kept at a low temperature and the split exit is open. Upon injection of the sample a liquid film of sample is created on the wall of the injector or on the packing material. This film starts to evaporate from its top side. The sample vapours formed are discharged from the injector via the split exit. After the entire volume is (slowly) injected, there is a short wait time prior to closure of the split exit. This time is called the 'additional solvent evaporation time'. In this additional evaporation time the small volume of liquid present in the liner is given time to evaporate. After the additional solvent evaporation time the split exit is closed and the injector is heated. Typical sample introduction rates vary between 25 and 250 µL per minute. The introduction of 100 µL hence can take up to 4 minutes. Because the components are retained in the liner during the entire time required to introduce the large volume and the actual introduction only takes place when the injector is heated, the long time required for sampling has no adverse effects on the injection bandwidth of the components. When using the speed controlled sampling mode the actual volume of liquid present in the liner at each time is very low. Hence, with this mode of large volume injection PTV liners with low internal diameters can be used. This in contrast to the situation with the Rapid injection mode where larger liners are advantageous because of their larger volume capacity. An advantage of narrow liners is that the splitless transfer of the sample components after elimination of the solvent is faster. This means that speed-controlled sampling is the preferred technique where unstable analytes are to be analyzed.

When using the speed controlled PTV large volume injection method, the volume that can be injected is not restricted to a maximum. During sample introduction a steady state is reached. In this steady state or equilibrium state the speed of introduction of the liquid sample into the liner equals the evaporation rate. Liquid is introduced at the top of the injector at a rate equal to the evaporation rate. In this way a steady state situation is created. This steady state is described by the following equation:

Liquid flow into the liner [g/min] = gaseous flow out of liner [g/min]

At first glance this equation might look extremely simple. Closer examination reveals however, that the system described by this equation is very complex. Many parameters affect the

equilibrium state described by the equation, the most important ones being: liner temperature, nature of solvent, carrier gas flow rate, dimensions and design of liner, pressure inside injector liner etc. The equation given above can only be solved analytically by introducing a number of (sometimes unrealistic) assumptions. Figure 11 shows the solutions of the equation for three solvents at two total injector gas flow rates. For more details the interested reader is referred to literature [7]. The figure shows the relation between the evaporation rate of the solvent (y-axis) and the temperature of the injector (x-axis). To reach the steady state, the rate at which the sample is introduced should equal the evaporation rate. Unfortunately the reliability of the data shown in figure 11 is not sufficient. This means that for practical use the data shown in this figure are of limited value only. This is mainly due to the fact that in the calculations the cooling of the liner that occurs upon evaporation of the solvent is not taken into account. To include this effect a correction factor has to be introduced. In practical speed controlled large volume experiments the actual evaporation rate is roughly 3 times lower than the value obtained from figure 11. This corrected value can be used as a rough initial guess during method development for speed controlled large volume injection. Further fine tuning of the sample introduction rate has to be carried out experimentally following the procedure described below.

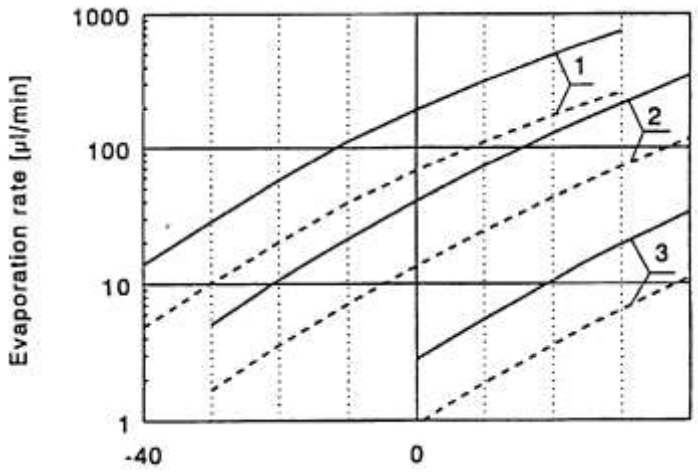


Figure 11. Evaporation rate of some widely used solvents as functions of the PTV initial temperature and the split flow rate.

