Konrad Grob

Split and Splitless Injection for Quantitative Gas Chromatography

Concepts, Processes, Practical Guidelines, Sources of Error

Fourth, completely revised edition

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Fourth, completely revised edition

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Preface

In the scientific literature and in commercial catalogs, methods are almost invariably described as "easy"; there seem to be no limitations and problems. If original papers reflect the euphoria of the inventors, this is understandable. That catalogs of instrument manufacturers do not mention weaknesses of a product might be attributed to the rules of business. Even review papers, however, tend to neglect problems, maybe because authors do not want to risk good relationships or have insufficient experimental support for criticism.

As a result of this, there is a frightening discrepancy between the rose-colored descriptions and the reality in laboratories. Published work, for instance, reports relative standard deviations that are far lower than commonly obtained in reality – errors by a factor of two are rather frequent, and probably more frequent than recognized. The frustration of the analyst is understandable. His position in relation to his boss, who might have never gone through the reality of chromatography, is weak, because he seems to be an especially incapable analyst.

For new techniques, a few chromatograms are usually provided as a proof that they work. Inventors cannot be blamed for not having tested them with all possible samples and under all conceivable conditions – an impossible task. Techniques routinely used by tens of thousands of users should, however, be investigated rather comprehensively to enable understanding of the mechanisms involved and systematic discovery of the critical samples and conditions. This means, primarily, investigation of possible imperfections – not out of malevolence towards the inventor or instrument manufacturer, but to prevent failures during applications involving particularly unfortunate conditions. The user should know about the problems so they can be foreseen or, if they occur nevertheless, to avoid his spending days in search of the source, finally to discover he was looking in the wrong place.

Instruments are usually evaluated by means of a few injections of some alkanes in a simple solvent. Such quick tests resemble Russian roulette: whether an instrument is shot or escapes alive is primarily a matter of luck. Real evaluation is far more demanding. Even today instruments differ significantly in their essential parts, which is why the critical details of injector design are a subject of this book.

The book also concentrates on weaknesses of the techniques because it is assumed that problems are the reason why the analyst takes a look at it. Overemphasis of problems bears a danger, however, that a reader starts wondering why reasonable results were ever obtained or why capillary GC has not been abandoned altogether. He must be reminded that most problems are important only for certain types of sample and conditions.

There is no doubt that capillary GC in general and injection in particular are demanding techniques. They are full of pitfalls, but also rich in possibilities for a creative analyst – and certainly never boring. The better an analyst masters it and the more he knows, the more he is likely to be fascinated and the better he realizes how much more could be made of it.

Hopefully many will pick up problems and incomplete concepts, work on the subject, and contribute to the further development of capillary GC. Around 200,000 people use capillary

GC and could, therefore, profit from such contributions. It is my impression that GC injection techniques are still far from being optimized to the point which could be reached. Thousands of analysts go through the same trouble and lose weeks of work because known problems have not been solved. Apart from the frustration, this results in unnecessary costs. The basic problem seems to be that nobody is willing to carry the burden of perfecting these techniques. We are all paid for specific work (my job is in governmental food control), rather than to help others. Because offering an improved split/splitless injector does not seem to be a way of improving sales of instruments, instrument manufacturers hesitate to invest in this direction.

This book was started as a revision of "Split and Splitless Injection in Capillary GC", published by Hüthig (Heidelberg) in 1993, which in turn was an update of "Classical Split and Splitless Injection" from 1986. The new material, primarily on sample evaporation, necessitated, however, a new structure and finally a large part of the book was rewritten. The CD-ROM, produced by Maurus Biedermann, was added because the videos on the processes occurring in devices imitating injectors cannot be replaced by a description. Programmed temperature vaporizing (PTV) injection, on the other hand, has grown into a field requiring more space than is available in this book.

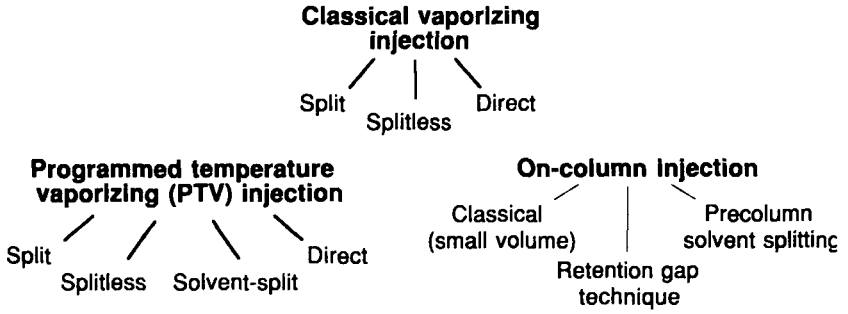
I wish to thank Ian Davies, Cambridge, UK, for converting Swinglish (Swiss English) into a more proper language, and Jonas Grob, one of my sons, for turning more than one million letters and many figures into attractive pages.

Fehraltorf, October 2000 Koni Grob

Survey of Injection Techniques

Is splitless injection a procedure during which you never touch the split outlet valve? If there is a danger of such confusion, please have a look at the following list of short definitions. Injection into GC capillary columns can be confusing, because there are so many different techniques. And if you ask why this is so, the answer is that each of these techniques is better than all others in some respects and has features some analysts do not want to do without. The following table provides a survey of the main injection techniques. It does not mention numerous others which have never become popular or have lost their importance, such as injection through a loop, capsule injection, and moving needle or other solid injection techniques.

Injection into Capillary Columns



Short definitions might be as follows:

Classical vaporizing injection. Sample evaporation in a permanently hot vaporizing chamber before transfer into the column.

Split injection. Only a small part of the vapor enters the column, the rest being vented. The technique of choice for rather concentrated samples, as well as for gas and headspace analysis.

