Journal of Chromatography, 294 (1984) 65–75
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 16,667

EFFECT OF DIRT ON QUANTITATIVE ANALYSES BY CAPILLARY GAS CHROMATOGRAPHY WITH SPLITLESS INJECTION

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(Received February 16th, 1984)

SUMMARY

In splitless injection involatile by-products ("dirt") have a significant effect on quantitative analysis. The transfer of medium to high boiling solutes from the vaporizing chamber into the column is hindered by the retention power of the dirt. The extent of the material transferred is not equal for clean (calibration) mixtures and dirty samples. Hence calibration with clean mixtures provides data (area per concentration for the external standard method, or response factors for the internal standard method) which must not be applied to the sample. It is proposed to calibrate by addition of standards to the sample. With such a quantitation procedure, systematic errors can be excluded and extremely dirty samples can be analysed by the splitless method.

INTRODUCTION

Many analysed samples contain high-boiling or involatile by-products, commonly called "dirt". On the other hand, studies were carried out on quantitative analysis with splitless injection with mixtures containing some standards in a pure solvent. The effects of the dirt have never seriously been studied. The results of investigations with clean mixtures, *e.g.*, refs. 1–3, may be too "optimistic", on the other hand there is often an exaggerated fear of dirt. Especially splitless injection tolerates very large amounts of dirt, provided the dirt does not introduce materials producing peaks which interfere with the peaks of interest. However, the analyst should be aware of the effects of dirt on quantitation to be able to design suitable procedures. Furthermore, he should know what to do if the system starts to degrade; the regeneration is often trivial.

In a previous paper[4] we described the effects of dirt introduced by the on-column technique, *i.e.*, that deposited into the oven-thermostatted column inlet. A relatively small amount of dirt in this area causes peak broadening and/or distortion, predominantly by the mechanism of band broadening in space. The column should be equipped with a retention gap[5,6], not only to reconcentrate the bands broadened in space, but also because it may be partly filled (in terms of retention power) by the dirt until some peak broadening becomes apparent. The amount of dirt which the retention gap will tolerate before peak broadening becomes apparent was found to

0021-9673/84/$03.00

range between about 1 μg for a very thin film column (0.07 μm) and about 100 μg for a thick film column (1 μm). The latter value corresponds to a single 1-μl injection of a solution loaded with 10% of dirt or, *e.g.*, ten injections of solutions with a 1% dirt level.

Whenever the amount of dirt in the column inlet becomes excessive, a section of the retention gap is removed the length of which corresponds to the length of the flooded zone (about 30 cm per microlitre of sample injected). This is particularly easy if the retention gap consists of fused-silica tubing.

The results of this previous study also apply to the splitless injection technique, although most of the dirt remains in the injector liner just below the point where the syringe needle releases the sample. Involatile by-products of the sample may reach the oven-thermostatted column inlet only if they are transferred as particles (droplets or aerosol). Nevertheless, the proportion of the dirt which gets that far is estimated to range between 1 and 20%. It is highly dependent on poorly understood factors influencing the formation of small droplets or aerosols. The dirt is deposited in the column inlet section near to the attachment to the injector. But if solvent is recondensed in the column, creating a "solvent effect", some of this dirt may be re-dissolved, flows with the solvent further into the column and spreads throughout the flooded inlet section as in on-column injection.

In on-column injection, dirt affects the peak shapes, and not, or only indirectly, the quantitative results. In splitless injection the situation is just the opposite: unless the column inlet has become very dirty, the peaks remain perfect in shape, but the quantitative results are unreliable.

The critical point in the splitless injection technique is the transfer of the sample vapours from the vaporizing chamber into the column. If the carrier gas flow-rate is low, *i.e.*, the transfer is slow, the sample vapours tend to be more rapidly diluted than transferred and even within extremely long splitless periods (closure time of the split exit) only, *e.g.*, 40% of the sample enters the column[7]. For a clean test sample with a solute of intermediate to high volatility, thus for a fully vaporized, unretained solute, the minimal carrier gas flow-rate to achieve at least a 95% transfer of the sample material was found to be about 2.5 ml/min (splitless period about 90 sec). The transfer is accelerated if the solvent recondenses in the column inlet. But with a carrier gas flow-rate below about 1.5 ml/min, even in this most optimistic of all cases the sample transfer is poor.

