Koni's Korner

EDITORIALS & ARTICLES FROM THE RESTEK *ADVANTAGE*

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Back to top of page

EDITORIAL

Assuring Accuracy of GC Results

Direct Verification within Methods

by Dr. Konrad Grob, Kantonales Laboratory, Zurich

Accuracy and reliability of analytical results are often a problem. At best, errors are just embarrassing. More often, however, analytical results determine whether a product is decreased in price or declared as adulterated. Water supplies may be closed. Thus, errors may have severe financial and/or legal consequences. No surprise that the boss taking action asks twice about the reliability of the results. All of us analysts know (luckily more often than the boss) how easily a result may turn out wrong. The problem has, of course, been recognized in most laboratories, and recently a whole avalanche of measures have been taken to remedy the situation. Some of the efforts have substantially improved the situation. For many types of errors, however, I am skeptical because they will hardly be eliminated by the general schemes as are the current trend. Their detection presupposes more work in the lab, improving the processes and techniques involved. Elements should be added to methods that enable immediate recognition of possible deviations for each analysis. Such direct verification will render results reliable.

In the past: standard deviations

In earlier times, results were primarily checked by repeatability. Statistically minded people wanted the analyses to be repeated many times so they could calculate the probability that the results were correct. It was assumed the values obtained would have a Gaussian distribution around the true result, i.e. there would be primarily random errors.

The more practical rule says that a result is OK if the same numbers are obtained three times. For an old hand the rule was refined. If the first of three results deviated excessively, he would make a fourth attempt and if this last result fitted results no. 2 and 3, the first would be disregarded - because the first determination tends to be wrong anyway.

This is all nonsense, of course! Totally wrong results can also be reproducible. Reproducibility quantitates random deviations, but not systematic ones, i.e. the minimum guaranteed uncertainty rather than accuracy. If results are poorly reproducible, we should conclude there is something fishy about them, but we cannot reverse this, deriving accuracy from a low standard deviation. Most of the severe errors are, in fact, due to systematic deviations, i.e. one of those many traps involved in an analytical procedure. Glassware was not cleaned properly, a batch of dichloromethane contained too much hydrochloric acid, injection desorbed material from a corner, the previous blank test has not been checked. The experienced analyst knows dozens of such stories and probably performed repeatability tests to get his salary at the end of the month rather than because he believed in their usefulness.

EDITORIAL

Food Technology -- Are We as Safe as We Think?

by Dr. Konrad Grob, Kantonales Laboratory, Zurich

Food technology is developing at an ever-faster rate. Clever treatments can make meat look more attractive or convert by-products to a well-paid food component; modifications or additions enable sales-stimulating health claims and new packaging materials allow longer storage. To the shareholders, the strategy of pushed innovation is presented as a means to increase profitability. To the consumers, it is presented as a means to make "improved" products. Innovation to find cheaper technology (e.g., the addition in wine of extracts from oak chips instead of storing the wine in oak barrels) is almost inevitable in a competitive economy.

I do not want to discuss the benefits of such innovation, but instead draw attention to the risks. Those responsible for the innovation say they conduct thorough "scientific" research and "rigorous control" of all risks, but when food control authorities ask specific questions, they usually discover the research was not done because it would have been too expensive or there was no time because of the intense competition. An example: it is about two cents (US) cheaper to remove the free fatty acids from a liter of raw edible oil by steam treatment compared to the older method of extraction with alkali. As steam treatment requires heating the oil at 240-260°C for a few hours, it seems obvious that the effects of such extremely high temperatures should be investigated. For instance, up to 60% of the essential linolenic acid is isomerized to various trans components. The industry never checked whether this can be tolerated. I hope the consumer profits from the two-cent savings -- he carries the risks anyway.

By eating we expose our bodies, perhaps our most valuable asset, to these products. Often only in the hospital (e.g., when cancer ruins our organism) do we start seriously thinking about such risks. Nobody knows how many cases of cancer, heart disease, obesity, and other diseases are caused by unwanted side-effects of food technology, but it might be many more than we think. During the last 15 years, Europe has dealt with the threat of bovine spongiform encephalopathy (BSE), also called mad cow disease. If BSE had been transferred from animals to humans equally as well as between certain animals, most of the British and a good portion of the continental Europeans could have died already or would die during the next ten years, resulting in hundreds of millions of deaths. It is amazing that we have seen only 90 victims so far. This case should teach us a lesson. It was caused by the use of "valuable by-products" (i.e., wastes from animal bodies) and a more "efficient" treatment using the knowledge of the time that heating to 120°C should rule out infection. However, this was wrong; one of these errors caused by our incomplete knowledge.

Every new process or component added to our foods introduces risks. Most risks are small, but there are thousands of them. For a food control laboratory, such as the one where I work, this is frightening. For example, in 1995 we were testing olive oil for adulteration with specially-treated sunflower oil when we were disturbed by an extra peak. It turned out to be bisphenol-A

diglycidyl ether (BADGE) released from the internal coating of the food can, present at a concentration of 80ppm. Considered as a suspect carcinogen, the Swiss legal limit was 20ppb. 80ppm for a possible carcinogen was astounding! Billions of food cans contained BADGE at levels exceeding 1ppm. Millions were removed from the Swiss market and authorities in the EU became active. Later BADGE was shown to be non-carcinogenic. Hence, we were lucky once again. However, it only took a few months to realize that the migrant from the food containers to the food usually forms a forest of peaks, with dozens of giant trees whose identities -- not to mention their toxicity -- nobody has investigated.

Many analysts know that food packaging materials often release forests of peaks, rendering their search for a given compound difficult. Usually they have no time to identify these peaks, or are even told not to do so. Many producers do not want to know about them because then they would carry the responsibility. It is a horror to any manager that a migrant from his product could be a potent carcinogen, and to have journalists or lawyers show him documents that prove he knew about it for years. Ignorance may be bliss to them.

With the fast changes in food technology safety is a delicate issue. We analysts have the most powerful tools in our hands to known that food safety does not meet the standards communicated to consumers. Some of us daily see the forests of unknown peaks, the artifacts from technological processes, and contaminants. The probability is high that among the ten thousands of compounds, a few are highly toxic. Many of the untested compounds we ingest in amounts that exceed those required to prevent pregnancy or to change our thinking (e.g., LSD or psychopharmaca). They might make us more beautiful, but, unfortunately,many more chemicals cause damage than help us. The problem is that we cannot spit them out once we notice that they are bad.

We analysts carry the burden of knowledge and responsibility to inform; even if it is to people who do not want to know. This is a difficult position. The analyst working for producers is the spoilsport of the technologists who are enthusiasts of their new product and of those who want to see the money coming in soon. The others working for the public usually get to hear that there is no regulation on the subject,that there is no sufficient proof of a harmful effect and, hence, that nothing can be done, or that there is no longer anybody around who could investigate the subject. In fact, mechanisms of economy get stronger, whereas authorities defending the public interests shrink.

I believe and hope that, in the end, we Europeans will escape BSE with less than 1000 human deaths. We have no idea how many diseases and deaths are due to minor risks caused by food technology, but we should take notice that the probability of being severely hit constantly increases. This makes one wonder if the game about higher profitability plays with our safety?

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EDITORIAL

The Value of Education

by Dr. Konrad Grob, Kantonales Laboratory, Zurich

In my last Korner, I concluded that only an education and qualification system could prevent chromatography from further decline. Instead of devoting time and effort to force better chromatography through quality management schemes, validation, accreditation, and bureaucratic piles of paper, I suggested that institutions invest in improving the quality of their employees. In this way, both the employees and the employers share the responsibility for improvement. Employers should realize that knowing how to manage a crimper for closing autosampler vials (despite what some instrument vendors may claim) is not "all" it takes to make a gas chromatography (GC) laboratory successful. At the same time, if analysts want to be considered valuable assets, then they should be ready to take an examination that affirms their education and training. How much education is necessary?

I received an overwhelming response to my article. Almost all of which confirmed my conclusion. For the sake of argument, though, I would have liked to hand over the microphone to somebody who disagrees. However, without refuting my point, I will take a moment to gain a perspective on the questions of 1) How simple is GC really? and 2) How much education is necessary? When observed from a safe distance, the work of a GC analyst appears simple. A gas chromatographer performing routine analyses should be able to help troubleshoot when results are not appropriate. The analyst should, in addition, be able to "rapidly" analyze x in sample y, and know how to select the right column, the injection technique, and all the many parameters finally determining whether the analysis will be successful. If capable of developing methods, the analyst needs to overview the possibilities and the problems to be expected; the clever choosing of strategies, tools, and conditions that may save trouble over years and reduce the time needed per sample by a factor of more than two.

No doubt analysts who are able to answer common GC questions achieve more reliable results because they can find the pitfalls. While other people waste several days because an analysis does not turn out adequately, these analysts find tests that can rapidly localize the problem. They know beforehand that aqueous samples are more difficult to analyze and should be injected in small volumes.

In industrial countries, every working day costs around $1,000. Eliminating three days of expense per month by improved troubleshooting saves $3,000 for that month. You easily can save several days per month by using better methods or improving performance of given methods. A knowledgeable gas chromatographer can prevent visits by the service engineer and avoid other delays disturbing production or delivery. If half of the profit generated by more competent work goes to shareholders, the analyst's salary should increase by $3,000 at least.

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EDITORIAL

Do We Need a Chromatography School?

by Dr. Konrad Grob, Kantonales Laboratory, Zurich

Sometimes things need to be said in a pointed way. The following is one of those things. If even less than 50% is applicable, then it is enough to sound an alarm.

The Slow Sinking of Chromatography

I grew up in the good old times when the world believed in chromatographers. We were proud of what we did, could work in our own way, but were also responsible for our results -- we were our own maestros, playing our chromatographs with the best of melodies. But, then misery befell us. It was suspected our results were faked. Bureaucratic methods like Good Laboratory Practices (GLP) were invented and, ever since, many of us must document every move we make. We wanted to work in the lab, not do office jobs on an uncomfortable lab chair, didn't we?

Confidence in our results was lost almost completely. This has its origin in embarrassingly poor results delivered by some labs, but also in the difficulty of the public to understand that our results may have a substantial margin. They may think that if an instrument is expensive, the results must be absolutely accurate. At court, results are no longer questioned on their accuracy,but on the paperwork behind them: when was the balance checked last, and is there a certificate proving that the hexane was not water. Shouldn't we feel offended by such general distrust? As a consequence, much of our work has become regulated in minute detail, with many ideas having a penetrating odor of stacks of paperwork and meeting rooms. Many methods grew to be more than 10 pages, half of which are taken up with titles and decimal numbering. They specify standard compounds in every detail, as well as simple manipulations like how to rinse a round flask. However, they all too often do not even mention the tasks that cause real problems, such as the details on how to perform injection techniques. Have we lost all of our competence or are some totally unskilled lab workers dictating our lab?

Lab Work Degrades

In many labs, work has become dull. It has degraded to the execution of recipes -- as mundane as making hamburgers, only having more frustrations and a lower success rate. Your results are accepted only if you have a validated method and a certified standard. Many methods could be greatly improved, saving large amounts of time and money, but changes presuppose such a large amount of rework that most people just keep quiet. It is performing analyses in chains. Many of the best chromatographers are no longer finding enjoyment in such work.

Commonly, lab supervisors are no longer in the lab. They are chemists who learned hardly more about chromatography than interpreting a van Deemter curve. For them, going into the lab might mean learning the basics from the supervised -- it is easier to design Excel spreadsheets

and manage quality from a safe distance. Analytical refugees because of a lack of chromatography education?

It is sad to see such an exciting field declining. GC would not be the first chromatographic technique to degrade. Thin layer chromatography (TLC) is an excellent method for many purposes, much faster and cheaper than some other methods. However, where could I learn the art of TLC today?