Clearly, the most critical components in a PTV large volume injection are the more volatile solutes. This irrespective of which PTV large volume injection mode is used. Volatile components are easily lost during the elimination of the solvent. If these components have to be analyzed, the solvent vent conditions have to be chosen carefully. Unlike the situation in the Rapid large volume injection mode, where components with a boiling point only some 10 to 40°C above the boiling point of the solvent can be quantitatively recovered, a much larger difference in boiling points is required when using the speed controlled injection mode. Looked to it from the positive point of view: if one is only interested in components with boiling points above that of roughly C_{18} large volume PTV injection is extremely easy. For such a sample very often the first experiment already gives quantitative recoveries.

Optimization of a PTV speed controlled large volume injection starts with a series of experiments to establish the maximum evaporation rate of the solvent. For these experiments a standard sample containing components covering a wide range of boiling points is required. An alkane standard disolved in the solvent to be used, is generally the easiest standard, although any other sample meeting the requirement of a large boiling point range can also be used. Once the standard is selected the next point of interest is the selection of the liner. Although speed controlled injections can, in principle, be carried out using empty liners, it is usual to employ a liner containing a packed bed, in order to create a sufficiently large surface for evaporation. A small amount of packing (bed height 10 to 15 mm) in a narrow liner is generally sufficient. In contrast to the situation in Rapid injection, where the maximum amount that can be injected is directly proportional to the amount of packing, much smaller packing volumes suffice for speed controlled injections. Suitable packing materials are again glass wool or Supelcoport. If very volatile components are to be analyzed or if one wants to introduce additional selectivity in the sample introduction process an active material such as Tenax of any other GC packing material can be used. After installing the liner into the injector the split flow has to be selected. Here again the simple rule is: the higher the split flow rate the faster is evaporation. Flows are usualy set between 100 and 250 ml/min.
Next, the initial temperature of the injector should be selected. Generally a good initial value is 15 to 40 °C below the boiling point of the solvent. The initial oven temperature is not really important, but it is wise however, to select the initial oven temperature some 10 °C above the PTV solvent vent temperature. This prevents possible recondensation of solvent vapours in the column. Once the split flow and the initial liner temperature have been selected, figure 11 can be used to determine the (uncorrected) evaporation rate of the solvent. The value determined this way should now be divided by a factor of three to correct for cooling of the liner. The last parameter to select is the additional solvent elimination time. In the first experiment too long a time is deliberately selected, *i.e.* 20 seconds. All conditions for a first large volume injection experiment are now selected.

The first real injection again is a 1 µL cold splitless injection of the standard mixture. This is the reference chromatogram. This chromatogram is recorded using the packed liner. If the components are amenable to thermal degradation or adsorption on the packing material, also a reference injection on an empty liner can be carried out. In this way possible thermal degradation or adsorption can be identified. Once the reference injection of 1 µL is completed the first large volume injection is carried out. A large volume of a sample diluted in proportion to the increase in injection volume (*e.g.* 125 µL of a 125 times diluted standard) is injected. This is done at the conditions identified above. A comparison of the large volume injection and the reference chromatogram now indicates whether (and to which extent) components are lost. If one is only interested in relatively high boiling components (elution temperature roughly above that of n-C_{18}) very often the first injection already gives good results. If, however, also more volatile components are of interest a more careful optimization is mandatory.