Splitless injection. Nearly all of the sample vapor is transferred from the injector into the column; the technique is performed with a split injector. Trace analysis of contaminated samples.

Direct injection. All the vapor is transferred into the column; performed with an injector without a split outlet. Trace analysis, usually involving instruments converted from packed column GC.

Programmed temperature vaporizing (PTV) injection. Injection into a cool chamber which is subsequently heated to vaporize the sample. Newer technique to replace classical vaporizing injection.

Solvent splitting. Most of the solvent vapor is vented; the solute material is transferred into the column in splitless mode. Usually used for large volume injection in trace analysis.

On-column injection. Injection of the sample liquid into the column inlet or an oven-thermostatted capillary precolumn. Technique providing the best results, but not suitable for highly contaminated samples.
Retention gap technique. Use of an uncoated precolumn to overcome band broadening resulting from sample liquid flooding the column inlet. Most important for large volume on-column injection and on-line coupled LC-GC.
Precolumn solvent splitting. Injection into a precolumn connected to a vapor exit through which most of the solvent vapor is released. Used for large volume injection or on-line transfer.

Contents

A Syringe Injection into Hot Vaporizing Chambers

B Sample Evaporation in the Injector

C Split Injection

D Splitless Injection

E Injector Design

A Syringe Injection into Hot Vaporizing Chambers

1. Introduction

1.1. Syringe Injection

There are several reasons for the **general success of the syringe** for sample introduction in chromatography:

- the flexibility with which the sample volume can be adjusted;
- the possibility of releasing the sample in a predetermined region of the vaporizing chamber;
- withdrawal of the device after depositing the sample;
- easy cleaning of the sampling device;
- easy construction of autosamplers – the sample can be picked from the vial closed by a septum using the same device.

This does not mean, however, that the syringe only has advantages, as will be shown below, but the alternatives engender just as many problems and inconveniences.

Alternatives

In fact, in the past, some alternatives have been tested, but none has become a serious competitor with the syringe. Systems based on **rotating switching valves**, similar to those used in HPLC, have been proposed several times (*e.g.* [1]). They are widely used for gaseous samples, but not for the liquids commonly analyzed. Samples have been placed in small **capsules** which were opened in the vaporizing chamber. For solid (solvent-free) injection solutions were placed in **glass tubes** of ca. 15 x 0.7 mm i.d., from where the solvent was evaporated in a manifold that could be evacuated. These tubes were then dropped into the vaporizing chamber from a rotating block situated above the chamber.

Complex Process

At first sight, the concept of syringe injection into the classical vaporizing injector seems to be obvious – the needle releases a liquid sample into the hot vaporizing chamber, where the liquid quickly evaporates such that only vapors reach the column entrance. On closer inspection, the process is more complicated.

1 The sample solvent (normally more than 99 % of the sample consists of volatile solvent) **evaporates at least partially inside the needle** because the latter enters a zone at a temperature far above the solvent boiling point. Fast autosampler injection is an exception to this.
2 Evaporation inside the needle produces a **spray effect** that largely determines sample evaporation inside the vaporizing chamber. It is, in fact, the prerequisite for sample evaporation inside empty injector liners.

Neglected Subject?

The problem of syringe injection into vaporizing injectors has long been neglected, although some analysts, mostly working with packed columns, have been aware of it since the sixties. Perhaps the complexity of the problem was the reason, hindering the discovery of simple, generally valid solutions. The discussion of how to inject a liquid sample also has a **touch of awkwardness**, comparable perhaps with teaching an adult how to eat Italian spaghetti without smearing the red tomato sauce over his face and tie. Evaporation inside the needle is, however, **often the major source of error in quantitative analysis**, and it might well turn out that introducing a sample in a volatile matrix into a hot injector is even more difficult than eating spaghetti properly in front of a very important person.

1.2. Sample Evaporation inside the Needle

It is tempting to think of sample introduction into the injector as a purely mechanical process executed by depressing the plunger of the syringe – an **injection as in medicine or liquid chromatography**. In cold on-column injection this is indeed the case, but in vaporizing injection it is the exception rather than the rule. Partial evaporation in the needle causes two main problems.

1.2.1. Inaccurate Sample Volume

Sample (solvent) evaporation in the syringe needle renders the amount of sample delivered into the injector unreliable (**Figure A1**). Syringes are conceived to inject an amount of liquid that corresponds to the volume read on the barrel of the syringe. The **liquid inside the needle** is not measured by the commonly used plunger-in-barrel syringes (of, *e.g.*, 10 µL) because it is supposed to remain there at the end of the injection.

If a solution in a volatile solvent is introduced into an injector at 250 to 300 °C, it is difficult to prevent some liquid evaporating and emptying the needle largely. Because of this, the **amount of sample injected is greater than that measured**. Because the volume inside the needle is 0.6-1 µL and

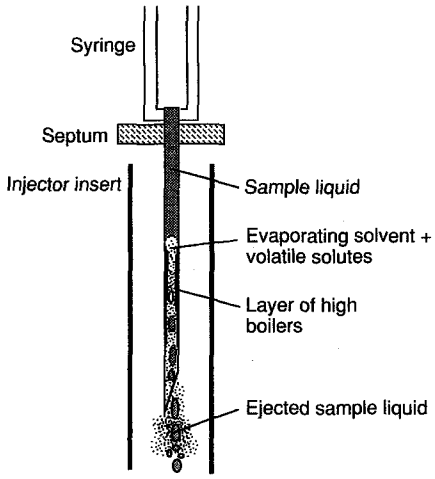


Figure A1
Basic problems caused by syringe injection of samples in volatile matrices into hot injectors.
a) Some of the sample material which should remain in the syringe needle at the end of the injection is expelled, increasing the volume of sample actually introduced above that measured on the barrel.
b) Part of the high-boiling solute material remains on the internal wall of the needle and is finally taken out of the injector with the syringe, resulting in a distortion of the sample composition (discrimination).

the sample size commonly injected between 1 and 2 µL, the needle volume is anything but negligible. Injection of a volume below ca. 0.6 µL is not possible if the needle volume is emptied.