Insufficient sample transfer in splitless injection not only causes loss of sample material (sensitivity), but is also a source of non-linearity, because the different solutes are not transferred for the same extents. Volatile solutes diffuse more rapidly away from the column entrance; high-boiling solutes tend to adsorb on the wall of the liner. Sometimes the quantitative results depend significantly on factors influencing the sample transfer rate (carrier gas flow-rate, recondensation of the solvent), which is explained by the varying proportion of sample material transferred.

Any retention of solute material on the wall of the injector insert increases the volume of carrier gas which must be transferred into the column to carry along a sufficiently high proportion of the solute material. Dirt, the subject of this paper, is probably the most important source of such retention.

EXPERIMENTAL AND RESULTS

First the discriminations in the analyses of a clean and a dirty sample were compared. The test sample consisted of equal amounts of C_{10}–C_{34} *n*-alkanes dissolved in *n*-hexane (50 ppm). To simulate dirt, various amounts of DC 200 were added, a relatively low-molecular-weight methyl silicone stationary phase. The carrier gas flow-rate was 3 ml/min and the splitless period 40 sec. Since the column temperature during the injection was 30°C and the solvent strongly recondensed in the column inlet, good transfer conditions were chosen. (For a fully evaporated solute a transfer of 95% would have been achieved in about 20 sec.) The injector temperature was 250°C. The mixture was analysed on a 20 m × 0.32 mm I.D. glass capillary, coated with SE-52 of film thickness 0.15 μm, temperature programmed from 30 to 310°C at 10°C/min. The sample volume, 1.2 μl (including the needle volume), was introduced by the hot needle technique[8].

As a calibration, the clean test mixture was injected three times. The average

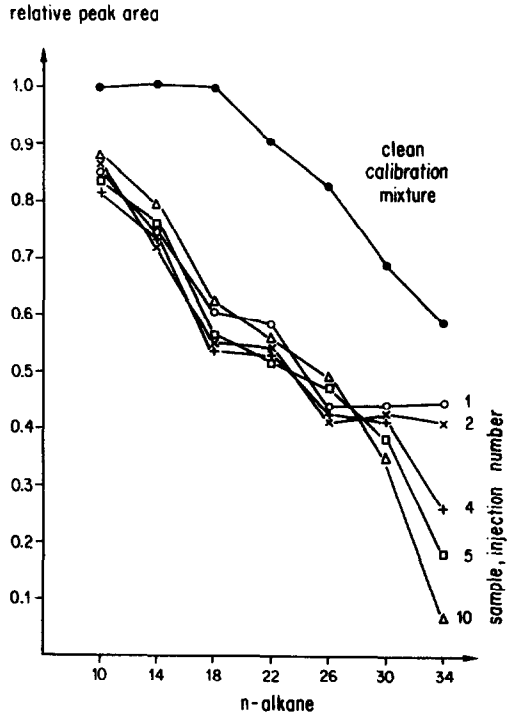

Fig. 1. Dependence of absolute and relative peak areas (discrimination) on the presence of dirt in the sample. The peak areas were normalized onto the C_{10} area obtained with the clean calibration mixture for two samples containing the same concentration of *n*-alkanes, one in pure hexane (calibration mixture), the other in *n*-hexane containing 5% DC 200 as dirt. Although relatively good transfer conditions were used, the dirt hindered even transfer of the volatile C_{10} into the column (injector temperature 250°C). The losses increased with decreasing volatility of the solutes, but they remained practically constant from the first to the tenth injection, although the initially clean injector became severely contaminated. C_{34} is an exception, obviously because for this solute the retention in the dirt layer of the insert wall is important.

peak areas were normalized on C_{10} as internal standard (value 1.0). The results shown in Fig. 1 (upper curve) indicate a considerable discrimination of the *n*-alkanes above C_{18}, which is primarily due to losses inside the syringe needle.

