

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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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.

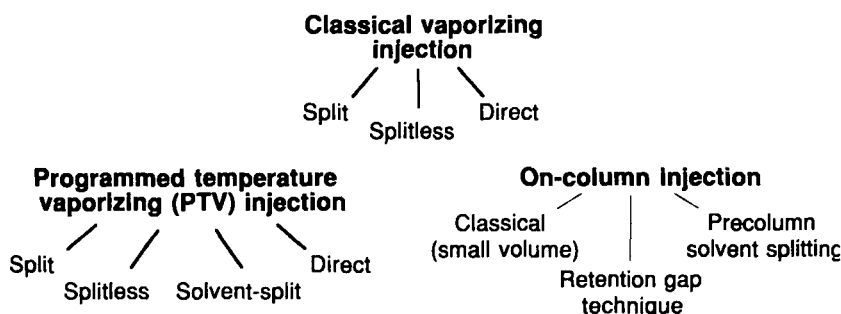
Fehraltorf, October 2000

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.

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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.

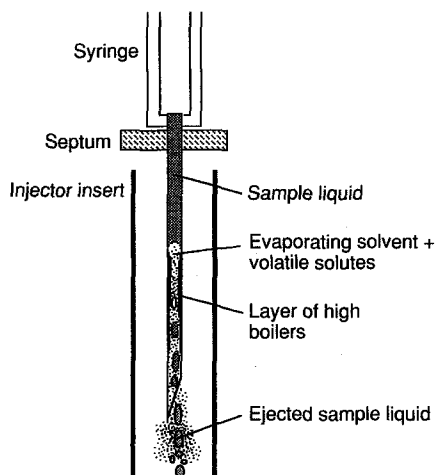
1.2. Sample Evaporation inside the Needle

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

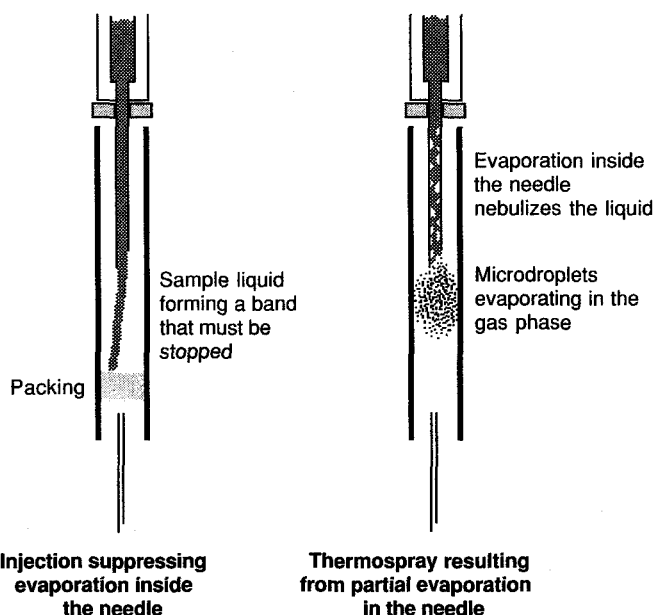
As sample evaporation inside the vaporizing chamber is linked with that inside the needle, Sections A and B are inter-related and are directed towards the following conclusions.

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.

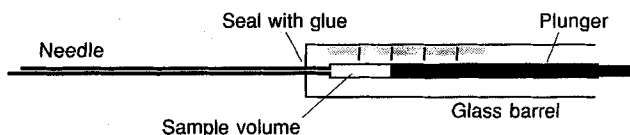


Figure A3
The most commonly used syringe with fixed needle and steel plunger.

2.1.1. Plungers

Steel plungers seal against the glass barrel by closely fitting dimensions: clearance between the plunger and the barrel is approximately $0.5\ \mu\text{m}$. 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 mm². 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.

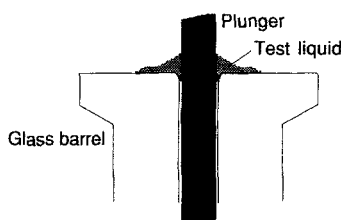
Test of Tightness

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**

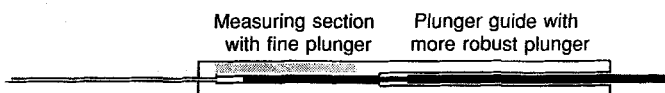
Test of the tightness of the plunger by application of a drop of liquid in the region where gas would leave.

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 μ L Syringes

As prices of syringes decreased, fewer 10 μ L syringes with plunger guides are used. For 5 μ L 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.