Quality Management, a Makeshift Solution

Much of quality management (QM) resembles a desperate support structure used to stop the decay of analytical chemistry; a sophisticated system to protect against a lack of competence. However, in reality, QM might even accelerate the decay as it chases away the good analysts by way of boredom and frustration. Many newcomers have no proper education and will hardly develop a passion for the work they encounter, and only look at peaks when asked to do so in **bold** letters. Many laboratories have lost their competence to create or modify methods. In the end, the pessimists are right: merely the most rigid and painstaking descriptions prevent people from doing the incredible things that many technical support services hear about every day. Analytical chemistry risks collapse, despite -- or maybe because of -- the rapidly growing QM systems.

A Need for Better Education

Is there anything we can do to stop this decline? The key problem concerns competence of the analysts. Chromatography is demanding and requires professionally trained people. Analysts must be masters of their field, motivated to do their work well and react promptly if something peculiar is observed. They should feel responsible for their results, but also be recognized for performing a difficult job. Supervisors must be knowledgeable leaders in chromatography, guide with ideas and suggestions, understand problems, as well as pick up and support the good ideas of their people. Emphasis must be moved from paper-leaden QM systems towards ensuring the competence of the analysts.

Many of the modern technologies suffer from lacking education. In new fields, the originators are the natural teachers, informally passing on their knowledge. When they leave, classical education should take over. However, universities are unable to offer this service for all the emerging fields and there is not enough room for chromatography in the teaching of general chemistry. Hence, new models of education are needed. We might also need an internationally recognized qualification system, so that well-trained chromatographers are recognzied as specialists in their field. The problem is serious and a great challenge for all who are willing to keep alive a field in which more than 500,000 persons are working.

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EDITORIAL

Working Safely with Hydrogen as a Carrier Gas

by Dr. Konrad Grob, Kantonales Laboratory, Zurich

Broad agreement is that hydrogen is the best carrier gas for capillary GC applications.

Nitrogen cannot seriously be considered as a carrier gas option, because diffusion speeds of the solute molecules are roughly four times lower than in hydrogen or helium, rendering the separation process exceedingly slow. Helium is the best alternative if hydrogen cannot be used, but hydrogen enables faster chromatography whenever inlet pressure exceeds roughly 0.7 bar, with a rapidly expanding difference when the required inlet pressure increases. Hydrogen is almost a must for high-temperature work such as triglyceride analysis, and analysis with long columns such as fatty acid methyl ester analysis on 100m columns. Also, hydrogen is available in unlimited amounts (using helium depletes limited natural resources). Hydrogen cannot be used with thermoionic detectors and some mass spectrometers, but the main argument against hydrogen concerns safety because it forms an explosive mixture with air. Can a lab manager take the responsibility for using hydrogen as carrier gas? Yes, if some simple safety measures are taken.

Exploding GC ovens

In the past, there have been many hydrogen explosions in GC ovens. I know of four in Switzerland,the latest one probably being 15 years back. I caused one myself in the seventies during production of glass capillary columns. I prepared about five columns a day using a procedure requiring five heat treatments in a GC oven. I could install a column in hardly more than a second, virtually without turning a screw. I set a high inlet pressure to remove the air and heated the GC at full power to 280°C. One day, a column had enough tension to pull the inlet out of the ferrule. Hydrogen ran into the oven at about 1 bar, without any restriction. The heating filaments were red-hot (2.5 kW,in an old instrument) and ignited the mixture. The explosion was heard through several labs. The instrument seemed to jump 1-2cm from the bench and lost the dust from its top. Opening the door, I was amazed to see the glass capillary column hanging a bit lower than before, but not being broken.The door did not open as smoothly as before, this being the only reminder of the event.

The other explosion in our lab occurred shortly after an on-column injection for triglyceride analysis. We changed the columns almost daily, and the inlet was installed rapidly, with a soft fitting used many times, tightening by fingers. As the syringe needle did not enter the column inlet easily, my coworker pushed a bit harder than usual. The needle went down and he did the injection. He started the program (with ballistic heating from the injection temperature of 70°C to 250°C) and switched on the recorder. He had just turned his back to the instrument when there was the bang. In fact, he injected into the oven, because the syringe had pushed the column out of the fitting. Again, there was no damage, neither to the instrument, nor to the

glass capillary column.

The two other explosions happened in other laboratories, in both instances because it remained unnoticed that the column had been removed from the instrument. Somebody wanted to bake out the column, set high inlet pressure and heated the oven at full power. There was no need for coffee to wake up that morning. The instruments were slightly deformed, but were used for another ten years at least.

4% Hydrogen, 630°C

We had many large leaks with broken columns, poor connections, and experiments more outrageous than can be described here - and there were no explosions. Beside some good luck, this is due to the fact that explosions of hydrogen are much less likely to occur than explosions of solvent vapors. A minimum of 4% hydrogen in air is required for an explosion to occur, and the mixture must be heated to about 630°C to be ignited (in contrast to around 0.1% and hardly over 200°C for vapors of many solvents). In fact, all the explosions I know of occurred with old instruments, characterized by high heating power and relatively small filaments, which turned red-hot when heating ballistically. Newer instruments with less heating power and larger filaments hardly reach the temperature for ignition. Also, the 4% concentrationis not that easily reached: concluding from the experience of many critical situations, it seems that the two most common risks in practice, breakage of the column or a leaking connection to an injector, hardly ever result in an explosion. All explosions I heard of were the result of unhindered flow of hydrogen from the injector into the oven.

Measures ensuring safety

Hydrogen sensors

Risks must be taken seriously, even when it seems unlikely that severe damage may occur. Since the early eighties we gradually equipped all of our instruments with hydrogen sensors, a small device available from various sources. Some air is picked from the zone of the oven ventillator and brought to a sensor detecting hydrogen in the concentration range of 0.1 to 1%. When 1% is reached, the gas chromatograph is switched to cooling, which stops the heating and purges the oven with ambient air. Usually a lamp blinks and an alarm signal calls for attention. A luxury version even replaces the hydrogen in the carrier gas line with nitrogen. The sensor also goes off if concentrations of solvent vapors in the laboratory are high, which eliminates afurther risk (independently of whether hydrogen or helium is used as carrier gas).

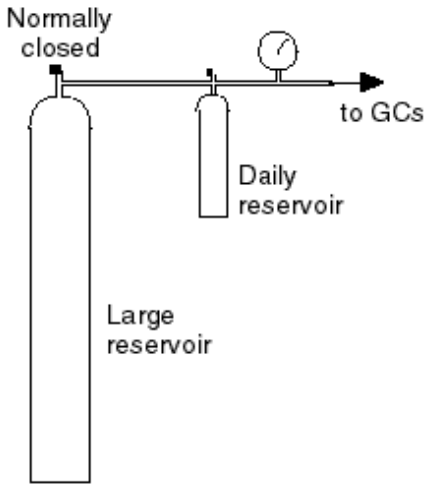
Flow-regulated carrier gas supply

Classical gas chromatographs regulate the carrier gas by pressure. When there is no column, such systems may deliver many liters of gas per minute into the oven. Hewlett Packard introduced a flow-regulated system,in the worst case limiting the flow into the oven to the rate adjusted for the application, i.e. column plus split plus septum purge flow rate. The maximum flow rate is 500ml/min. Flow regulation strongly reduces risks, but is not considered to be safe. A rough estimation provides some clues. A GC oven has an internal volume of around 40 liters. The explosion limit of 4% is reached when the oven contains 1.6-liter of hydrogen. If the system leaks by 500ml/min, it takes little more than 3 minutes to render the oven atmosphere explosive. If the imposed flow rate is 100ml/min only, however, the critical limit is calculated to be reached in 16 minutes. This assumes a tight oven, which is not realistic; hydrogen diffuses

extremely rapidly and the oven atmosphere is exchanged in far less time than 16 minutes. Hence, with a leak of100ml/min, the hydrogen concentration in the oven cannot reach the explosion limit. Potentially dangerous flow rates exceeding 100ml/min are of interest for split injection with high split ratios. If the high flow rate is turned down 1 minute after the injection, this eliminates this risk and also conserves carrier gas. In splitless injection, the adjusted flow rate will be substantially below 100ml/min anyway. In on-column injection,the flow rate during analysis is far below the critical minimum. It depends on the construction of the injector seal, however, whether the flow rate can be limited to a few milliliters per minute: during injection, a rotating valve leaks at a far higher flow rate, and pressure would collapse when limiting the flow rate to less than about 100ml/min. Hence, flow regulation or a restrictor limiting flow in a pressure-regulated system can exclude a dangerous hydrogen concentration in the oven.

Intermediate storage in small cylinders

Figure 1: Gas supply system ruling out large losses.
Gas is consumed from a daily reservoir and the well-observable manometer provides control over the gas consumption.



Rather frequently a column is dismounted without switching off the carrier gas supply. Other times a septum leaks to such an extent that the hot carrier gas can burn fingers held many centimeters above the septum. In both these situations, hydrogen may leak into the laboratory at high flow rates. The risk of a laboratory explosion is minimal, however. A small laboratory contains maybe 50,000 liters of air. 4% of hydrogen corresponds to 2000 liters, i.e., to the content of a full 10-liter cylinder at 200 bar pressure. Admittedly, mostly 25- or 50-liter cylinders are used, but since the laboratory atmosphere is exchanged many times per hour, these cylinders would have to be emptied in minutes to reach the explosion limit. Again, hydrogen is far less dangerous than solvents, for many of which 50-liters of vapor is the limit, corresponding to a spill of around 250ml of liquid solvent. And, since the solvent vapors tend to form a "lake" above the floor,the explosion limit is reached locally even more rapidly. A simple safety measure rules out large scale loss of hydrogen and is also a warning of massive leaks: The main reservoir, maybe a 50-liter cylinder, is not directly connected to the laboratory gas supply, but via a small daily reservoir. The large tank is closed (except when almost empty). The gas is consumed from a 3- to 10-liter cylinder, adjusted to the daily consumption. A 3-liter cylinder filled up to 50 bar contains 150 liters of hydrogen. If, for instance, three gas chromatographs consume 100ml/min each (25ml/min for the FID), the cylinder must be refilled every 25 hours, which suits when these instruments work day and night. If filled to 20 bar only, it would contain an amount well fitting the consumption of a working day. A manometer or an electronic readout is positioned such that everybody will see it. If the daily reservoir must be refilled on the same day, this is a warning and a leak will be detected long before the large reservoir is emptied. The manometer can be equipped with an alarm indicating low pressure. In Switzerland, this is a standard installation found in many laboratories for more than 20 years.

Hydrogen generators

Hydrogen generators are an alternative to the small daily reservoir. They deliver the gas at limited flow rates and totally avoid the necessity of storing gas. If split injection must be possible at high split flow rate, however, rather large generators and/or one for every few instruments are needed.

Conclusion

I understand the dilemma of the laboratory manager: he or she may recognize that hydrogen is preferable and cheaper than helium, but does not want to take risks. Can these risks be managed? Hydrogen sensors rule out explosions in the oven, and daily reservoirs or hydrogen generators eliminate risks in the laboratory (as well as costs caused by large losses). Checking tightness of the gas plumbing every 6-12 months is also advisable whether hydrogen or helium is used.

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EDITORIAL

Carrier Gases for GC

by Dr. Konrad Grob, Kantonales Laboratory, Zurich

Probably more than 90% of the present GC instruments run with helium as carrier gas. Some people use hydrogen or nitrogen, maybe because the first ones are hidden pyromaniacs (some GC ovens actually exploded) and the second still have nitrogen mounted on the instrument from the times they worked with packed columns. These gases serve to produce wind through the column to move our solutes forward. The solute molecules evaporate from the stationary phase surface, i.e. enter the open space of the capillary column, are hit by a carrier gas molecule and start traveling down the tube. After a short distance, however, they touch the sticky surface of the stationary phase and go through another partitioning process. Does the choice of the carrier gas interfere with this? Yes, it does, through its diffusivity and viscosity. You want to know why hydrogen is the best carrier gas?