Then a sample with identical concentrations of the *n*-alkanes but with 5% of DC 200 was injected several times. The obtained peak areas were normalized onto the peak area of C_{10} in the clean calibration mixture. The C_{10} areas were 10–20% below those of the calibration mixture (Fig. 1), indicating a sample transfer of only 80–90% of this volatile solute. Losses of the higher alkanes (relative to the clean mixture) increased to about 40%, but the differences between the first and the tenth injection were surprisingly small up to C_{30}. The transfer of C_{34} must have been increasingly hindered by the accumulated DC 200.

Finally the clean calibration mixture was injected three times into the now dirty injector. The results were practically identical with those of the first three injections of the clean mixture into the still clean injector insert. Only C_{34} showed a decreased relative response, from 0.6 to 0.35.

The influence of dirt on the sample transfer is noticeable at surprisingly low dirt concentrations, as shown by analogous experiments with different concentrations of DC 200 in the sample. The injector insert was cleaned and the system re-calibrated before each series of injections. Table I shows the average peak areas measured for the second to the fifth injection of the dirty mixture relative to that of the calibration mixture. Thus, all peak areas were corrected by the discrimination determined for the clean sample. Values below unity mean a discrimination due to the dirt. The presence of 100 ppm of DC 200 reduced the peak areas of the higher boiling *n*-alkanes by 15%. At 0.1% dirt the losses were 30%. High DC 200 concentrations (1–20%) produced similar results, with losses of about half of the solute materials, again with the exception of C_{34}. It is interesting that the column was not noticeably affected by this many injections, introducing a total of nearly 5 mg of DC 200.

The second sample had already been used for studies of the effects of dirt in on-column injection[4]: the sterols and components of similar retention times ("sterol fraction", including tocopherols and triterpenes) in edible oils and fats, analysed by direct injection of the slightly diluted oil. The concentrations of the peaks of interest in the oil are between 10 and 1000 ppm. The triglycerides represent a high-boiling

TABLE I

EFFECT OF INVOLATILE BY-PRODUCTS IN THE SAMPLE ON SAMPLE TRANSFER IN SPLITLESS INJECTION

Peak areas of a test sample with various concentrations of dirt (DC 200) compared with the areas obtained from the clean mixture.

% dirt	*Relative peak areas of n-alkanes*						
	10	*14*	*18*	*22*	*26*	*30*	*34*
0.01	1.0	1.02	1.01	0.84	0.86	0.86	0.82
0.1	1.0	1.0	0.96	0.80	0.70	0.74	0.68
1	0.87	0.81	0.80	0.56	0.53	0.55	0.54
5	0.86	0.69	0.57	0.61	0.56	0.63	0.44
20	0.88	0.78	0.62	0.50	0.52	0.32	0.12