1.2.2. Discrimination against High Boilers

Discrimination resulting from **selective elution from the syringe needle** is often even more troublesome. When the analyst withdraws the plunger after an injection, he might find little liquid hanging on the tip of the plunger. It is tempting to conclude that most of the needle volume has been transferred into the injector and that a nominal injection of, *e.g.*, 1 µL in reality introduced 1.6-2 µL.
While this conclusion may be correct for the solvent and the most volatile solutes, components with an elevated boiling point are likely to be only transferred partially; of these an equivalent of only, *e.g.*, 1 µL was injected – the exact amount cannot be determined visually. Thus, **high-boiling sample components enter the vaporizing chamber in amounts which are too low relative to the others**, and hence are "discriminated" against compared with the volatile material.

Overdosage of Volatiles

It may be objected that one should speak of "overdosage" of the volatile components rather than "discrimination"

against the high boilers because, in fact, **too much of the volatile material** is analyzed. However, such terminology has not become popular.

Samples of Broad Range of Volatility

Discrimination by selective elution from the needle is a severe problem for samples containing components of a wide range of volatility, particularly when some have **elevated boiling points**; it is mostly negligible when all solutes are volatile, and absent if gases are injected (including headspace analysis). Discrimination is one of the main reasons why the volatility of **internal standards** should be similar to that of the solutes of interest.

1.2.3. Poor Reproducibility

Deviations because of partial elution from the syringe needle call for compensation by means of calibrated correction factors (often wrongly termed "response factors"). The deviations are, however, usually poorly reproducible both within a series of injections of the same solution (random error) and between injections of different solutions, such as the calibration mixture and the samples. This results in **increased standard deviations** and possibly **systematic errors**.

1.2.4. Degradation of Labile Solutes

Degradation of labile solutes on the **hot metallic needle surface** or on the layer of contaminants deposited on the internal wall of the needle may be another problem. GC instruments are constructed such that the sample does not make contact with metal surfaces, but if a component evaporates from the needle wall, such contact is intense.

1.3. Conclusions

As sample evaporation inside the vaporizing chamber is linked with that inside the needle, Sections A and B are interrelated and are directed towards the following conclusions.

1.3.1. Fast Autosampler?

In the second half of the nineteen eighties, Hewlett-Packard introduced the fast autosampler which avoided sample evaporation inside the needle. For some time this seemed to be the solution of the problem, although it meant that manual injection was no longer equivalent – the **autosampler was no longer an automated version of manual injection**, but a different technique often producing significantly different results.

Handicapped Evaporation in the Injector

This conclusion was questioned again when it became obvious that the fast autosampler not only solved a problem, but also created a new one – it rendered sample evaporation inside the vaporizing chamber more difficult (*Qian et al.* [2]). **Figure A2** anticipates the conclusions of Sections A and B; there is a dilemma – performance regarding syringe introduction is traded against evaporation performance inside the vaporizing chamber.

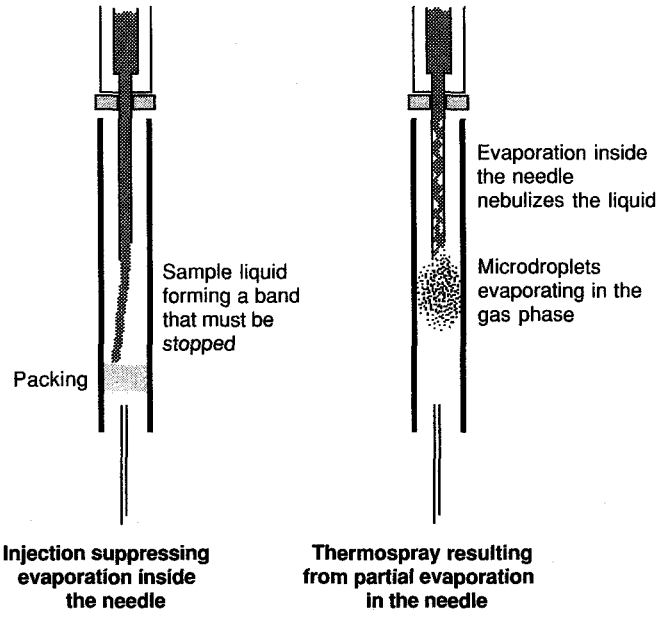


Figure A2
The dilemma regarding sample evaporation: fast autosamplers avoid evaporation inside the needle, but render vaporization inside the liner difficult. Slower injection causes partial evaporation inside the needle, which improves vaporization inside the liner by production of a thermospray.

1.3.2. Suppressing Evaporation inside the Needle

With regard to the accuracy of the sample volume injected and the composition of the sample analyzed, the **best techniques for introducing the sample** into a hot chamber are those preventing sample evaporation inside the syringe needle. This can be achieved by

- injection at a velocity such that heating and evaporation of the sample inside the syringe needle is avoided (fast autosampler),
- injection of samples in high-boiling solvents, or
- injection through a short needle.

Programmed temperature vaporization (PTV) and on-column injection are also solutions to this problem.

Band Formation

Injection suppressing evaporation in the needle causes the sample liquid to leave the needle as a band (jet). As this band moves at high velocity and covers long distances in hot chambers, it must be stopped by a **packing** (such as deactivated glass wool) or by obstacles (Section B). This may lead to losses of high-boiling, adsorptive, or labile solute material.

1.3.3. Thermospray

The most gentle sample evaporation inside the vaporizing chamber is obtained when some solvent evaporation inside the needle nebulizes the sample liquid at the needle

exit. The resulting microdroplets readily evaporate while suspended in the carrier gas. This avoids contact with adsorptive or contaminated surfaces.
Because vaporization inside the needle often causes uncontrolled elution, the technique must be optimized such that **transfer from the needle is as complete as possible**. Sample volumes will be too large, however, and discrimination against high boilers cannot be totally avoided.

