# Koni's Korner

## Certification of injectors and injection techniques? Comments on splitless injection by readers.

*by Dr. Konrad Grob*

In the spring and summer 1996 issues of *The Restek Advantage,* I posed the question of whether the most frequently used injection technique in capillary GC, splitless injection, is as mature as one tends to think. I traced its history to show that there has never been the systematic optimization and testing many think should have happened. Nobody felt responsible: Users assumed that instrument manufacturers provide exhaustively tested injectors and working instructions, whereas instrument companies just produce what they think "science" wants. But who is "science"?

Optimization of a technique as complex as splitless injection is work of such a volume that it cannot be accomplished on a single Friday afternoon, when the work of the week is completed. One of the open questions concerned sample evaporation in splitless injection. Should the liner be empty or packed? Should it have a constriction at the bottom? I was hoping for contributions by those routine users who must have found an answer in one way or another, but only received more general comments, three of which I want to bring up here.

**Who introduced splitless injection?**

Leslie Ettre was upset by my saying that my father introduced/invented splitless injection. Indeed, non-splitting injection was used from the very beginning of capillary GC, in particular before splitting was invented. I want to apologize for not having mentioned this. In response to him, my definition of "splitless" injection is not any non-splitting injection technique, but that of using an injector with a split outlet which is closed during the splitless period. At least in Europe, "direct" injection has always been distinguished from "splitless" injection.

**Accelerated transfer through increased flow**

E.H. Foerster, from Southwestern Institute of Forensic Science in Dallas, Texas, found that the analysis of low concentrations of certain active drugs (he named alprazolam, trazodone, and quinidine) was possible by split, but not by splitless injection (4 mm i.d. liner with glass wool). He could improve the results from splitless injection approximately four fold by increasing the carrier gas inlet pressure (gas flow rate) during a 1 min. transfer period after injection. He explained this by the reduced residence time in the injector during split or accelerated splitless injection. The same argument was used by Hewlett-Packard in favor of what they termed "pressure pulse".

An increase of the flow rate by a factor of four is possible only if initial inlet pressures are modest and, nevertheless, does not seem overwhelming: it reduces reaction time by a factor of four. However, the effect could be more than proportional, since the sample liquid deposited onto the packing initially forms an island cooled to the solvent boiling point. A high flow rate might remove the solute material from these surfaces before they have reached the injector temperature again. If evaporation occurred in the gas phase, the fog of the non-evaporated solute material could have been transferred into the column before it settled onto the packing material. Unfortunately, Mr. Foerster did not compare the performance of the packed liner with that of the empty liner, because gas phase evaporation is usually still most gentle (but not always complete).

**injector overloading**

Gary Kellog, from the Springfield, Missouri Public Health Department, drastically illustrated the effect of overloading too small vaporizer chambers. "Last February we received a new GC/MS system, including a Varian 1078 temperature programmable split/splitless injector. At about the same time, I received my first copy of *The Restek Advantage* including your article on injector design and sample introduction. I had never used a split/splitless injector before. The old instrument was set up with a flash vaporization injector with a 0.53 mm ID column, and it didn't take long to realize that the old operating parameters would not work on the new system. When I began to calculate the vapor volumes and the liner volumes (54 mm x 0.8 mm ID with 9 mm column installed height, methanol as solvent), it was obvious that a lot of my sample was going into places other than the column. Due to the limited size of the 1078's liners (54 mm long), I chose the largest ID liner offered (3.4 mm), added a 1 cm plug of deactivated fused silica wool placed above the installed column height, and began to experiment with the temperature programming on the injector. I also switched to a lower vapor volume solvent, with a higher boiling point to take advantage of solvent effects (toluene)."

Gary Kellog used a mixture of pesticides to compare the peak areas obtained by the old conditions (0.8 mm i.d. liner, 250°C) with those he introduced recently (3.4 mm i.d. liner, injector programmed from 200 to 300°C). The detector, column, injection volume, and other conditions

| | Peak area x $10^6$ | | |
|---|---|---|---|
| **Compound** | **0.8 mm i.d.** | **3.4 mm i.d.** | **Difference** |
| alpha HCH | 0.63 | 4.16 | 7.85 |
| diazinon | 0.69 | 6.34 | 9.19 |
| heptachlor | 0.49 | 3.98 | 8.12 |
| endrin | 0.41 | 2.32 | 5.66 |
| p,p'-DDT | 0.72 | 5.00 | 6.94 |
| coumaphos | 0.69 | 2.08 | 3.01 |

were identical. Results were obtained with toluene as the solvent, which must have improved them substantially. From a long list of results, I just want to cite a few.