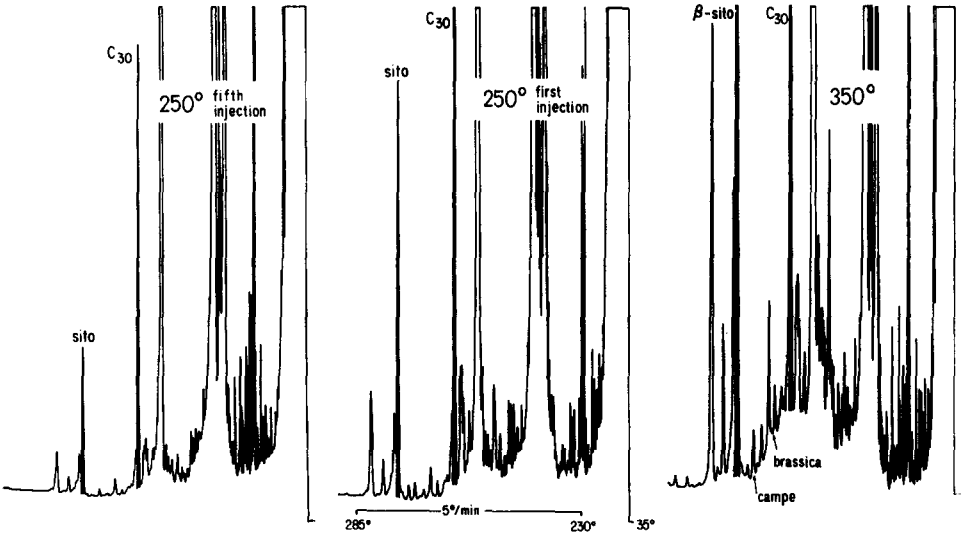

Fig. 2. Chromatograms of olive oil injected splitless as a 20% solution in hexane onto a 6-m apolar capillary column. Peaks: C_{30} = *n*-triacontane; β-sito = β-sitosterol; brassica = brassicasterol; campe = campesterol. The last two compounds are of interest in the detection of additions of rape-seed oil to the olive oil. With the injector at 350°C (right chromatogram), a virtually complete solute transfer from the injector into the column is achieved, but the "hump" underneath the C_{30} peak, due to free acids, indicates degradation of triglyceride material. The first injection into a clean injector at 250°C gave a poor solute transfer. During the fifth injection into the now dirty injector only about 10% of the solute material entered the column.

by-product of very high concentration in the injected sample. Olive oil diluted 1:5 in *n*-hexane (Fig. 2) was injected splitless (1.5 μl) onto a 6 m × 0.30 mm I.D. glass capillary column coated with 0.4 μm of SE-54, equipped with a 1 m × 0.32 mm I.D. retention gap in the inlet. Carrier gas inlet pressure (hydrogen), 0.1 atm, resulting in a flow-rate of 6 ml/min; splitless period 30 sec; temperature programme from 230 to 300°C at 5°/min, after an injection at about 35°C. After each injection the triglycerides were eluted from the column at inlet pressure 0.5 atm and 340°C.

If the injector temperature is kept above about 300°C, the triglycerides leave the injector (probably primarily after the split valve is re-opened again). If the injector temperature is only 250°C, the triglyceride material accumulates in the insert, polymerizes and forms a dark brown layer.

The first chromatogram in Fig. 2 was produced with an injector at 350°C. The transfer of the solute material (sterol fraction) was virtually complete (determined by comparison with on-column injection). At an injector temperature of 250°C the first injection gave a four-fold reduction of the peak areas. The following injections resulted in a further decrease in the amount of material transferred. With the fifth injection (Fig. 2), about 2.5 times less material was detected as for the first injection, and about 10 times less than when the injector was kept at 350°C. Only about 10% of the sample reached the column —a 10:1 split injection rather than a splitless injection, although good transfer conditions were selected!

For this particular problem the use of a very high injector temperature is a valid solution. This would contradict the widely voiced argument that the injector

temperature in splitless injection should be kept relatively low, because there would be far more time available for evaporation than in split injection. However, we were less interested in this particular case than in the phenomena to be expected when injecting dirty samples.

A clean calibration mixture containing equal amounts of *n*-triacontane (C_{30}) and stigmasterol (100 ppm in acetone–hexane) was compared with a 20% solution of olive oil in *n*-hexane, spiked with the same C_{30}–stigmasterol mixture to a concentration of 100 ppm. Stigmasterol was found in the original olive oil at a concentration of 35 ppm. The mixture was analysed on a 15 m × 0.3 mm I.D. glass capillary column coated with SE-52 of film thickness 0.15 μm; carrier gas flow-rate, 3 ml/min; splitless period, 50 sec; injection at ambient temperature, followed by a temperature-programmed elution between 230 and 270°C. The injector temperature was 250°C. The clean C_{30}–stigmasterol mixture was injected twice into the injector with a freshly cleaned insert, followed by injections of the oil sample. The C_{30} peak areas measured for the oil sample decreased in the first injection to a quarter of the area obtained with the clean mixture and then stabilized at between 10 and 20% of that area (Fig. 3). The C_{30} areas determined for the clean mixture in injections 13, 14 and 16 were about 4 times smaller than with the clean insert (injections 1 and 2), but twice the area obtained with the oil sample.