Diffusivity

Diffusivity provides a measurement for the diffusion speed of a solute vapor in a given gas. For helium and hydrogen, diffusivities are similar, but that of nitrogen is about four times lower (see Table I).

Table I: Relevant characteristics of carrier gases[1]

Carrier gas	Viscosity at 50°C [kg/s m]	Diffusivity (butane, 100°C [m2s])
Hydrogen	9.4	$6\ 10^{-6}$
Helium	20.8	$5.5\ 10^{-6}$
Nitrogen	18.8	$1.5\ 10^{-6}$

The diffusion speed of the solute in the carrier gas determines the speed of chromatography. A solute molecule evaporating from the stationary phase surface into the gas stream should begiven enough time to diffuse back to the stationary phase (Figure 1) before having gone farin order to undergo another partitioning process - it is these contacts which differentiate between different substances, and a large number of contacts are needed to obtain the best separation. We get more of them if the solute diffuses more rapidly and/or when we give itmore time, i.e. reduce the gas velocity. However, there is a limit: giving it more time for the diffusion towards the stationary phase (radial diffusion) also provides more time for spreading within the open bore of the column, i.e. for band broadening through longitudinal diffusion. This is why there is an optimum gas velocity: it provides a maximum number of contacts with the stationary phase with a minimum of band broadening in the gas phase.

This kind of logic applies to all gases. In fact, all

carrier gases provide similar separation efficiencies - provided conditions are adjusted correspondingly. The time needed is different: since diffusion in hydrogen and helium is much faster than in nitrogen -- for (wanted) radial as well as (unavoidable) longitudinal diffusion -- GC is 2-3 times faster with the former. If we users of hydrogen wait for one hour, users of nitrogen should wait for 2-3 hours to get the same performance. Nitrogen is for those who own a comfortable arm chair in the lab or who are afraid of the result. Usually users of nitrogen are not really that patient and run their chromatography at similar speed as others using hydrogen and helium. Table II shows what they get. It compares separation efficiencies measured in terms of Trennzahl (TZ) indicating thenumber of peaks which could be fully separated between two components to be defined, in this case, the alkanes C13 and C14. At the gas velocities most commonly used with hydrogen (40-60 cm/s), nitrogen produced hardly more than half as many peaks. When using hydrogen, the same result could have been obtained from a column roughly 3 times shorter in a third of the time. To give an impression of how the chromatograms look like, an example is shown in Figure 2. At halved velocity, nitrogen provided good performance also.

the column.

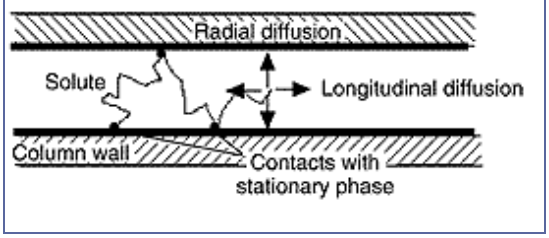
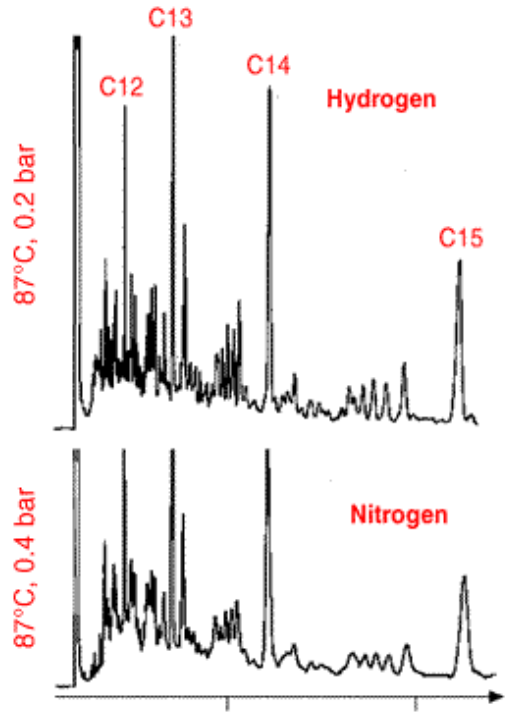


Table II: Separation efficiencies in terms of separation numbers (Trennzahl, TZ) for the n-alkanes C13 and C14 and a 12m, 0.25mm ID column coated with a methyl silicone.

Gas velocity	Hydrogen	Nitrogen
50 cm/s	24	13
40 cm/s	25	15
30 cm/s	23	17
20 cm/s	20	23

Figure 2: Separation of a kerosene fraction using hydrogen or nitrogen as carrier gas at the same average gas velocity (40 cm/s).



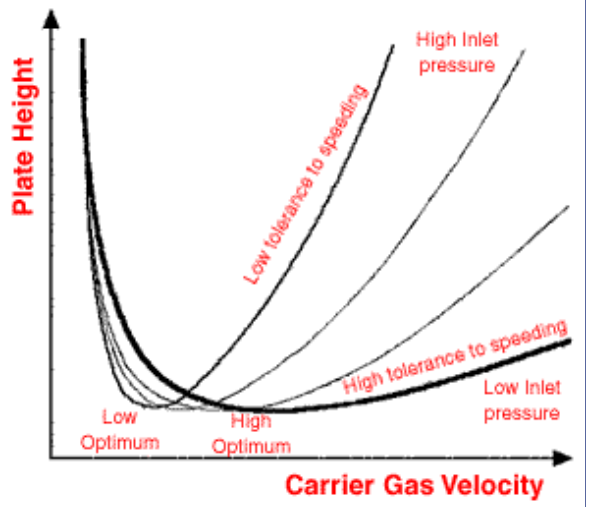
In this application, nitrogen just requires extra time. However, long retention times also produce low peaks, i.e. poor sensitivity (see Figure 2). Additionally, do not try to run triglycerides or

other labile compounds with nitrogen as carrier gas: they are largely degraded during the long run time required.

Viscosity

The other difference between the carrier gases concerns the viscosity that determines the inlet pressure required for a given gas velocity. High inlet pressures strongly compress the gas in the column inlet, which causes the problems shortly outlined below. This explains why hydrogen is preferable to helium. You have certainly seen the h/u curves, also called van Deemter curves, plotting HETP (plate height) against gas velocity. Their peculiarity: the best is at the bottom, i.e. the optimum gas velocity is at the lowest point of the curve; the larger the plate heights, the worse the separation. The curves say that separation is poor when the gas velocity is below the optimum velocity (left of the optimum in Figure 3,the result of excessive longitudinal diffusion) and that it worsens again beyond that optimum(the curve rising at the right, the result of insufficient radial diffusion).

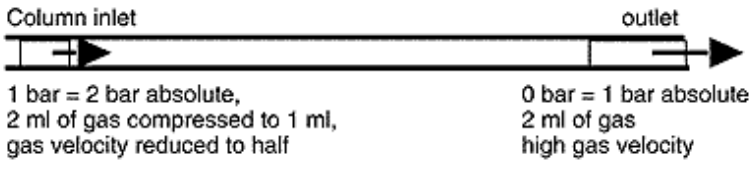
Figure 3: High inlet pressures cause the optimum gas velocity to be low and the loss in separation efficiency when exceeding this optimum to be high.



For columns of a given diameter, the optimum velocity is highest when the column is short. This is because inlet pressure is low. For hydrogen or helium, with about the same diffusivity, the optimum is almost the same, i.e. around 40-50 cm/s. Further, the losses in performance upon speeding, i.e. using excessive gas velocity, are relatively small. The longer the column,the higher is the inlet pressure required. This shifts the optimum gas speed to lower values and, as if there were a strict educator behind the chromatographer, speeding is punished more strongly when the velocity must be low anyway. Hence, using a column of doubled length requires more than twice as much run time, because the gas velocity must be lower. In thisrespect, helium is worse than hydrogen because its viscosity is about twice as high: the higherinlet pressure requires a lower gas velocity and if you do not obey, the punishment is harder.

What is the reason for this? If the column head pressure is, e.g., 1 bar, corresponding to 2 bar absolute pressure, the carrier gas in the inlet is compressed to half the volume compared to the column outlet (assuming the latter is at ambient pressure, 1 bar absolute, Figure 4). Hence the plug corresponding to 2 ml in the outlet is only 1 ml and is half as long. To displace 1 ml, half the velocity is required compared to displacing 2 ml at the outlet. Hence optimization must compromize between a low velocity in the inlet and a higher one at the outlet.

Figure 4: Compressibility of the carrier gas causes the gas velocity in the inlet to be lower than in the outlet.



Conclusions are against intuition. From short columns we know that 40-50 cm/s are best. In the last, e.g., 15 m of a long column, pressure conditions are the same as in a short column, i.e. the optimum gas velocity and tolerance to speeding must be the same. The problem resulting from the compressibility of the gas is obviously in the inlet of the long column. We are tempted to assume that it is related to the fact that the gas velocity is 20-25 cm/s only and would conclude that a compromize should be chosen between maybe 30 cm/s in the inlet and 70cm/s in the outlet in order to result in some 50 cm/s as an average. Experiments show that this is wrong: the best average velocity is only 20-25 cm/s. Hence the system wants an even lower velocity in the inlet: about 10 cm/s. And it insists in that: it forces to choose a velocity at the outlet lower than found to be optimum, and if you do not obey to the 10 cm/sin the inlet, punishment is hard. A rapid glance into the above h/u curve shows that 10 cm/s would provide extremely poor performance at the column outlet. Thus the correct conclusion is that optimum velocities are far lower in a compressed gas. This is not really new: GC with vacuum at the outlet, e.g. with GC-MS, is even faster.

Nitrogen has only drawbacks and is not suitable for capillary GC. Helium is as good as hydrogen if inlet pressures are below about 50 kPa, but requires slower GC at higher inlet pressures (for longer columns), the difference being roughly a factor of two when 150-200 kPa must be applied for helium.

The most important argument against the use of hydrogen concerns safety. **The next "Korner" will report on how our lab solved that problem.**

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EDITORIAL

Certification of injectors and injection techniques?

Comments on splitless injection by readers.

by Dr. Konrad Grob, Kantonales Laboratory, Zurich

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 fourfold 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 aVarian 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 (54mm x 0.8mmID with 9mm 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 (54mm long), I chose the largest ID liner offered (3.4mm), added a 1cm plug of deactivated fused silica wool placed above theinstalled 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 (0.8mm i.d. liner, 250°C) with those he introduced recently (3.4mm i.d. liner, injector programmed from 200 to 300°C). The detector, column, injection volume, and other conditions 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.

	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

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 600-700µl 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 ananalyst 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 of354µl, 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 1µl 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 syring eneedles, 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 every 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 dealon 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.

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EDITORIAL

Why Uncoated Capillary Precolumns Enable Injection of Large Volumes

by Dr. Konrad Grob, Kantonales Laboratory, Zurich

This issue of Koni's Korner deals with uncoated capillary precolumns or desolvation precolumns. The development of the retention gap technique for introduction of large volumes of sample was an exciting experience of which I would like to give a summary here. Uncoated precolumns are used for two totally different purposes: for on-column injection of large volumes and as a garbage bin (guard column, disposable inlet) for the analysis of samples with non-evaporating by-products ("dirty" samples).

The "Retention Gap"

During the first two years of using on-column injection, we were puzzled by occasional splitting of peaks that eluted at several tens of degrees above the oven temperature during injection. In 1981, using glass capillaries, we saw how the injected sample liquid moved rapidly along the capillary wall and deeper into the column. In a 0.32mm ID column, 2µl easily "flooded" 50 centimeters of the column inlet. Even worse, sample liquids not wetting the stationary phase (e.g. solutions in methanol on apolar silicones) just left a droplet here and there (as water on a window pane) and entered the column up to several meters for every microliter injected. It was obvious this would not produce the sharp initial bands required. Flooded zones of 20-40 cm seemed to be the maximum for avoiding noticeable peak broadening. A paper by W.L. Saxton (HRC 1984) confirmed this conclusion. This enables injections up to 1-2µl of a wetting sample.