2. Syringes

Here syringes suitable for vaporizing injection are described. Catalogs of syringe suppliers provide useful further information. A summary of the subject has been published by *Hinshaw* [3].

2.1. Plunger-in-Barrel Syringes

Figure A3 shows the front of the most commonly used microsyringe with a fixed needle. The needle is sealed into the glass barrel by means of a droplet of epoxy glue. The sample volume to be injected is measured in the barrel of the syringe and **does not include the liquid inside the needle**. Measurement assumes that the needle remains filled with liquid.

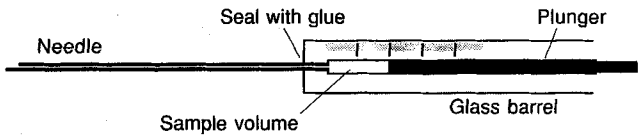


Figure A3
The most commonly used syringe with fixed needle and steel plunger.

2.1.1. Plungers

Steel plungers seal against the glass barrel by closely fitting dimensions: clearance between the plunger and the barrel is approximately 0.5 µm. Because the glass barrels and steel wires cannot be fabricated with the appropriate precision, plungers are **adjusted individually** by immersion in acid. This explains why plungers should not be exchanged from one syringe to another (if they seem to fit, they might not be tight).

PTFE Tips

Plungers with a PTFE tip have been less successful. They enable the production of syringes with exchangeable plungers at lower cost, but tightness usually becomes a problem after prolonged use.

Tightness of the Plunger in the Barrel

Moderately high pressures are encountered when the needle is inserted into the injector and the carrier gas inlet pressure is high. Far higher pressures can, however, be reached during depression of the plunger, because the cross section of the latter is only ca. 0.2 mm^2. Force on the plunger corresponding to 100 g (which is clearly more than normally applied) relates to 50 bar or **5 MPa**.

Maximum 80 % Withdrawal of Plunger

Tightness of steel plungers without PTFE tips depends on the **position inside the barrel** – the further the plunger is withdrawn, the shorter is the tight section. This is why it is sometimes recommended that the plunger is not withdrawn by more than about 80 % of the syringe capacity. This means that in a 10 µL syringe, the tip of the plunger should not be behind the 8 µL mark.

Viscosity of the Sample

Tightness also depends on the **viscosity of the medium** between the plunger and the barrel – seals are tight up to far higher pressures when there is a film of liquid instead of gas; the type of liquid (usually the solvent) also has a strong influence.
Syringes with capacities of 50-500 µL are available with steel plungers fitting tightly in the barrel (as for 10 µL syringes), as well as with "gas-tight" plungers equipped with PTFE tips. Steel plungers are more reliable because they are not deformed during prolonged use, as are PTFE tips. If they are used for injection of gases, however, tightness is critical because of the low viscosity of the gas.

Test of Tightness

In case of doubt, the tightness of the fit of the plunger in the barrel should be tested. For **injection of liquids** a solvent of low viscosity, such as hexane, is picked up and pulled backwards out of the needle into the barrel. The needle is inserted into an injector with a high gas pressure inside. If there is leakage, the meniscus of the liquid moves upwards and liquid accumulates in the region where the plunger leaves the barrel. The test becomes sensitive when the plunger is inserted a short distance only into the syringe and when waiting for a time longer than during a normal injection.
The most sensitive test involves a **dry syringe**. The plunger is pulled out of the barrel and allowed to dry. The needle is introduced into an injector, causing a stream of carrier gas to flow backwards through the syringe and dry it. The plunger is then re-introduced to the level to be tested and a drop of a solvent of low viscosity (such as hexane) is placed around the plunger where it enters the glass barrel (**Figure A4**). Some liquid flows into the narrow gap between the plunger and the barrel. Escaping gas (leakage) is sensitively detected by visual observation.

2.1.2. Plunger Guides

With manual injection, death of syringes most frequently results from **deformation of the plunger** – when not de-

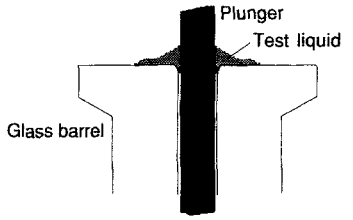


Figure A4
Test of the tightness of the plunger by application of a drop of liquid in the region where gas would leave.

pressed concentrically, the steel wire is bent. Plungers cannot be re-straightened properly, because there remains a deformation that rubs on the glass wall. This hinders fast depression (as required for hot needle injection). Grayish sludge containing fines from the plunger and the glass soon further hinders the movement of the plunger. The plunger guide was introduced as a solution to this problem.
The plunger guide can also be of advantage for the injection of **samples in highly volatile matrices**, because warming of the barrel by the fingers can be avoided.

Elongated Barrel

SGE elongated the glass barrel by adding a region of wider bore in which a thicker rear part of the (also elongated) plunger moves (**Figure A5**). Only this robust thicker section leaves the barrel. Hamilton produces removable metal plunger guides working on the same principle. One drawback is that the syringe is heavier and more difficult to handle with one hand only.

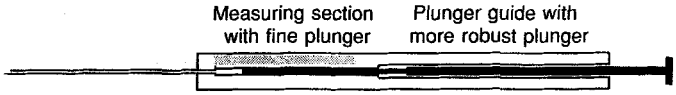


Figure A5
Syringe with plunger guide.

5 µL Syringes

As prices of syringes decreased, fewer 10 µL syringes with plunger guides are used. For 5 µL syringes, however, the use of a guide is recommended. Their plunger has only half the cross section and is bent correspondingly easily.