Such deviations of the absolute peak areas are important if quantitative analyses are based on the method of external standardization, as discussed below. But many analyses are carried out according to the method of internal standardization, where the transfer of only 10–20% of the solute material strongly reduces the sen-

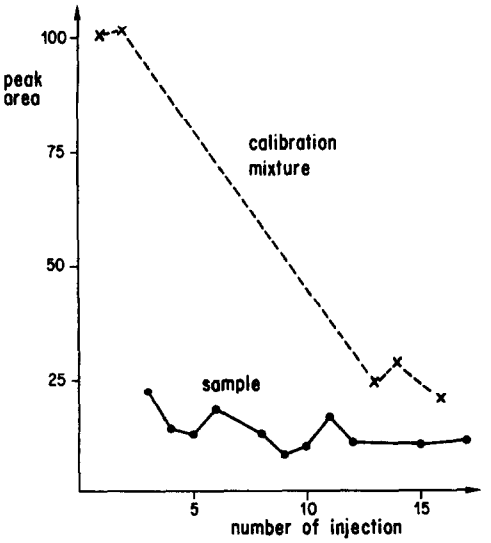

Fig. 3. The classical external standard method often leads to systematic errors. Peak areas obtained for 100 ppm of C_{30} are far larger in a clean calibration mixture (hexane) than in a 20% olive oil solution spiked with the same concentration of C_{30} (injector with clean insert at the first injection, 250°C). After ten injections of the dirty sample the C_{30} area is reduced to a quarter of the original size, but is still twice that in the dirty sample. A calibration with injections 1 and 2 would have caused a ten-fold error, with injections 13, 14 and 16, a two-fold error.

sitivity, but does not directly affect the quantitative results. C_{30} may be regarded as the internal standard for quantitation of the sterols, of which the concentration of stigmasterol is known (135 ppm). Thus, an area ratio may be calculated for C_{30}/stigmasterol in the clean mixture and the oil sample (areas of stigmasterol corrected to a concentration of 100 ppm). Results are given in Fig. 4 for the same sequence of injections as in Fig. 3. The area ratio calculated for the clean mixture injected into the clean insert was 1.4 (part of which is due to the fact that stigmasterol was only about 85% pure). The area ratio in the oil sample increased to about 3. The clean mixture injected subsequently (into the now dirty insert) gave an area ratio of about 2.5. Hence it approached but did not fully reach that of the oil sample (similar to the proportion of the transferred material, Fig. 3).

DISCUSSION

Reasons for poor transfer

The differences in the quantitative results obtained with the clean and the dirty mixtures are assumed to be due to two mechanisms, the relative importance of which depends on the applied conditions such as the carrier gas flow-rate and the recondensation of the solvent in the column inlet. A third mechanism, concerning losses inside the syringe needle, might be of some importance also, but is excluded from this discussion.

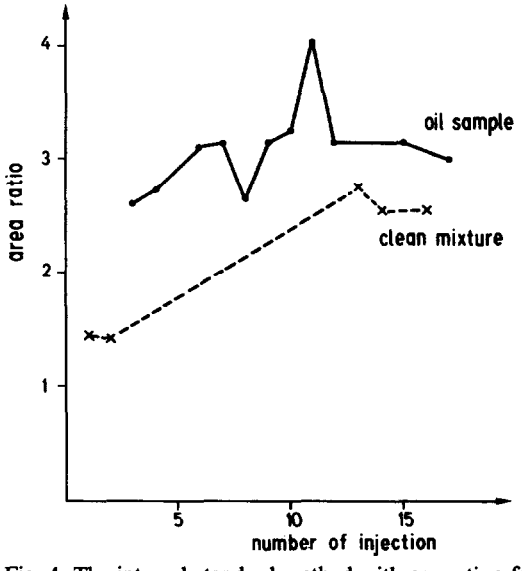

Fig. 4. The internal standard method with correction factors determined by use of a clean calibration mixture also yields erroneous results. The area ratio of C_{30} and stigmasterol obtained by the calibration mixture in pure hexane differs from that of the 20% oil solution (calculated for equal concentrations). The expected area ratio according to the flame ionization detector response and the purity of the stigmasterol used is about 1.4. If dirt is present, C_{30} enters the column to a greater extent than stigmasterol (lower retention in the somewhat polar, partly degraded triglyceride material). Hence correction factors for the clean and dirty mixtures may be different, whereby the results are influenced by the dirt in the sample and on the glass insert. As in Fig. 3, calibration by injections into the dirty liner (injections 13, 14 and 16) would have resulted in smaller errors (15–20%) than calibrations with a clean insert (factor of 2).