During these experiments, we were puzzled by certain columns that did not produce broad or split peaks even when we injected 5µl. It took some time and several cups of coffee to discover these were the columns which we had prepared with 0.5-1m of uncoated inlet. We realized that straightening the end sections of the columns, which is the main problem in using glass capillaries, did not damage the stationary phase because the inlet was uncoated. Indeed, when the sample liquid was spreading in the uncoated inlet only, peaks were sharp. The explanation was rapidly at hand. Solutes pass much more rapidly through the inlet if the latter is uncoated (low retention power) and are focused at the entrance of the coated section. Actually they pass through the inlet at low temperature, are stopped in the inlet of the separation column, and wait there until temperature has increased further to enable the separation process to start. It took some scratching of my beard to give this child a name, also because English is the third foreign language in my country. We finally called the uncoated inlet with negligible retention power a "retention gap."

The maximum injection volume

Having learned this, we wanted to explore the usefulness of the retention gap, i.e. how long an

uncoated precolumn could be and what would be the limit to the injection volume then. It could be experimentally confirmed that the focusing effect, hence the shortening of the initial bands, was about equal to the ratio of the retention powers in the uncoated inlet and the coated column. Thus, the longer the retention gap or the thicker the coating in the separation column, the more efficient was reconcentration. The retention power of an uncoated inlet corresponded to that of a column coated with a film of around 1nm thickness. Hence, combined with a separation column with a 1µm film of stationary phase, the initial bands would be shortened by a factor of 1000. This was breathtaking: as some 20cm of residual band length can be tolerated in the separation column, the initial band could be 200m long -- presupposing, of course, that the uncoated column inlet was that long. We were more modest and first used a 5m uncoated inlet to inject twice the total volume of an ordinary 10µl on-column syringe. As this was immediately successful, we had a 100µl on-column syringe made, prepared a 50m deactivated glass capillary and connected it to a 15m separation column. Eagerly we injected 200µl ofa very dilute sample. The first observation was that the pen of the recorder did not want to return from the solvent peak. The minutes passed and the fear grew that we had flooded the whole gas chromatograph. But finally, after some 35 min., the pen came down very rapidly. Many extremely sharp peaks followed (mostly solvent impurities), showing that reconcentration of the initial bands had worked. With a column temperaturecloser to the solvent boiling point, the width of the solvent peak was reduced to hardly 10 min. This was a milestone we celebrated with a cake.

The next step (after having carried out the food analyses we are paid to do) was to determine the lengths of the flooded zones per injection volume orhow much could be injected into an uncoated precolumn of given size. For example, a 10m x 0.53mm ID or a 15m x 0.32mm ID precolumn had a capacity to safely retain 80-100µl of sample liquid. Using 60m x 0.32mm ID precolumns, we could, in fact, inject 400µl.

Concurrent solvent evaporation

We immediately started using the technique for our work, e.g. for the analysis of surface and ground waters. The gain in sensitivity and the advantages forsample preparation were spectacular. Although, as expected for on-column techniques, the samples needed to be reasonably clean to avoid excessively rapid contamination of the precolumn. Some practical problems had to be solved, of course. First of all, a method was needed for joining the uncoated precolumn with the separation column. After having a hard time with butt connectors and fused joints, the press-fits were a great relief (1986). In 1984, we started transferring whole HPLC fractions on-line into GC, comprising 200-350µl of (normal phase) eluent (HPLC served for sample preseparation or clean-up at high resolution). Since transfer of even larger volumes was desirable (some 400-800µl), we returned to some basic development work. The sample liquid in the flooded precolumn provides solvent effects to focus the volatile sample components. However, not all of the solvent is needed for this. As the sample was introduced at conditions causing a large proportion of the solvent to evaporate simultaneously (partially concurrent evaporation), the first peaks were still sharp and perfect in size, but for a given precolumn the transfer volume could be increased several times or the precolumn could be shortened. When samples were introduced at a speed such that all solvent evaporated concurrently, an uncoated precolumn of merely 1-3 m in length could receive virtually unlimited volumes of sample -- at the expense, of course, of the solvent effects: components eluted below about 150°C were lost. In 1985, we introduced a 10,000µl volume -- but it took 83 min. This was good enough for a record, but the solvent peak required nearly 1m of chart paper! Furthermore, the FID soon became black like a chimney.

The early vapor exit

On the four automated LC-GC instruments, which perform more than half of our analyses today, partially and fully concurrent evaporation are still the key techniques. However, a further improvement was important: the early vapor exit. When more than 50-100 µl of solvent are introduced, discharge of the vapors through the whole column becomes slow and the flame detector turns into waste incinerator. A separate outlet is needed for the solvent vapors. The earlier this exit is positioned, the shorter the path is for the vapors and the faster is their release. On the other hand, the inlet must be long enough to retain the solutes, i.e. to achieve solvent/solute separation. The latter is achieved either by solvent trapping in the flooded zone (uncoated precolumn) or by the (less efficient) stationary phase trapping in the coated ("retaining") precolumn (see Figure 1). Partially concurrent evaporation provides solvent trapping and usually produces perfect peaks even for components eluted immediately after the solvent. For fully concurrent evaporation, however, just stationary phase trapping is available (often reinforced by phase soaking), which restricts the analysis to solutes eluted several tens of degrees at least above the column temperature during solvent evaporation. With the early vapor exit, evaporation rates went up to typically 100-400µl/min. The new record for concurrent evaporation (from 1989) stands at 20,000µl of a hexane solution introduced in 20 min.

The future

Presently the injection of volumes larger than 10 µl is a subject at most meetings dealing with capillary GC. Two approaches are in the focus of the interest: Programmed Temperature Vaporizing (PTV) injection by the solvent split technique and large volume on-column injection. The PTV technique is relatively robust regarding the injection of "dirty" samples,but the most volatile as well as the high boiling and labile components tend to be lost. The on-column technique avoids such losses and the results are highly quantitative, but the uncoated precolumn is sensitive to contamination by non-evaporating sample byproducts and to attack by aggressive components like water (humidity).

The future will show which technique wins, but the end of the development has not been reached yet. I believe that the on-column/retention gap technique provides the better basis and can still be improved. The first step has been made by the European leader in GC instrumentation, CE Instruments. Because the adjustment of appropriate conditions requires some understanding of the background, a computer-guided instrument for sample volumes up to 250 µl was designed. A standardized precolumn system ("Uncoret" -- composed of an uncoated and a retaining precolumn in one piece,15m x 0.53mm ID) is used and the software has evaporation rates for the most commonly used solvents in its memory. It automatically adjusts the autosampler injection speed and closes the vapor exit at the appropriate moment for the analysis. In Europe, a good number of instruments are in use for trace analysis of fairly clean samples, and probably about half of them are in commercial laboratories doing water analysis.

The next step is the addition of a small bore, probably permanent, hot vaporizing chamber above the precolumn system (Figure 1) that serves as a filter for retaining "dirt" and for vaporizing non-wetting samples. This adds to the on-column system the robustness against "dirt" of the PTV but maintains the better and more reliable means for solvent/solute separation. In summary, GC is an excellent technique for trace analysis, but the small injection amounts (typically 1-3µl) are as appropriate as wooden wheels on a sports car.

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EDITORIAL

The GC Separation Process

A simple model for non-mathematically minded chromatographers

by Dr. Konrad Grob, Kantonales Laboratory, Zurich

There may be moments when you sit in front of a gas chromatograph, waiting for a peak and ask yourself how the peak travels through the system. You may have studied textbooks to answer this question and probably have found some mathematical concepts. If they satisfied you, please stop reading here; you might find the following text too dilettante.

I am not satisfied with mathematical descriptions. They usually start with theoretical plates in the explanation. But when you try to imagine a column as a distillation tower with tens of thousands of evaporation steps, you are quickly frustrated. You should not take theory as literally as that. Since I cannot see the process with my own eyes, I try to imagine what it is like.

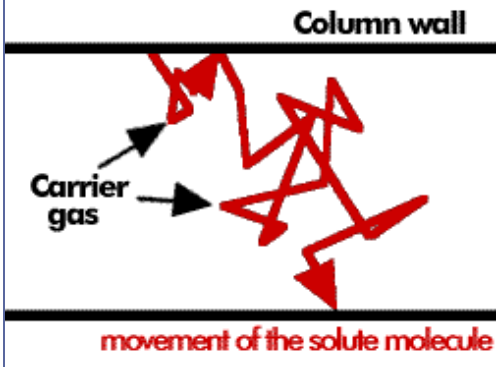
In my simple model, every molecule goes through a three-step cycle, thousands of times. Each cycle is different, but averages of these many times are similar to those of other molecules of the same species.

The Three Steps

For the description of Step 1, let us start with a molecule that just evaporated from the column wall into the gas phase. The molecule flies a small fraction of a millimeter until it hits a particle of the carrier gas, changes direction and either picks up or loses energy. It has no eyes and no intention where to go. It flies back and forth, as well as towards the center and towards the wall of the tubing. The carrier gas moves it forwards, but gas flow is not like swimming in a river. More than 99% of the space in the gas phase is empty and does not move. Flow in the gas phase merely means that more of the particles flying past are directed toward the detector, not in the other direction. After a time, which is short in one instance and long in another, our solute molecule hits the stationary phase, where it is likely to remain attached like a fly on a flypaper.

Step 1:

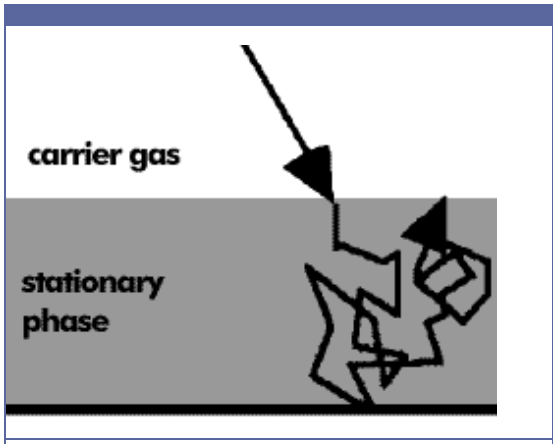
Between contacts with the stationary phase, the solute molecule diffuses through the gas phase in an irregular way.



Step 2:

The solute molecule is attracted to the surface of

the stationary phase by intermolecular forces, but since it continues moving, it still has some chance to free itself and take off again. If success is not immediate, however, it is grabbed and dives into the liquid (Step 2). It goes up and down, maybe even several times to the support surface. Sooner or later it returns to the surface of the stationary phase film, from where it may dive back into the flood - or take off into the gas phase.



Step 3:

At the stationary phase surface, the molecule either pulls itself free and evaporates or returns into the liquid, repeating Step 2.

Step 3: At the surface of the liquid, the solute molecule finds itself in a cavity formed by stationary phase that pulls it back into the liquid. The molecule has kinetic (thermal) energy and vibrates: it tries to tear itself free and to escape into the gas phase (to evaporate). Its energy may be high or low, depending on the last collisions with the stationary phase molecules. If its movements are sufficiently violent, the molecule will take off into the gas phase. Otherwise, the liquid takes it again and Step 2 is repeated (maybe many times).

The average kinetic energy depends on temperature: The higher the temperature, the more violently the molecule moves and the more often it is able to take off, i.e. Step 2 is repeated fewer times until evaporation succeeds. Hence, the solute will arrive at the end of the column in a shorter retention time. Increase in temperature accelerates the GC process. Higher temperature also accelerates diffusion (Steps 1 and 2), but it is primarily Step 3 that renders GC so temperature-dependent.

Where does separation take place?

Diffusion in the gas phase (Step 1) does not significantly contribute to separation, since all types of molecules behave similarly. Nor does it occur in the layer of the stationary phase (Step 2). Separation occurs through Step 3, and is related to the probability of take off: Because intermolecular forces differ, different molecule types have different chances to pull themselves free. As an average, one molecule may take off once out of 5 times it is at the surface. If another does once out of 5.1 times only, the two may end up being separated if the process is repeated sufficiently many times.