Reinforced Plunger Neck

Because a high proportion of all plungers are bent when they reach the zero position (they are pushed excentrically into the barrel), SGE produces syringes of standard length, but with reinforcement of the last section of the plunger that enters a specially designed nut at the rear of the barrel. The plunger button is reinforced also. This facilitates fast depression of the plunger as needed for the "hot needle" technique.

Flexible Plunger

SGE also offers a syringe with an elastic plunger which cannot snap off or be deformed permanently.

2.2. Plunger-in-Needle Syringes

Plunger-in-needle syringes keep the **sample inside the needle**. The plunger is equipped with a thin wire protruding into the needle to displace the liquid (**Figure A6**). The barrel of the syringe indicates the position of the wire inside the needle, but does not make contact with the sample. All the liquid is displaced.

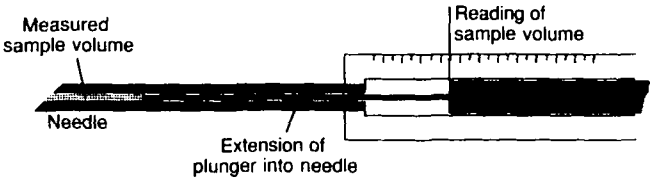


Figure A6
Plunger in needle syringe.

1 µL Syringe

Plunger-in-needle syringes of 0.5 to 25 µL capacity are available commercially, but only the 1 µL syringe has found widespread use. It enables accurate measurement of ten times **smaller sample volumes** than standard 10 µL syringes, *i.e.* as little as 0.05 µL, and suggests itself for the injection of non-diluted samples. Standard needles are 56 or 70 mm long. 56 mm needles have a 90° cut at the outlet; the internal and external diameters are 0.15 and 0.70 mm, respectively. 70 mm needles have a 17° tip; internal and external diameters are 0.15 and 0.47 mm, respectively.

Problems

There are several problems connected with injection into hot chambers; they will be discussed in Section A9. Cleaning is more difficult and there is no visual control of whether or not air bubbles are included in the sample plug.

No Withdrawal of Plunger

One should resist the temptation to take a look at the fine tungsten wire serving as the plunger – after the plunger has been fully withdrawn from the syringe, it is **extremely difficult to insert it again**.

2.3. Syringe Needles

2.3.1. Dimensions

Needle diameters are standardized by "**gauge**". Those most important for GC are listed in **Table A1**.

- The **internal diameter** is kept as small as possible to minimize the inner volume of the needle (extra volume being transferred when the needle is heated). On the other hand, the needle should not cause build up of an excessive pressure drop, because this hinders sucking up the sample liquid, particularly when volatile solvents are involved.
- The **outer diameter** is a compromise between robustness and a minimized effect on the septum.

Length

Standard syringes are equipped with needles **51 mm long** (2 inches, including the section glued into the glass barrel). As will be shown later, split injection at low split ratios and splitless injection often require longer needles, commonly

Table A1
Diameters of the most important syringe needles and internal volumes for needles of 51 mm length.

Gauge	Diameters (mm) internal	external	Internal volume (µL)	Main use
22	0.41	0.72	6.73	Headspace
22S	0.15	0.72	0.90	Autosampler
23	0.64	0.34		Autosampler
23S	0.64	0.15		Autosampler
25S	0.15	0.52	0.90	Autosampler
26	0.26	0.46		
26S	0.13	0.47	0.68	Manual injection

71 mm (3 inches). For injection with band formation, 3.7 mm (1.5 inches) needles are most suitable.
Gas syringes for **headspace analysis** should have an 80 mm needle with a side port hole. Syringes with needles of custom length are available at a small extra cost.

2.3.2. Needle Tips

Beveled Tips

The standard style needle tip for injection through a septum, the beveled point, is polished at an **angle of 17-20°**. The tip is bent slightly inward, *i.e.* towards the center of the tubing, for better displacement of the septum and to reduce the chance of the needle cutting away a particle of the septum material.
The tip is **easily bent**, *e.g.* after the syringe is dropped on the floor. The deformation is more easily felt by sliding the fingers over the needle tip than seen by eye. It affects the way the liquid exits the needle (see Section B3.2) and scrapes a hole into the septum. Needle tips should, therefore, be regularly checked.

Conical Style

Syringes for autosamplers, in particular, are often equipped with conical style needles – cut squarely, but polished to a cone with an 8° angle. If they always pierce the septum at the same position, they are supposed to **reduce septum coring** (and resulting deposition of particles inside the vaporizing chamber).

Side Port Hole

The tip of needles with a side port hole is closed to a rounded point. About 1 mm back, there is a small hole in the side wall. This needle style practically rules out cutting of septum particles and is unlikely to be plugged, which is particularly suitable for **headspace syringes**, because other needles tend to be plugged. They have, nevertheless, never become popular.
For injection of **liquid samples**, release through the side port influences sample evaporation and distribution within the vaporizing chamber – sometimes advantageously, sometimes not.

2.3.3. Fixed versus Removable Needles

Most manufacturers offer syringes with fixed needles, cemented into the barrel at a position corresponding to the zero graduation, or removable needles, tightened against the barrel with a small PTFE ferrule. When a fixed needle is damaged, the entire syringe must be replaced; this is probably the only argument in favor of the removable needle.

Problems with Removable Needles

Prices of syringes with removable needles are substantially higher, and this investment is justified only when the needle is ruined rather frequently. Furthermore, connection of the needle to the barrel can be a problem, firstly, because it usually retains some air and **encourages bubble formation**, like a boiling stone, when picking up volatile solvents. Secondly, some connections have significant **dead volume** – sample material enters this by diffusion, particularly when the syringe is lying around after the injection with sample liquid remaining in the critical region. Because rinsing with solvent does not clean dead volumes, this readily generates "memory effects".