In the process of optimization of a speed controlled PTV large volume injection the recoveries that are found for volatile components relative to those found for non-volatile species are important. If poor recoveries are found for all components (both volatile and non-volatile), then liquid sample is being lost via the split exit. In this case the injection rate exceeds the

evaporation rate. The solution is to now reduce the rate of sample introduction by a factor of 2. If on the other hand, only volatile components are lost, at some stage in the process of injection the injector liner is being vented to dryness. To solve this problem either the temperature can be reduced (by *e.g.* 10°C) or the rate of introduction can be increased (*e.g.* by a factor of 2). At this stage of method development the additional solvent evaporation time should not yet be reduced. At some point in method development it is seen that from the one error (only losses of volatiles) one turns into the other error (losses of both volatiles as well as non-volatiles). The optimum liner temperature (or introduction rate) occurs between these two points. Once these conditions have been identified the additional solvent elimination time can be reduced in small step to minimize losses of volatiles during this stage of large volume sampling. If too short a solvent elimination time is applied peak distortion in the middle region of the chromatogram can again occur (see figure 10). An example of the optimization of a PTV speed controlled large volume injection is given in figure 12.

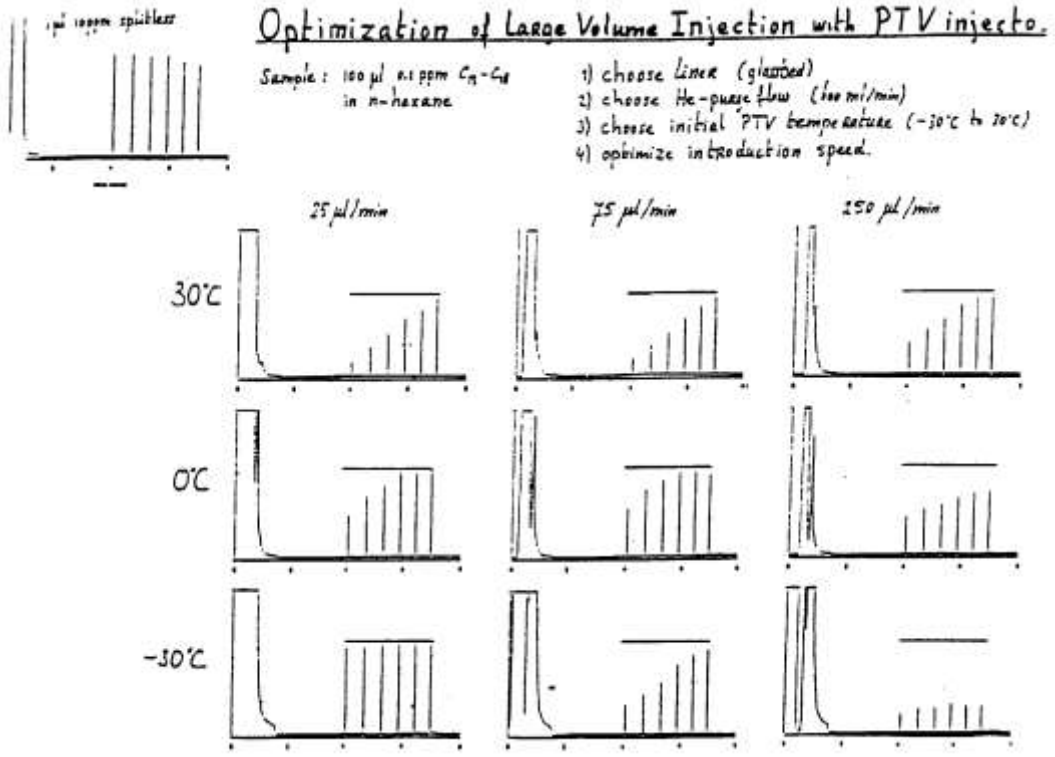


Figure 12. Example of the optimization of a PTV speed controlled large volume injection (100 µL of a standard containing n-alkanes C_{13} to C_{18} in hexane).

Even after careful optimization of a speed controlled PTV large volume injection, it will prove extremely difficult to avoid the loss of volatile sample constituents. When hexane is used as the solvent, only components with boiling point above that of roughly n-C_{13} can be recovered quantitatively in a speed controlled injection. Solutes with lower boiling points will, even under

optimized conditions, be (partly) lost. In general, however, the losses that occur are reproducible, which implies that also solutes that are partially lost can be analyzed accurately.