Selectivity

Selectivity of the stationary phase works through its influence on the probability of take off. For instance, if the stationary phase contains cyano groups and if two solutes differ by adouble bond, the additional interaction with the double bond hinders take off and adds cycles of Step 2 until the molecule is able to make the next jump through the column. According to this model, selectivity of a column is determined by the properties of a thin surface layer of the stationary phase only; the bulk could consist of any other liquid. If, for example, a few nanometers of a polar stationary phase could be deposited onto a normal film of an apolarphase, the column should show high polarity. Maybe one day, this concept can be used to produce good columns

with a stationary phase of poor diffusivity.

Column diameter

If you were to re-invent capillary GC, what would you optimize? You recognized that every time a molecule takes off from the stationary phase surface, it contributes to the separation. You will, therefore, try to obtain a maximum number of these events. This has to do with Step 1: jumps between two contacts with the stationary phase should be short in order to make maximum use of the column length available. Your first idea is probably to slow down the carrier gas in order to provide the molecule more time to find its way back to the stationary phase surface.That is correct, but there is a limit to this, because the molecule also moves longitudinally, spreading the band as long as the molecule is in the gas phase. In fact, this is why there is an optimum gas velocity (see van Deemter plots). You can gain more if you reduce the capillary diameter. If the distance to the stationary phase surface is shorter, the molecule will touch the latter more frequently, i.e. there are more contacts with the stationary phase per unit time and there is less time for longitudinal diffusion until the molecule returns to the flypaper. In fact, narrow bore capillary columns have always been more efficient. Why then did the wide or megabore columns become so popular?

Column capacity

Steps 1 and 3 have obvious purposes: transport of and differentiation between substances. What is the usefulness of Step 2? Diffusion in the ocean of stationary liquid is the most time-consuming step of GC, as shown by the following estimation: if a peak is isothermally eluted after 10 min. and the gas hold-up time is 30 seconds, molecules travel 30 seconds in the carrier gas and dive in the stationary phase for 9½ minutes (minus the few seconds they spend altogether at the surface trying to take off). 9½ minutes are spent to periodically give them a chance to perform Step 3. If the film were 10 times thinner, every Step 2 cycle would be 10 times shorter and the same number of opportunities for Step 3 could be obtained in less than 1 min; including the gas hold-up time, the run time would be less than 1½ min. If the film were just thick enough to embed the molecules for take off, GC would be much faster. However, this creates a practical problem:insufficient column capacity. Step 3 assumes that during take off the solute molecule is surrounded by stationary phase only. If solute/solute interaction interfered, the probability for take off would be altered. Since the solute concentrations in the center and at the borders of the solute band differ, take off would occur under inhomogeneous conditions, resulting in the well known broadened and asymmetric overloaded peaks. The ocean of liquid has the effect of diluting the solute. It removes solute molecules from the surface layer. Indeed, if the film is ten times thicker, ten times more can be injected to achieve the same solute concentration in the liquid, holding back the molecule in Step 3.

Retention time and elution temperature

Increase of film thickness proportionally prolongs retention times in isothermal runs because each cycle of Step 2 takes longer. Hence, columns with a ten times thicker film should be used with ten times longer retention times (lower program rates) in order to achieve the same separation processresulting from Step 3. In reality, however, users tend to select conditions resulting in similar retention times and increase elution temperatures by roughly 15 degrees for a factor of two in film thickness. This involves a trade between Steps 2 and 3. Temperature increase slightly accelerates diffusion speeds (reducing the duration of Step 2), but the main effect is an increased probability for take off (Step 3). If diving into the stationary phase takes twice as long, this is compensated by a doubled probability of take off. It is paid for by reduced

selectivity: if the probability to evaporate from the stationary phase surface becomes high, small differences in the structure of two solutes have less influence on the retention time and the two peaks will get closer together. In fact, thick film columns provide lower resolution, not because separation efficiency in terms of theoretical plates is lower, but because relative retention times (alpha values) are smaller (J. Chromatogr. 207 (1981) 291).

Conclusions

I find it exciting that a model as simple as that described is capable of correctly describing the principal phenomena observed in GC separation. This helps us to understand our daily observations, making the process taking place in the tiny capillary column behind the oven door much more vivid. The above considerations have not been brought to an end, and I would not be surprised if such simple models would turn out to be fertile ground for developing new insight and techniques.

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EDITORIAL

Sample Evaporation in Splitless Injection

A Problem?

by Dr. Konrad Grob, Kantonales Laboratory, Zurich

My last "Korner" expressed doubts about GC techniques being as well optimized as one would think. This is because nobody feels responsible and no institution is willing to pay employees to solve problems for the approximately 200,000 other users of capillary GC. Many of the existing designs and working rules emerged from specific circumstances and interests rather than thorough investigations. This "Korner" questions such a rule.

Have you ever been puzzled by the fact that most standard methods recommend the use of a packed injector liner for split injection and an empty one for splitless injection? Usually an explanation is given: the residence time in the injector is much shorter for a split injection than for a splitless injection. Is this a satisfactory answer for you? It is not for me.

Quality assurance requires a lot of time to be invested into checking the accuracy of the equipment. Sources of error, which are more demanding to understand and check, are frequently neglected, even though these errors are often the source of more severe errors than, for example, the balance, pipette, or oven temperature. Sample evaporation in splitless injection belongs to them.

Origin of the Rule

The rule that liners for splitless injection should be empty was introduced by my father in the early seventies. He wanted to avoid the retention of solutes on a packing material, which can hinder the transfer of higher boiling and adsorptive components into the column. In fact, during the splitless period, the gas phase of the vaporizing chamber is exchanged at the most twice and minimal retention results in loss. The material reaches the column only when the split outlet isopened and is largely vented through that exit. My father's experience was with manual injections. Furthermore, high accuracy was not his first concern. His rule survived until today without ever having been seriously questioned. There are, however, reasons to have another look at it. I would like to present the problem to experienced users, hoping for responses, which I would like to publish in a future "Korner."

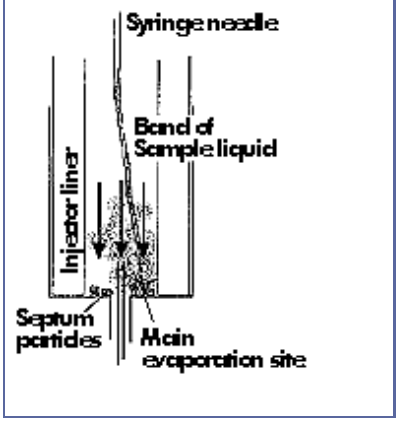
The problem of sample liquid "shot" to the bottom of the injector chamber

Minimization of retention power in the injector is an important aspect, but not the only one to be considered. A previous "Korner" described the problem of sample evaporation inside a hot injector: if the sample liquid leaves the syringe needle as a narrow band, as water leaves a tap without a hose,it moves at the velocity of a fast car and arrives at the bottom of an empty liner in about a millisecond-far less than required to receive the heat for evaporation. As the sample

liquid hits the bottom of the chamber, it may be rejected toward the center, but it is more likely to stay, possibly to be sucked up by septum particles accumulated there. Usually the column entrance is positioned slightly above this "waste bin" of the injector (see Fig. 1) and receives little of the material "shot" to the bottom since the carrier gas comes from the top.

The evaporating solvent produces a volume of vapor that easily expands towards the center of the chamber. Since temperature at the evaporation site remains near the solvent boiling point, solutes hardly have a chance to follow. They are vaporized afterward. However, their vapor volume is so small that it is unlikely that it will reach the column entrance: 10 ng of solute produce less than 1 nl of vapor. Hence, the vapors remain at the bottom of the chamber until the split outlet is opened and they are vented. Also in splitless injection, the sample must be vaporized above the column entrance.

Figure 1: Incomplete sample evaporation above the column entrance results in loss of solute material.



Splitless injection was conceived for sample evaporation in the gas phase between the needle exit and the column entrance, which, as we know today, presupposes nebulization at the needle exit. Nebulization presupposes partial evaporation inside the needle: the liquid explodes and small droplets are rapidly slowed down by the carrier gas. Evaporation in the gas phase largely avoids adsorption on surfaces and, hence, allows even high-boiling and other difficult compounds to reach the column unhindered. So far,my father's rule is accurate.

Problems arise when samples are not properly nebulized, as is expected, if (1) the sample is dissolved in a high-boiling solvent or (2) one of high surface tension, (3) if it contains an elevated concentration of non-evaporating by-products, and (4) if a fast autosampler is used, suppressing evaporation inside the needle.

"Dirty" samples

Many samples injected by the splitless method are "dirty." We often notice that the same concentration of a component produces a smaller peak in a "dirty" sample than in a mixture of standards. One percent of non-evaporating material was found to result in approximately a 15% loss for the C10-alkane and a 40% loss for C22; losses for C30 sometimes exceeded 90% (J. Chromatogr. 294 (1984) 65). Hence, peaks in "dirty" samples were too small, and the higher-boiling components discriminated more than the volatiles. If a clean mixture of standards is used for calibration, the analysis of a "dirty" sample is correspondingly inaccurate. Glass wool between the needle exit and the column entrance eliminated this matrix effect (Chromatographia 18 (1984) 517). We assume that droplets of non-evaporating by-products carry the sample material to the bottom of the injector.

Fast autosamplers

Fast autosamplers do not reproduce the conditions of manual injection for which the empty liner was designed. Injection is performed in such a short time that evaporation inside the syringe is avoided. The sample leaves the needle as a band of liquid, and, since nebulization is suppressed, it is "shot" to the bottom of the injector (J. Qian et al., J. Chromatogr. 609 (1992) 269). Solute degradation on the metal surfaces at the bottom of the injector results not from the chemical activity of these surfaces, but from how the sample material gets there.

Tests on completeness of evaporation

Have you observed the problem described above? If so, how large are the resulting deviations? The following testing procedures may help:

On-column injection

The most comprehensive control of results obtained by splitless injection compares with on-column injection. One of the samples analyzed is injected a second time by the on-column technique. If no on-column injector is available on the instrument, the column is dismantled from the vaporizing injector. After waiting 20-60s (decompression of the gas in the column will cause backflow), 1-2 µl of sample is injected into the column inlet. Use either an on-column syringe with a thin needle or a short piece of 0.53mm i.d. precolumn to enable injection with a standard syringe.

Conditions ensuring nebulization

You may want to test whether conditions for nebulizing the sample would improve your results. Remember what supports nebulization:

- Partial vaporization inside the needle (i.e. use "hot needle" injection), no fast autosampler.
- Use a low-boiling solvent of low surface tension, such as pentane or ether (i.e. substitute at least 90% of a more difficult solvent).
- Use a high injector temperature (above about 240°C).
- Inject a modest volume of sample (e.g. 1µl reading on the barrel).

Clean sample

Both tests, mentioned above, are not suitable for checking the effect of non-evaporating sample by-products. Very "dirty" samples cannot be injected on-column and may not be nebulized even when dissolved in pentane. Compare absolute and relative peak areas in a clean mixture of standards and the "dirty" sample with a number of components covering the chromatogram of interest. If peaks are smaller in the sample than in the calibration mixture and if the later eluted components suffer more, this fits the mechanism described above.

Packed inlet

Position a small amount of glass or fused silica wool just above the column entrance in order to stop sample liquid. If the wool increases peak areas for the "dirty" sample, or for a sample injected in a difficult solvent, or for one that is introduced by a fast autosampler, you have "caught the worm."

Conclusions

Unfortunately, interpretation of the test results is complicated by interfering mechanisms. Peak areas of a 1 µl splitless injection might be nearly twice those of a 1 µl on-column injection because the needle is empty. Losses inside the needle will, on the other hand, reduce the peak areas, discriminating against the high boiling solutes. Packing material may adsorb solutes. Polar

by-products may deactivate them again, increasing the areas for the "dirty" samples. Hence conclusions must be drawn with some care.