2.4. Cleaning of Syringes

Before investing much effort in sophisticated procedures for cleaning syringes, it is useful to consider some general rules which help minimize the effort required. Such rules might even become **parts of validated methods**, because the reliability of the results easily depends on them.

2.4.1. Basic Rules

Classify the Cleaning Required

When performing series of analyses, **it is usually sufficient to remove 99 %** of the material from the previous sample, because solute concentrations vary by less than a factor of 10. Such cleaning is readily achieved.
At the opposite end of the scale of difficulty, a syringe might first be used to prepare a standard solution, measuring a **neat substance**. Afterwards it is used for injection in **trace analysis**, in which picogram quantities of the same component, levels maybe 100,000,000 times less, are detected. Cleaning the syringe to remove 99.9999999 % of the material is virtually impossible.

Use the Same Syringe

Use the same syringe throughout a series of analyses (as autosamplers inevitably do). This renders the requirements **more transparent**. It rules out introduction of materials from other sources (such as from the preparation of a standard solution). It also ensures that the sample always leaves the needle in the same way – small deformations of the needle tip may have a strong effect on the evaporation process (*e.g.* through a spray effect).

Estimate the Required Cleaning Effect

If the samples contain the solutes of interest in amounts varying by not more than one order of magnitude (*e.g.* analysis of the fatty acid composition of edible oils), 99 % cleaning is sufficient. In the analysis of pesticide residues, a high concentration might be 100 times above the lower detection limit

of the method, *i.e.* 99.9 % cleaning guarantees that subsequent samples will be free from residues, *i.e.* there is no memory effect.
Efficiency of 99.9 % is probably about the limit of reliable syringe cleaning by autosamplers or manual injection without special precautions. If higher efficiency is required, a blank must be run after the analysis of every sample.

Beware of Concentrated Samples

A common experience, *e.g.*, in residue analysis, is that a highly concentrated solution of a standard is injected to find the peak of interest (setting up the method). The samples analyzed subsequently are all positive. As the analyst recognizes that his results are puzzling, he runs a blank and confirms the carry-over. It is concluded that injection of highly concentrated solutions should be avoided and that blanks must be analyzed before running the first analysis.

Separate Syringes for Adding Standard

Although addition of standards by use of a 10 µL syringe is not highly accurate, it is frequently used in the interest of working with small sample sizes and vials. The danger of this procedure is that the same syringe is subsequently used for injection of the sample. As the standard solutions are usually **100-10,000 times more concentrated**, cleaning is demanding.

Label Syringes

It is convenient and advantageous for the reliability of the results to use **different syringes for different purposes**. It might be necessary to label them to rule out confusion, *e.g.* by use of colored rings at the top of the glass barrel. Alternatively, syringes with especially short needles can be used for purposes other than injection.

Silylation Reagents

If samples contain high concentrations of derivatization reagents, such as for silylation or acylation, the syringe must be **cleaned immediately after injection**, since hydrolysis by humidity from the air easily plugs the needle otherwise.

2.4.2. Cleaning Procedures

Movement of the Plunger

The most simple cleaning procedure is moving the plunger up and down. The effectiveness of this procedure is limited by the **volume of liquid inside the needle**, which is moved up and down without really being replaced – it is merely mixed with the solvent or the subsequent sample.
Because **turbulence** caused by transition from the narrow-bore needle into the wider barrel provides most of the mixing, the liquid should be withdrawn as fast as possible. Rapid suction also prevents all the material deposited on the syringe wall dissolving in the first small amount of liquid entering the needle; this is most difficult to remove afterwards. Use of autosamplers shows that reliable 99.9 % cleaning is achieved in this manner.
Sample material **between the plunger and the barrel** is not efficiently removed. The amount is, however, small – if there is a 1 µm gap between the barrel and the plunger, this

volume amounts to ca. 0.7 % of the whole internal volume of the barrel and 99.9 % cleaning efficiency is, hence, hardly endangered.

Solvent or Subsequent Sample?

Often syringes are not cleaned with solvent, but with the subsequent sample. Whether or not this is acceptable is determined by the tolerable carry-over. Material from the first sample corresponding to a volume of about 1 µL might be transferred into the following sample. If it is assumed that the component of interest was present at a concentration 100 times higher in the first sample and that the volume of the second sample is 10 mL, contamination reaches 1 %. If the sample volume is only 1 mL (autosampler vial), contamination reaches 10 %. Cleaning with the subsequent sample is, hence, acceptable **if 90-99 % cleaning efficiency is sufficient**.

Discharge Backwards

When performed manually, cleaning efficiency can be substantially improved by passage of a plug of liquid backwards out of the syringe. Some 5 µL of liquid is sucked into the syringe and the plunger is removed from the barrel. At this moment, the 5 µL are in the upper region of the barrel. The **syringe is then shaken sharply** such that most of the liquid leaves the barrel. In this way, the poorly exchanged plug is removed and the whole channel in the barrel is rinsed. The plunger can be immersed in solvent to clean its outside before it is brought back into the syringe.

Vacuum

A source of vacuum can be used to **suck solvent through the syringe**. The plunger is pulled out of the barrel, the needle immersed in a suitable solvent, and the vacuum applied. Soft **rubber or silicone tubing** connecting to the vacuum is suitable – if the rear of the syringe is pressed against it, sufficient tightness is obtained. The plunger is again rinsed before being re-inserted. A weak vacuum is preferable, particularly for a volatile solvent, because a strong vacuum causes evaporation instead of rinsing.

Pressurized Solvent

Syringe cleaners are available consisting of a solvent container connected to a source of pressurized gas. They are equipped with a **septum** through which the syringe needle is introduced. The plunger is removed, opening the way for the solvent to rinse the syringe needle and the barrel. The solvent may need frequent replacement, not least because septum particles tend to accumulate and release silicone components which show up in the chromatograms.