For experienced users there are different methods to minimize or eliminate losses of volatiles in PTV (speed controlled) injection. A first method is the use of a liner packed with an active adsorption material. This material will than (selectively) retain the components of interest. Well known active packing materials are Tenax, Volaspher, Carbotrap etc. When using these materials it should be realised that they limit the possibility to analyze high molecular weight solutes. For example in case of Tenax it will be very difficult to desorb components above roughly n-C_{30} at the maximum desorption temperature the Tenax allows. A second possibility to minimize losses of volatiles is by the use of a so-called 'keeper' or 'co-solvent'. This is a higher boiling solvent that is added to the main solvent in a relatively low concentration (*e.g.* 5%). The co-solvent forms a stable liquid film in the liner that only slowly evaporates. Volatile components will be effectively retained by this film.

1.3.5. Selection of the PTV large volume injection mode

Despite the fact that several large volume injection techniques have been studied for over 10 years, their use is still not common practice in (routine) laboratories. The main reason for this is that most of the techniques require carefull optimization, use expensive instrumentation and lack ruggedness. For widespread acceptance of large volume injectionm the technique should be simplified and made more rugged and the instrumentation used should be made cheaper. This can be achieved by using PTV based methods for large volume injection. By using PTV injectors equipped with packed 3.4 mm. i.d. liners, sample volumes up to some 150 µL can be injected 'at-once'. The optimization of the key parameter, the solvent vent time, requires only a very limited set of injections. Large volume PTV Rapid or “at-once” injection is rugged, both with regard to small fluctuations in instrumental parameters, as well as with regard to the introduction of non-volatile matrix constituents. This should be the first technique of choice in developing a new PTV large volume application. Speed controlled injection is slightly more complicated to optimize but shares the advantage of a good ruggedness. In table 2 an overview of the characteristics of PTV 'at-once' and PTV speed controlled injection is given. This table provides the guidelines for the selection of either of these two PTV large volume injection modes.

Table 2. Comparison of PTV Rapid and PTV speed controlled large volume injection.

	PTV Rapid	PTV Speed controlled
Preferred liner i.d.	3-4 mm	1-4 mm
Sample volume (µL)	< 150	>150
Main optimization parameters	Vent time	Injection speed
Ease of optimization	+	+/-
Ruggedness	+	+
<u>Applicability</u>		
Volatility range	> n-C_9	> n-C_{13}
Labile components	-	+/-
Polar solvents	+	+
'dirty' samples	+	+

EXPERIMENTS PTV LARGE VOLUME INJECTION Direct 'at-once' injection

Sample: Alkanes C_7 to C_{18} in hexane
Target volume: 100 µL (if allowed by injector)

1. Optimization of the splitless transfer

a. What is the initial PTV temperature you are going to use?
b. What heating rate are you going to use?
c. What is the final PTV temperature you will use?
d. What splitless time will you select?
e. What splitflow will you use?

2. Finding the maximum volume of the liner

a. What liner type do you use (diameter, packing etc)?
b. Give two methods to determine the liner capacity?
c. You select the simple method. Is the split flow on during the determination?
d. You perform the measurement four times. How long do you wait between two subsequent experiments.
e. Is the temperature of the injector during these measurements important?
f. Is the way you inject of importance?
g. What safety margin do you use?

3. Finding the optimum solvent elimination time (use peak width method)

a. What initial oven temperature are you going to use?
b. What would be the solvent elimination time in the first experiment?
c. How do you judge from a chromatogram whether the solvent elimination time applied is too short, OK or too long?

4. The reference chromatogram

a. How do you investigate whether thermal degradation or irreversible adsorption of components occurs in the liner?
b. How do you determine the recoveries of the large volume experiment.

Once all of these questions have been answered you can start the experimental work.

EXPERIMENTS

1. Find the maximum injection volume.
2. Determine the evaporation time for a large volume injection.
3. Record a reference chromatogram.
4. Optimize a large volume experiment and calculate the recoveries for the various alkanes.

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