Sample evaporation could be forced to occur above the column entrance by the means shown in Fig. 2: A short plug of deactivated glass or fused silica wool is positioned just above the column entrance in order to prevent non-evaporated sample from dropping to the bottom of the chamber. Alternatively, a liner is equipped with a constriction at the bottom, and the column is installed in the orifice. However, these solutions also have drawbacks: Wool is adsorptive and particularly problematic for trace analyses commonly performed with splitless injection. Second, septum particles and other non-evaporating materials now accumulate above the column entrance andmay retain the sample components. With the classical arrangement, they were not in the way.

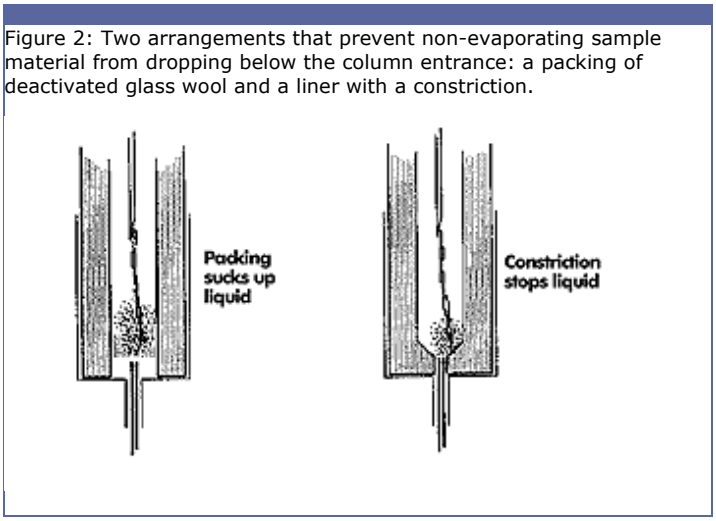


Figure 2: Two arrangements that prevent non-evaporating sample material from dropping below the column entrance: a packing of deactivated glass wool and a liner with a constriction.

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EDITORIAL

Are GC Techniques Really Optimized?

by Dr. Konrad Grob, Kantonales Laboratory, Zurich

Users tend to think that basic GC techniques have been investigated in all details and sanctioned by a competent committee. In fact, why should one think about the design of an injector liner, after splitless injection has been used for more than 25 years? Modern companies invest 5-10% of their profits into research and development. Hence, bigger instrument manufacturers must have many labs with numerous people optimizating techniques. As splitless injection is probably the most widely used method of sample introduction in capillary GC, manufacturers must have tested their injector with all types of samples before releasing a new instrument. True or not? It would be difficult to find out. I have not seen behind the walls of all the instrument manufacturers, but I have witnessed most of the development of splitless injection. I have come to the conclusion that the above views are awfully naive. There wasn't the idealist who invested many years to perfect splitless injection, nor an employer financing such a project. No instrument manufacturer had a single person working even just one year in extracting the knowledge from the literature available and checking all possible uses.

Splitless injection was shaped through a number of incidents and particular circumstances with only a few people involved. There were misunderstandings and errors; conditions were changed (such as carrier gas flow rates lowered or the injection process accelerated) without properly taking notice of the consequences. Some assumptions survived over decades without ever having been questioned. No one person took the responsibility for providing the analyst with an optimized technique.

"Invention by Accident"

Splitless injection was introduced by my father in 1968. He did not "invent" it by developing a concept in his mind and putting it into practice. He simply forgot one morning to open the split vent before performing what should have been a split injection. Peaks turned out to be very large (since all sample material entered the column). More surprisingly, all peaks were perfectly sharp. Everybody at that time was convinced that something like splitless injection would be impossible because the slow transfer of the components into the column created broad initial bands. Under other conditions, peaks were as broad as expected, and it took him about four years to determine the parameters required to produce sharp peaks, i.e. to understand the concepts of solvent effects and cold trapping.

Working in his spare time in the cellar of the school house (he was a teacher), my father had no means to modify the injector. Circumstances thus dictated that the new technique worked with the split injector available. It primarily had to solve his problems in trace analysis and was not developed with the interest of today's maybe 200,000 chromatographers in mind. For instance, he was not interested in highly accurate quantitative data. His work was supported by a cigarette

company to find out why smoke is harmful, not to develop an injection technique.

Size of the vaporizing chamber

Because my father realized that a larger vaporizing chamber would be needed for storage of the sample vapors between their formation and transfer into the column, he had an injector made by a local mechanical shop. The design of this injector was described in J. High Resolut. Chromatogr. 1 (1978) 57. Since 1 µl of liquid transforms into 100-400 µl of vapor (further enlarged by mixing with carrier gas), an 80 x 4 mm i.d. chamber was selected with an internal volume of about 1 ml. There were long discussions concerning the geometry of the liner. A longer, more narrow chamber was preferable because it reduced mixing with the carrier gas and improved the transfer of the vapors into the column because of the higher gas velocity. However, this would require a very long syringe needle to allow the release of the sample near the bottom of the chamber. Because of its length, the syringe needle would be awkward and difficult to use.

This injector almost immediately became the standard for Carlo-Erba instruments. The other manufacturers continued to introduce injectors with chambers of merely 1-2mm i.d. (with an internal volume of 0.06-0.25ml) for another decade. Few seemed to ask where the sample vapors would go. Nobody seemed to know or care to prove if a 2mm i.d. liner provided enough sample vaporization space. Quantitative work performed with splitless injection during those years was often embarrassingly poor. Some authors concluded that "the splitless injector acts like a non-linear splitting device and delivers unpredictable and irreproducible quantities of individual components on to a WCOT column." Other authors published papers where more than 3µl of methanol (which has a vapor cloud of 2.5ml) had been injected into a 2mm i.d. liner with an internal volume of 0.25ml. Letters to the editor reacting to such elementary shortcomings made instrument manufacturers aware of the importance of the size of the vaporizing chamber.

Injection Rate

My father and I are also responsible for an error introduced in 1978. In order to enable injection of larger samples, we recommended introduction at a rate adjusted to the transfer of the vapors into the column, i.e. 1 µl in approximately 10 seconds. As published in 1979, we soon became aware that slow injections result in extremely large losses of higher boiling components inside the syringe (sample evaporation takes place in the syringe needle). However, there are still autosamplers slowly injecting into hot injectors.

Length of syringe needle

The syringe needle must be long enough (70-80mm) to bring the center of the vapor cloud just above the column entrance. The vapors must expand backward to make the best use of the liner volume available and ensure that the carrier gas plug between the sample vapors and the column entrance transfers into the column before the sample vapors.

Carrier gas flow rate

In the early days, splitless injection was used with hydrogen carrier gas flow rates of 2-4ml/min. As shown in 1981, 2ml/min. is the lower limit ensuring complete transfer from 4mm i.d. liners into the column, i.e. accurate splitless work. Many analysts continue to ignore this fact. For instance, GC-MS units have become popular with analysts with carrier gas flow rates limited to less than 1ml per minute due to their limited vacuum pump capacity. These MS units are

primarily used for trace analysis with splitless injection, but nobody shows concerns about the effect low injector flow rates have on splitlessquantitative results.

Injector Designs

There are more design characteristics known to be critical but neglected in many of the instruments presently used. The split outlet line should have a small internal volume to prevent the sample from being pushed into it by the pressure wave initiated by sample evaporation. In order to prevent loss of vapors, no flow should pass over the top of the vaporizing chamber during the splitless period. The use of an empty, straight injector liner, as recommended by my father, made sense as long as sample evaporation inside a hot syringe needle supported nebulization of the sample at the needle exit. However,with the introduction of fast auto samplers, conditions have changed and sample evaporation must be reconsidered. This will be the subject in one of my next "Korners."

Conclusions

There has never been a comprehensive, professional investigation resulting in a convincing design of the splitless injector. In contrast to most other products marketed, such as cars or airplanes, the supplier carries no responsibility. Analytical chemistry relies on the knowledge of the analyst. He is responsible for choosing the right instruments and using analytical techniques correctly. Unfortunately, reality is often different, as demonstrated by unoptimized splitless injector designs and improper operating parameters.

I do not have a simple solution to offer, but some consequences seem obvious:

1. Users must realize that many injectors and splitless method parameters have never really been optimized and are prone to error.
2. It would take a lot of money and a concerted effort by all instrument manufacturers and analysts to perfect the splitless injection technique.
3. Maybe combined forces will be more successful. Analysts should publish their observations as well their ideas on what can be improved. If thousands struggle alone in their laboratory, frustration accumulates while problems remain unsolved.
4. Instrument manufacturers will optimize injector design if customers make it a priority.
5. Quality management puts tough requirements on the accuracy of oven temperature (which has little effect on reliability of quantitative results), but accepts injectors that disregard elementary requirements.
6. Certified methods commonly describe in detail how a sample is prepared, but do not specify how to perform splitless injection properly.

Capillary GC is immature because numerous technical aspects have not been adequately investigated. If this work is not done in the near future, poor quantitative results will invalidate the techniqueof capillary GC.

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EDITORIAL

Sample Evaporation in Hot GC Injectors

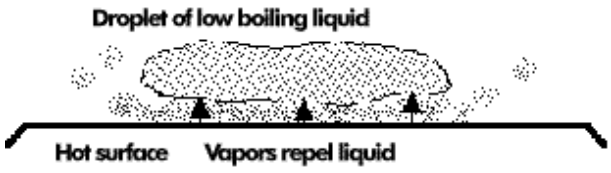
by Dr. Konrad Grob, Kantonales Laboratory, Zurich

Is sample evaporation in a hot GC injector something you have to think about? Injector temperatures seem to guarantee almost instant evaporation of solutions in volatile solvents. However, appearances are deceptive. Not even vaporization of the solvent is ensured, and as long as not all of the solvent is evaporated, sample components cannot evaporate. Sample liquid "raining" onto (or rather, by) the column entrance is not wanted.

The Leidenfrost phenomenon

(See fig. 1)
The problem of solvent evaporation has to do with the short time available for sample evaporation inside the injector and the Leidenfrost phenomenon. Have you ever seen what happens to a droplet of water falling onto a hot electric cooking plate? Was there the sharp hiss and the water was vaporized? No! The droplet became flat as a small disk and hovered a fraction of a millimeter above the plate. It may have moved nervously, jumping around the hot griddle. Evaporation took many seconds. If this experiment was repeated with a drop of edible oil, you'd observe a totally different behavior: the oil dropped onto the plate, adhered to it, and evaporated more rapidly than the water -- although (or rather because!) the boiling point is much higher.

Figure 1: The Leidenfrost phenomenon: a cushion of vapor repels liquids from surfaces the temperature of which is above their boiling point.



According to the "Leidenfrost phenomenon", liquids cannot touch a surface with a temperature above their boiling point because evaporation forms a cushion of vapor preventing contact. The higher the surface's temperature is above the boiling point of the liquid, the more rapid evaporation occurs. But, since more vapor is formed, the liquid is repelled further above the surface.

The solvent vapors separating the sample liquid from the hot surface of the injector liner have two important effects. First, they render the liquid highly mobile -- it glides away from hot surfaces. Secondly, they insulate the liquid from the hot surface. Since heat transfer is the time-determiningstep of evaporation, low boiling liquids may evaporate slower than higher boiling ones.

Time available for evaporation

The time required for evaporating the sample (first of all the solvent) is determined by the transfer of the heat consumed. For 2 µl of hexane, it was calculated as several hundred milliseconds[1], while 2µl of water require several seconds. Is this time available? It depends on how the sample liquid moves through the injector.

During manual injection, the plunger is depressed at a speed of around 1-2 m/s. However, as the liquid enters the narrower needle, it is accelerated to 15-30 m/s (some 50-100 km/h) and leaves the needle at this same speed at least. Fast autosamplers cause it to exit at speeds even far above those fast cars can achieve. If the injector liner is empty and the sample continues to travel at this speed, the column is reached in far less than 0.1-3 m/s -- which is 100-10,000 times less than needed for sample evaporation. To achieve full vaporization, the sample liquid must be slowed or stopped above the column entrance.