The results show drastic (66-89 %) losses of solute material with the small vaporizer chamber, but also that losses are different for each component. This was no surprise. The usable volume in this vaporizing chamber was 23µl. 1µl of methanol (which he usually used) must have produced 600-700p.l of vapor (@ 250°C injector temperature & intermediate inlet pressure). Even if the needle was only partially emptied, the injector was overloaded more than 40 times. 1µl of the toluene actually used produces only about 200µl of vapor. Losses of solute material are usually smaller than those of the solvent, because solutes may be deposited onto surfaces cooled by the evaporating solvent-but the process is poorly controlled. It is as if an analyst would spill more than 90 % of the solution during titration and then be surprised that results are poorly reproducible. There is no pool of liquid running out of the GC instrument, which in turn explains why so many people "spill" in the GC inlet without noticing it.

Gary Kellog's new injection technique might perform correctly, although it involves unusual conditions. He introduced his solution in toluene (b.p.110°C) into the PTV at 200°C. Standard working rules would require an injector temperature at, or below, the pressure-corrected solvent boiling point, in order to prevent rapid expansion of the vapors. He calculated that the chamber has a usable internal volume of 354µl, which should be sufficient to store the vapors even when considering that they will mix with the carrier gas present in the injector. A 2µl volume (or 1 µl of a solvent producing more vapor), however, would again be too much. Further, he applied some glass wool, which might retain the solutes when solvent vapors expand out of the injector chamber.

**Confusing injection conditions**

**Gary** Kellog plans to carefully test his injection conditions, maybe by comparison. with on-column injection. However, does it really make sense that every gas chromatographer develop his own conditions to get his sample into the column?

The comment by Gary Kellog demonstrates how chaotic injection in capillary GC still is. In HPLC, injection just requires filling of a sample loop without air bubbles and that the sample solvent is not too strong an eluent. It is standardized and essentially the same for all instruments. It is totally different in GC. Every instrument manufacturer seems to be proud of producing something different than the others and giving their injector another name. Did you ever count the names given to temperature-programmable injectors? Manuals do not provide sufficiently clear and safe rules on how to operate the device and warnings on where the limitations are. Confusion among the non-specialists is inevitable.

Why didn't anybody tell Gary Kellog that his old injector cannot be used in the way he used it-and how many others continue to do the same? Why didn't he know that with his new injector he can inject up to about 50µl (quite regardless of the vapor volume formed), provided he keeps the chamber below the solvent boiling point for the time of solvent evaporation?

**Why are injectors and injection techniques not validated?**

Today, splitless injection is frequently performed with too small vaporizing chambers, too short syringe needles, poorly suited carrier gas supply systems, excessively large samples, by the cool instead of the hot needle technique (or vice versa), by slow instead of rapid injection, with too low carrier gas flow rates, wrong column temperature during the sample transfer, too short splitless periods, packings in the liner at the wrong site, and without information on what all the critical parameters are. Properly written methods should specify all these conditions in at least as much detail as they specify sample preparation by saying that the flask must be rinsed twice and the solvent combined.

Analytical methods are validated in order to demonstrate the reliability of results. Chemicals, balances and pipettes are usually of certified quality and performance. Users check them ever so often. GCs are also checked. Oven temperatures are measured-as if this would be a critical parameter. Methods describe all steps of sample preparation in great detail, but when they reach the injection of the sample into GC, they become extremely short. Their authors would say that they cannot write as many versions as there are instrument manufacturers. True. But many users would badly need instructions, especially if their instruments work properly at best under special conditions.

The quality management people might not have realized the potential of the errors occurring during injection, as shown by the above example, it is many times larger than that of a balance. How can they validate methods if one of the principal sources of error remains out of control? Maybe they did realize the problem, but felt unable to make valid suggestions. Methods cannot be validated for all the different injectors on the market, nor can they require the use of an injector from a particular manufacturer. They must assume a properly working injection system and the application of validated working rules for that system. These rules do not exist. At least for the time being, the concept of validation reaches its limit at this point. It underlines that capillary GC is not a simple technique and it relies a great deal on the expertise of the operator.

**3 Final Points**

1) Does it really make sense that every gas chromatographer finds his own way to get his sample into the column?

2) How can methods be validated if one of the principal sources of error, injection, remains out of control?

3) Methods cannot be written in as many versions as there are instrument manufacturers.

I welcome your feedback.
Reach me by e-mail at
Konrad.grob@attach.ch.