Drying the Syringe by Vacuum

Other syringe cleaners (*e.g.* Hamilton, SGE) heat the needle and evacuate it. The needle is introduced through a septum into a **chamber that can be heated to 380 °C**. The plunger can be moved backwards and forwards to move the vapors, or removed completely to allow passage of a stream of air.

Such a device **cannot eliminate high-boiling or involatile material** – on the contrary, once lacquered at the high temperature, it can no longer be removed even by use of solvent, as observed for injector liners. Hence the syringe should be rinsed with solvent before introduction into this type of cleaner.
The device is particularly effective for **plunger-in-needle syringes**, because the whole part in contact with sample liquid is heated.

Drying in the Injector

An equally efficient method simply uses a normal vaporizing injector. The plunger is removed and the syringe needle is inserted through the septum. With a low pressure in the injector a **stream of carrier gas purges** the volatile material from the syringe. Because the syringe is purged outwards, no material enters the injector.

2.4.3. Plugged Needles

Needles may become plugged, *e.g.*, after injection of silylated or trifluoroacetylated samples containing high concentrations of residual reagent. When the syringe is left for some time, hydrolysis forms a plug near the tip of the needle. Plugged needles **should not be cleared by applying high pressure** to the plunger because all too easily the barrel cracks (pressures exceeding 100 bar are easily reached).

Cleaning Wire

New syringes sometimes contain **thin wires** in the needle which can be used to unblock the needle. Hamilton and SGE supply thin tungsten wires for the same purpose.

Heating

A rapid method involves warming of the needle at the site where blockage is assumed. Some solvent is placed in the barrel from the rear – by removing the plunger and introducing solvent by means of another syringe with a long, thin needle. Modest pressure is then applied to the plunger while the needle is warmed gently in a yellow flame (*e.g.* cigarette lighter). The plug softens and is displaced by the solvent, which flushes the needle. The needle must not reach high temperatures, however; otherwise it turns permanently soft.

2.4.4. Blocked Plungers

The plunger moves with difficulty if **gray sludge containing the fines of abraded glass and metal** accumulates between the plunger and the barrel. Solvent usually does not remove it.
Although against the advice of syringe manufacturers, pulling the plunger through the **fingers** removes such material rather efficiently and can solve the problem if repeated.
If this does not help, the sludge must be removed by use of **hydrochloric or phosphoric acid**. Immediately afterwards, the syringe and the plunger must be thoroughly rinsed with water and a solvent, such as ethanol or acetone. Alkali must be avoided because it attacks the glass.

3. Evaporation Inside the Needle

When the plunger of the syringe is pulled upwards after a manual injection of a solution in a commonly used solvent, hardly any liquid is seen clinging to its tip (plunger-in-barrel syringe). This implies that not only the volume of sample read on the barrel of the syringe was injected, but also that which should have been left inside the needle. What we see by eye, however, is the sample solvent, which is the sample component of least interest.

Manual Injection

This section deals with sample (solvent) evaporation inside the needle and transfer of solute material as it occurs with manual injection or with autosamplers which imitate this. It does not apply to autosampler injection at such a speed that evaporation inside the needle is suppressed.

3.1. The Three-Step Model

At first, the problem seems to be the fate of the liquid remaining inside the needle after the plunger was depressed. A closer look reveals that things might be more complicated.

Assumptions

Below we consider the injection of **1 µL** of liquid measured on the barrel of a syringe equipped with a 71 mm **needle of 1 µL internal volume**. We assume that the liquid is withdrawn into the barrel of the syringe before introduction of the needle into the hot injector.

1. Evaporation of the First Liquid

The first 1 µL of liquid injected, which is actually that left in the needle and not that observed when measuring the sample volume, encounters a needle wall which has been heated above the solvent boiling point, primarily during passage through the septum. **Violent evaporation** is initiated – vapors formed along the needle wall push some of the liquid out of the needle. Overpressure is built up, increasing the boiling point of the solvent; when the liquid leaves the needle, it explodes into small droplets, driven apart by the vapors (**thermospray**).
The evaporating solvent leaves high-boiling material on the needle wall, because the temperature of its environment does not exceed the (pressure-corrected) boiling point of the latter (left in **Figure A7**).

2. Cooling of the Needle Wall

Consumption of heat by the evaporating solvent cools the surface of the needle wall. When its temperature falls to the solvent boiling point, the **sample liquid wets the wall** and the following **liquid passes without evaporation** (center

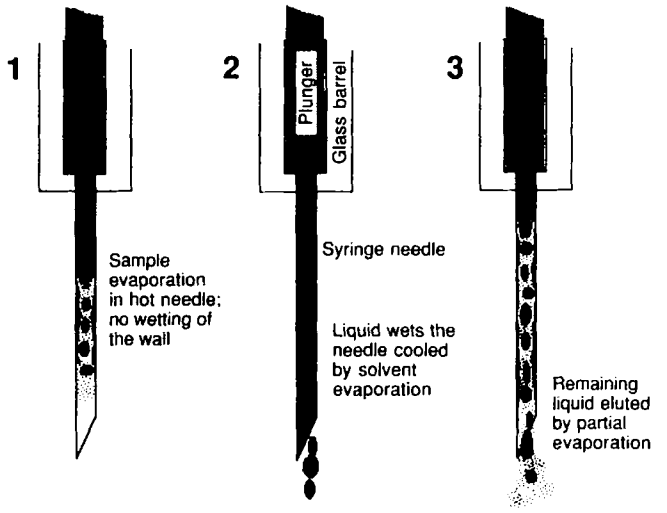


Figure A7
Three steps during injection through a hot syringe needle.

in Figure A7). The liquid might even re-dissolve the material previously deposited on the needle wall and carry it into the injector.
This picture obviously simplifies – only continuing solvent evaporation keeps the needle temperature at the boiling point. When the surface is wetted again after the formation of some vapor, however, the essential point is still achieved: **transfer without loss of high-boiling material**.