Nebulization of the sample liquid

(See fig. 2)
If samples are injected by a technique involving a hot syringe needle, partial evaporation inside the needle often nebulizes them. The resulting fine droplets are rapidly slowed to the gas velocity and reach the column after several hundred milliseconds only (depending on the gas flow rate). Visual experiments have confirmed that most organic solvents are nebulized when injected by the hot needle method (preheating the needle inside the injector before rapidly depressing the plunger). Nebulization in an empty liner provides gentle evaporation in the gas phase hardly involving any contacts with adsorptive and maybe dirty surfaces. Even high boiling, polar, and labile components are vaporized rather well.

Figure 2: Sample evaporation involving nebulization at the needle exit.

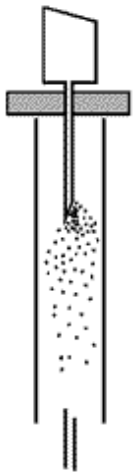
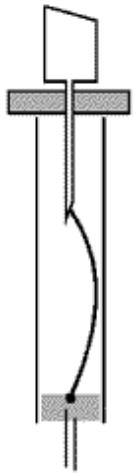


Figure 3: Non-nebulized sample liquid must be stopped, e.g., by glass or quartz wool.



Stopping sample liquid by packing material

(See fig. 3)
Nebulization does not occur with fast injection auto-samplers. The sample liquid forms a thin band, like water running from the tap, and moves almost without resistance. It must, therefore, be stopped above the column entrance by other means, which is all but simple because of the Leidenfrost phenomenon.

Heat consumption by evaporating liquid cools the source of the heat. If cooling is strong enough to reduce the surface temperature to the sample (solvent) boiling point, the liquid can contact the surface. This occurs with obstacles of a low thermal mass, such as glass or quartz wool. The liquid cools the nearest fibers it encounters and falls into the wool just as children jump into a haystack. Hanging in these fibers, the sample forms an island with a temperature corresponding to the solvent boiling point until the solvent is evaporated.

The smallest amount of wool which forms a short plug without major gaps (1-3 mg) serves the purpose. Additional amounts merely aggravate the problems -- adsorption and degradation of labile compounds. There are two concepts for placing the packing -- situated near the exit of the inserted needle, the packing will always receive the liquid and the solutes will always evaporate from its surface. This renders the process reproducible, but susceptible to the activity of the packing. Placed just above the column entrance, the packing rather serves as a safety net: nebulized samples will evaporate in the gas phase above the packing and pass the latter easily (adsorptive surfaces have less effect on passing vapors than on material evaporating from them). If the sample is only partially nebulized or not at all, the packing acts as a net underneath the acrobat in the circus. Packings of low thermal mass would be the most convincing solution to sample evaporation if they were inert.

Recently, Restek sent us some carbon material (Carbofrit™) with the suggestion to test it as liner packing. Initially, I didn't even want to try it because carbon is usually highly retentive and catalytically active. As we nevertheless gave it a chance, we were highly surprised -- it exhibited low retentive power and good inertness.

Liners with obstacles

Injector liners containing solid obstacles, such as baffles or an inverted cup (Jennings cup), were conceived to enhance mixing the sample vapors with the carrier gas and stop "shooting" sample liquid. The inverted cup forces the gas flow to reverse directions twice, which seemed to guarantee that non-evaporated sample material would not pass. There was no solid proof, however, because it is difficult to derive from chromatograms what happened inside the injector. Recent visual experiments provided more direct evidence. Because of the Leidenfrost phenomenon, the sample liquid is able to curve around hot solid obstacles and change direction rather sharply. For instance, it performed perfect slalom aroundthe baffles, hardly being slowed. When the obstacles stop the sample liquid, it is for different reasons than what the originators thought. The main effects are due to the fact that liquids are hindered to enter narrow channels (again, the Leidenfrost phenomenon). The inverted cup of the Hewlett-Packard liner usually stopped the sample liquid, provided the sample volume did not exceed 1.5µl. The most effectiveliner was, however, the "laminar liner" from Restek[2].

Conclusions

There are three principal concepts to achieve sample evaporation:

1. Sample evaporation in the gas phase of an empty liner provides the most gentle conditions, but presupposes partial evaporation inside the needle.
2. Well designed obstacles stop "shooting" sample liquid.
3. Packings with low thermal mass render vaporization most reliable, but evaporation occurs from a surface.

All three concepts may turn out best suited. You have to try.

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EDITORIAL

Why 5cm syringe needles for capillary GC?

by Dr. Konrad Grob, Kantonales Laboratory, Zurich

GC is a complex technique. All too often the analyst stands in front of his instrument, surprised about a result, maybe annoyed about a problem, and at a loss for an explanation for what he observes. Often, not even his colleague is able to explain. Another of these GC mysteries? Probably he would have the knack of it if he knew the many details involved in the analytical process. We make numerous choices without being aware of them, overlook variables clinging to the illusion that they had been thoroughly investigated in the past and that an international committee has decided that this or that is the correct choice. The length of the syringe needle is one such frequently neglected detail and is an example of a parameter which has never received proper attention.

Many years ago, the manufacturers of GC syringes looked upon their customers and noticed that there was no agreement on how long syringe needles should be for conventional vaporizing (split or splitless) injection. Some said 1.5 inch (the needle protruding 37 mm from the glass barrel), others 3 inch (71mm), or even longer. So, father syringe producer decided to compromise and have it in between: 2 inch (51mm). Whether or not he died in the mean time, that's how it still is. Some disagreed, but since it seems to be more important that GC is simple than that it is well optimized, the subject was commonly neglected. The subject of needle length seems not to be of sufficient scientific status to justify closer investigation.

As you can check by a few experiments, the length of the syringe needle and the depth by which a long needle is inserted into the injector often have an important impact on quantitative analysis. The reasons are explained below. It is concluded that they need to be adjusted to the situation. The length of the syringe needle determines from which point inside the liner the sample expands during the evaporation process. It may, however, also influence vaporization itself.

Headspace Analysis:

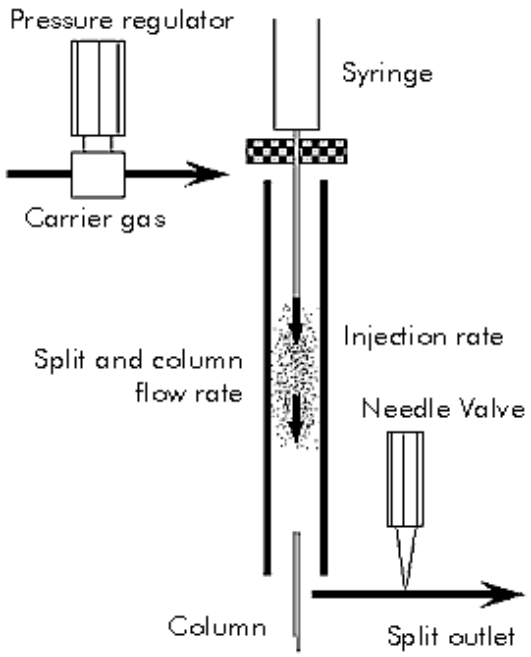
We start by looking at gas or headspace analysis, because the situation is particularly simple since no vaporization interferes. However, the same principles will also apply to liquid samples. We refer to (manual or automated) injection with a gas-tight syringe of 0.5-1 ml capacity.

Usually an amount of gas phase is injected that approaches the internal volume of the vaporizing chamber. For instance, a 4mm ID liner of 80mm length has an internal volume of 1ml. A 500µl sample mixes with carrier gas to form a vapor cloud of close to this volume (inlet pressure compresses the cloud, but increased temperature causes it to expand). Care must be taken to release the sample from the syringe needle in such a way that it ends being positioned inside the chamber.

Gas and headspace samples are usually injected in the split mode in order to achieve sharp initial bands. Depression of the plunger at normal speed introduces the sample at around 0.5-1 ml/s, i.e. 30-60 ml/min. If the sum of the split and the (comparably small) column flow rate corresponds to the rate of injection, expansion of the sample downwards replaces the gas flow from the rear. Gas supply is stopped; the gas phase running off originates from the syringe (assumption of a pressure-regulation/needle valve system, Fig. 1). At higher split flow rates, the sample is diluted with additional carrier gas from the rear. Under these conditions, basically unlimited volumes of sample can be injected without overloading the injector. A short syringe needle merely entering the vaporizing chamber (2-3 cm) serves the purpose, but longer needles are no drawback.

Since headspace analysis is mostly trace analysis, the split flow rate is usually substantially below the 30-60ml/min mentioned above. This leaves the choice of injecting at a correspondingly reduced rate or temporarily storing the vapor cloud inside the vaporizing chamber. The latter corresponds to common practice. If more sample is injected than gas runs off at the same time, carrier gas must be displaced within the injection system. Appropriately designed injectors with a pressure regulator at the rear and a needle valve in the split outlet have a relatively large internal volume in the gas supply and a small one in the split outlet, causing the sample to expand backwards (Fig. 2). Long syringe needles are required such that the sample expands from a point near the column entrance towards the rear. If the liner is 80mm long, the column enters by 5mm, and the injector head is some 12mm high, the syringe needle should be around 80mm long. The commonly used 5cm needles enter the liner by less than 4cm and merely exploit the upper half of the chamber. 500µl thus injected already overfill the injector liner, i.e. cause sample material to be expelled through the septum purge outlet or to penetrate the carrier gas supply line.

Figure 1: Injection at a rate equal to the flow rate of the gas passing through the liner: the flow from the carrier gas supply is substituted by that leaving the syringe needle.



Systems with flow-regulated carrier gas supply and a back pressure regulator in the split outlet (e.g. Hewlett Packard) behave differently. Pressure increase by injection causes the back pressure regulator to open widely and increase the split flow rate. The sample cloud expands downwards (Fig. 3). As the volume of the injector can only be exploited by releasing the sample at the top of the chamber, the syringe needle should be no longer than 2-3 cm (or a longer needle should

Figure 2: Headspace injection at a low split flow rate, using gas supply by the pressure regulator/needle valve system: the sample should expand from the column

be introduced only partially). A drawback of this type of gas supply is the split flow rate during the splitting process is rather ill defined.

Split Injection of Liquid Samples

Split injection of liquids resembles gas/headspace injection except that the rate of vapor formation cannot be controlled. Injection must occur rapidly in order to avoid excessive evaporation inside the syringe needle. 2µl of a solution in a volatile solvent, such as dichloromethane, creates some 0.9ml of vapor in maybe 0.5, i.e. vapors are formed at 1.8ml/s (108ml/min). With a split (and column) flow rate of 108ml/min at least, the situation of Fig. 1 applies, i.e. the syringe needle should merely enter the vaporizing chamber. It leave smaximum room between the needle exit and the column entrance for sample evaporation and mixing across the vaporizing chamber. If the split flow rate is lower, i.e. vapors are formed more rapidly than gas is discharged, a long or a short needle is best suited, depending on the carrier gas supply system involved (Fig. 2 or Fig. 3).

Samples with high boiling matrices, such as many undiluted liquids, evaporate slowly; discharge of the vapors is a problem only if the split flow rate is extremely low. Such liquids are easily transferred to the wall of the liner (no repulsion by vapors). If an empty liner is used (preferably of narrow bore, e.g. 2mm), short syringe needles render such transfer more reliable as the risk of shooting the sample liquid by the column entrance becomes small.

Splitless Injection

In splitless injection, the sample vapors must be stored in the vaporizing chamber until they are transferred into the column, which may take over a minute. Before being diluted with carrier gas, 2µl of a solution in hexane produce around 500µl of vapor, in dichloromethane as much as 900µl, which shows that the internal volume of an 80mm x 4mm ID liner must be fully exploited.

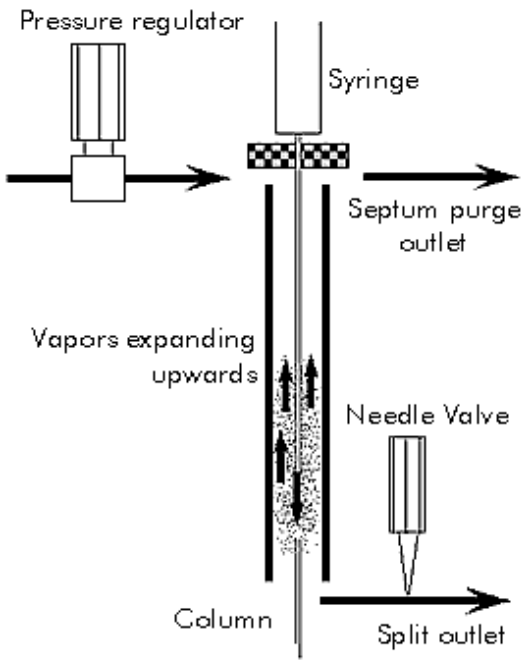


Figure 3: Sample expanding downwards in the instance of a system with flow regulation/back pressure regulation.

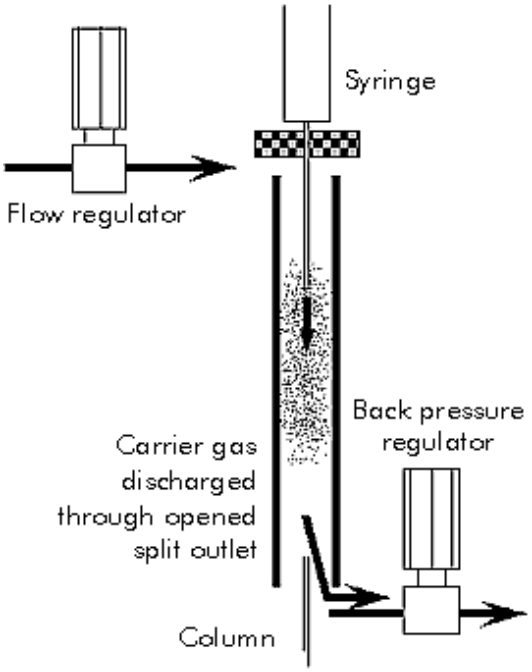
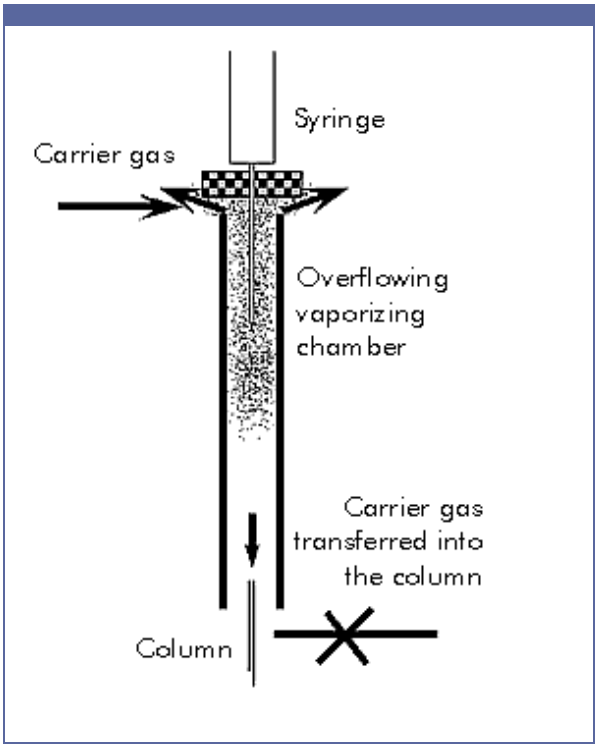


Figure 4: 5cm syringe needles are too short for splitless injection as the chamber is overfilled even with small sample volumes & some 400µl of carrier gas must be transferred into the column before sample vapors get



As the split outlet is closed, there is only one way of filling the vaporizing chamber: from the bottom to the top, displacing the carrier gas backwards. The syringe needle must be adjusted to situate the center of sample evaporation slightly above the column entrance. The distance between the needle exit and the column entrance must account for the distance the droplets travel before evaporating, i.e. 1-2 cm. For the usual geometry of the injector this means using 3 inch (71mm) needles (or rather the vaporizing chamber was designed such that standard 3 inch needles would fit). There is a second reason for depositing the sample close to the column entrance. As shown in Fig. 4, a 5cm syringe needle leaves a distance of some 40mm to the column entrance, representing a plug of some 400µl of carrier gas. Before substantial amounts of sample vapor reach the column, this gas must be discharged into the column, i.e. during 10-20s primarily carrier gas is "injected." Knowing how difficult it is to achieve complete sample transfer in splitless injection, this is certainly not the kind of problem we need.

Sample Evaporation Inside the Needle

As if the subject were not of sufficient complexity yet - the length of the syringe needle also influences sample evaporation. Parts of the sample may be vaporized inside the needle during injection or when the needle content is eluted after the plunger is fully depressed. On the one hand, this often causes problems as more is injected than measured and preferential vaporization of volatile components discriminates against high boilers. On the other hand, it helps nebulizing the sample liquid at the needle exit, which is the prerequisite for sample evaporation in the gas phase of the injector (the most gentle vaporization process, since there are no contacts with packing materials adsorbing or degrading solutes). There is more vaporizationinside long needles accentuating these advantages and disadvantages.

Conclusions

The 5cm needle for vaporizing GC injectors is a typical compromise: it is between the desirable long and the desirable short needle, but is hardly ever desirable as such. The following table suggests optimum needle lengths.

Optimum Needle Lengths

Injection Technique	Gas Supply System	
	Pressure reg./ needle valve	Flow reg./ back pres. reg.
Splitless	71mm	71mm
Split (flow rate >100ml/min.)	25mm	25mm
Split (flow rate <100ml/min.)	71mm	25mm
Split, high boiling matrix	25mm	25mm

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Method validation

A more modern trend is to rely on "method validation" and "ruggedness" of a method. A method should be studied and described in such detail that errors are practically ruled out. It is tested by other laboratories in the hope that still undetected problems are recognized.

Method validation is certainly an important step ahead, but I still see considerable room for improvement. Often being directed by management, it tends to involve exceedingly complicated procedures, committees, meetings, complex statistics, and, of course, much paper and computer work. Commonly, none of these managers executed the method themselves frequently enough to become aware of the really critical steps. Resulting methods tend to be detailed in how to weigh the sample and what glassware to use, whereas more delicate steps, such as the separation of phases in an extraction step or injection into a GC, just comprises a few lines, if described at all.

Such method validation has at least one important advantage: everyone is safe, because imperfections are sanctioned by a recognized scientific body. Nobody loses face if results are wrong or can be prosecuted for "his" error. Weaknesses of a method are, in a way, socialized.

Certification

The most recent achievement is a highly complex, intellectually convincing construction called "certification." Again, the efforts brought some improvements, but reality does not always look as convincing. The lab people received a heavy load of extra work: the balance must be checked every so often and forms must be filled in triplicate for all managers, certifying that the lab is in good shape. Chemicals must be delivered with extensive paperwork in order to make sure the substance in the bottle is what the certificate says. Many methods, chemicals, and instruments are eliminated because they no longer "comply" to one of the many "standards." Unreasonable constraints and complications demotivated many lab people. A number of labs even did steps backwards, as some of the analyses are no longer possible. Awkward methods are applied because modifications became exceedingly complicated. Workers get careless because they lose interest and no longer feel responsible.

"Certification" was again imposed from outside the lab and seems like a somewhat helpless attempt to solve problems by intellectual force and general systems. It neglects the serious problems due to those particularities of techniques and samples that are left outside the "certified" area. Of course, there might have been a poorly calibrated balance somewhere, but its contribution to analytical problems was negligible.

Errors to be fought

Purposeful quality assurance is primarily work in the lab, tough, but unspectacular fighting with problems. It has little to do with brilliant concepts and there are no sweeping solutions. A given sample may require a longer syringe needle or some packing in the liner for splitless injection. The following four types of problems may be distinguished.

Systematic errors

Random errors are easily recognized by reproducibility tests. Detection of systematic errors is more difficult, because the analyst must devise special checking procedures, which in turn presupposes a lot of knowledge and experience. Systematic errors occur, for example, when a

column shows varying adsorptivity or when the system behaves differently to the calibration solution than the (maybe "dirty") sample in split and splitless injection.

The extraordinary sample

The strategy of method validation assumes every sample analyzed corresponds to the sample(s) used for testing the method. In reality, however, maybe one out of twenty samples differs in a detail not thought of. Derivatization may, for instance, work beautifully under "normal" conditions, but the exceptional sample may contain a by-product hindering the reaction. In fact, validation of the method cannot consider all the possible extraordinary samples.

Incidental errors

In routine work, every so often the "analytical devil" catches a victim. The internal standard may be partially degraded because the solvent contained more peroxides than normal (concentrations at the ppb level may be sufficient for dilute solutions), the air is more humid during packing of a cleanup cartridge, or (during injection) a minute gas bubble causes more sample to be eluted from the syringe needle than normally. Even the most rigorous "certification" procedure is not immune to all this.

Analytical mysteries

Office managers may not accept it, but practical experience demonstrates that occasionally a result is wrong and no explanation can be found. I do not deny the principle that everything has a reason, but due to the impracticality of researching all these cases, the matter may remain a mystery.

Methods controlling each result: direct verification

No doubt, methods must be checked carefully before being applied, but they are unlikely to become foolproof. Knowing that, more should be done for the verification of each single or at least each group of results. The description of a method should include a list of the potential problems and how resulting deviations can be specifically detected. The latter requires the methods include verification elements. It has become common practice to reanalyze reference materials or spiked samples, which checks the system and the method as such, but does not prove that every single sample has been analyzed correctly. Most conclusive verification is obtained by controlling elements included into each analysis, such that the chromatogram obtained not only provides the result, but also enables to check for, e.g., extraction efficiency, degradation of a component, or yield of derivitization. Such "direct" verification enables the detection of samples behaving "abnormally" or any action of the "analytical devil." Such verification must be tailored to each method and may, for instance, include the following elements:

- To check the yield of a derivatization, a second internal standard not undergoing derivatization is added to each sample.
- A chemically stable component is added to the internal standard solution in order to check for degradation of the latter.
- Degradation of labile components is checked by addition of two internal standards, one being stable, the other of similar lability as the component.

- Adsorption is monitored by adding an inert and an adsorptive standard.
- Volatile and high boiling internal standards are added in order to monitor discrimination during GC analysis.
- Internal standards are added before and after a critical extraction or preseparation, checking for losses.
- Preseparation is controlled by adding components which should be removed and others that must be included in the window of the compound(s) of interest.
- Results are calculated in different ways and compared, e.g. using an internal and an external standard procedure.

Chromatograms obtained by methods designed for directly verifying results may well contain three or more internal standards. This renders evaluation of results more complicated, but computer reports may include all the necessary calculations and even a concluding statement on whether or not the analysis went OK.

Less repetition of analyses

Direct verification renders most duplicating analyses unnecessary. If a result is unexpected, the critical steps of the procedure can be checked, presumably confirming the result in most instances. If a result is wrong, the analyst knows where the deviation occurred and where to improve. This may save days of dull repetitious work and groping through the dark.

Real quality assurance

It must be admitted that the accuracy and reliability of analytical results is often a problem. Great efforts have been taken to improve on this, but all too often proposed "quality assurance" did not get beyond marginal aspects, such as measuring temperatures of all the heated parts of a GC instrument, which is mostly a waste of time and distracts from the true problems. Efficient quality assurance must get the knack of the really hot spots of each method. Potential sources of deviations must be determined and, as far as possible, checked by control elements built into the analysis of the sample. If such direct verification indicates that results are correct, we know that the detector was heated, that the internal standard solution has not been degraded, and that all the other steps and items involved were OK.

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