3. Expulsion of the Remaining Liquid

When the plunger reaches the bottom, the syringe needle is filled with the second microliter of liquid (that observed in the barrel). Before the needle can be withdrawn, its surface is again **heated above the solvent boiling point**, causing the content to undergo partial evaporation; a mixture of vapor and droplets is ejected into the vaporizing chamber. Again high-boiling solute material from the evaporating liquid is left on the needle wall.

Effect on Discrimination

Steps 1 and 3 in Figure A7 result in loss of high-boiling solute material as a result of incomplete sample evaporation on the needle surface. If the internal wall is sufficiently cooled to enable step 2, however, **losses occurring during the first step are recovered**.

Sufficient Cooling for Wetting?

It is largely speculation whether cooling is sufficient for a step 2. If we assume that the plunger is depressed at a velocity of 1 m/s, liquid enters the needle during a period of 15 ms. Partial evaporation of 2 µL of liquid absorbs a considerable amount of heat, but the heat capacity of the needle far exceeds the heat consumed (the mass of the needle exceeds that of the sample by a factor of about 25). The sample can,

therefore, **cool a thin surface layer at best** and, because of the high thermal conductivity of the metal, the cooling process must be very rapid if it is to be quicker than the rate at which heat is supplied. This also means that the temperature increase in step 3 is rapid – too rapid to give us a chance of (manually) withdrawing the needle before evaporation starts again.
Experimental data on losses in the needle suggest that liquid does wet the needle wall if the sample volume exceeds a certain minimum and depression of the plunger is fast. The videos on the CD, on the other hand, do not support this since a band of liquid should then be expected to leave the needle.

3.2. Models of Evaporation inside the Needle

Losses of high-boiling solute material depend on the specific nature of the injection. It is helpful to consider the three models below which describe how the solutes can leave the needle. First we concentrate on the liquid remaining inside the needle after depression of the plunger.

3.2.1. Distillation from the Needle

If the sample evaporates fully, only vapor leaves the needle. Vapor is expelled because of the **expansion in volume accompanying evaporation** (a factor of 100-500). According to the most simple model, transfer should be almost complete, as the volume of vapor remaining in the needle is less than 1 % of the original liquid content (0.6-1 µL of the 100-500 µL of vapor formed). This assumes that all of the sample is vaporized at once.
If a needle temperature of 200 °C is assumed (in an injector thermostatted at 250 °C), the distillation model would predict that of the *n*-alkanes only those with a molecular weight below that of *n*-undecane should reach the injector. It is, however, obvious that this does not accord with common experience.

Theoretical Treatment

Guha [4] studied the effects of sample evaporation inside the needle both theoretically and for some test mixtures, assuming complete evaporation and a distillation-like model. He used basic gas laws to calculate the effect of needle size, injector temperature, carrier gas inlet pressure, and sample volatility on the amount injected. The conclusion was that representative sampling could be achieved only by use of plunger-in-needle syringes without dead volume in the needle.

3.2.2. Gas Chromatography in the Needle

The above distillation model is inadequate, because it is **not generally necessary that the solute vapor reach atmospheric pressure** to leave the needle. In particular, the material deposited on the needle wall near the exit of the needle is well flushed out of the needle by the passage of the vapor of the volatiles (solvent).

Small Vapor Pressure Suffices

Transfer of high-boiling material through and finally out of the syringe needle resembles a gas chromatographic process – the needle is the capillary column, the condensed sample material and the contaminants from previous injections on the needle wall are the stationary phase, and the vapor of the sample (solvent) evaporating in the rear of the needle is the carrier gas (see upper scenario in **Figure A8**).

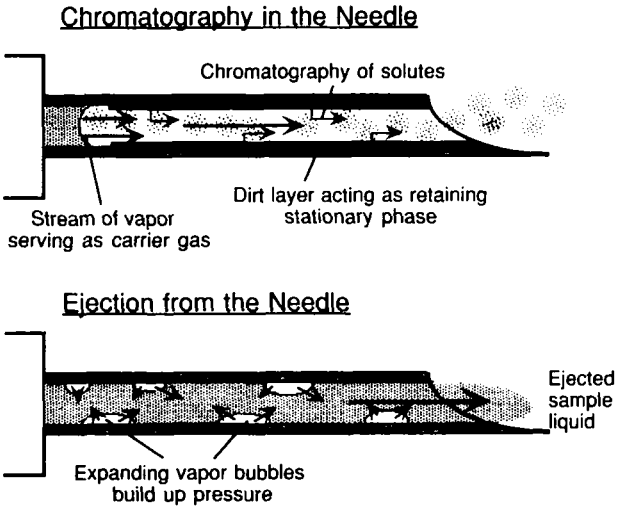


Figure A8
Two models describing the elution of the sample from the syringe needle at the end of the injection.

The components are partitioned between the gas (vapor) phase and the liquid phase on the needle wall in accordance with their vapor pressure. A **small amount evaporates**. This vapor is immediately **removed by the stream of solvent vapor**, which prompts more solute material to evaporate, etc. This model correctly predicts that the solutes eluted may include components which boil at temperatures far above that of the needle.

3.2.3. Ejection from the Needle

The above models require fairly gentle evaporation conditions inside the needle, in particular an amount of time which is usually not available. This gives rise to a third mechanism, which again is not realistic in the extreme form.
Rapid depression of the plunger might introduce the plug of liquid into the syringe needle at a speed such that no significant evaporation occurs until the plug is fully introduced. Violent evaporation on the needle wall then forms bubbles of rapidly expanding vapor, building up high pressure and discharging the liquid through the center of the needle. **Ejected liquid carries all dissolved sample material out of the needle, irrespective of volatility**. Losses and discrimination are restricted to the amount of solution evaporated on the needle wall.