The sample leaves the syringe needle as a mixture of droplets and vapours. If the sample is clean, the droplets evaporate within a short time or distance from the needle exit. On the other hand, if the sample is dirty, the droplets cannot fully evaporate. Even when the solvent and the volatiles are evaporated, small droplets still remain. These droplets of DC 200 or olive oil behave as a liquid phase in a gas chromatographic partitioning process. They release the volatile sample constituents and retain or extract the high-boiling solutes from the gas phase in the vaporizing chamber. Visual observations in heated glass tubes suggest that the droplets condense on the wall of the injector insert within less than a second. They carry along the high-boiling solutes and fix them to the dirt layer (Fig. 5).

If 1 mg of dirt is deposited on the wall of the insert over a length of 3 cm, the average film thickness of the dirt layer is about 3 μm (phase ratio, β, 300). The linear gas velocity through the injector during the splitless period is usually below 5 mm/sec. Hence the glass insert becomes a chromatographic column with a considerable retention for solutes of medium to high boiling points. Much of the retained solute material does not reach the column entrance during the splitless period. It is released when the split exit is re-opened, and is split according to the gas flow-rates into a small proportion of material which enters the column and a major part which is purged through the split exit.

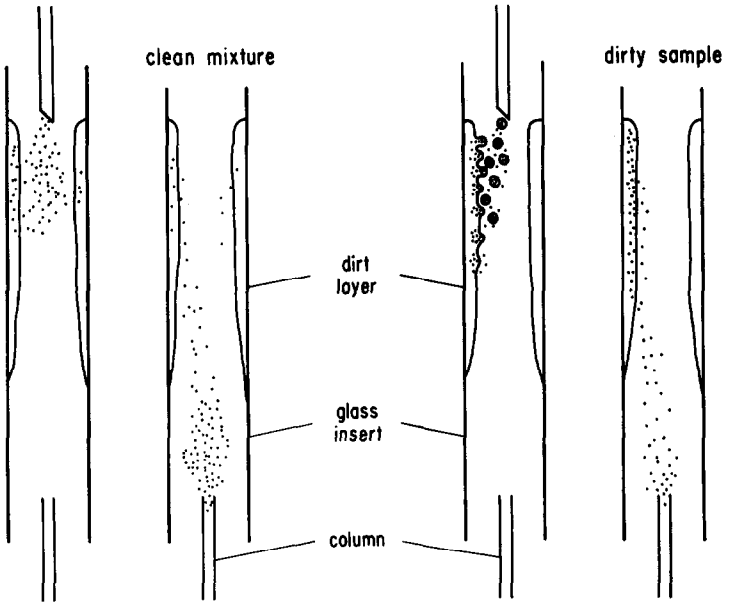

Fig. 5. Why is a smaller amount of the solute material transferred from the injector to the column if a dirty instead of a clean mixture is injected? The involatile dirt of the sample cannot evaporate. It forms droplets which adsorb higher boiling solute material and fixes it to the wall of the (possibly dirty) insert. From there only a limited amount of the solute material reaches the column entrance during the splitless period. The rest is desorbed when the injector is in the split mode again. The solutes in the clean mixture evaporate rapidly and a proportion enters the column before coming into contact with the dirty insert wall. The extent to which it is adsorbed by the dirt layer depends on the transfer speed.

Clean and dirty samples do not behave in the same way during a splitless injection. The results of the experiments with DC 200 (Fig. 1) have shown that the solute transfer is higher for a clean than for a dirty sample, whether the injector is dirty or not. Furthermore, the area ratio of stigmasterol/C_{30} was closer to the response factor of the flame ionization detector in the case of the clean than that of the dirty sample (Fig. 4). This indicates that some of the high-boiling solute material of clean samples enters the column before there is a partitioning with the dirt layer on the glass liner. If the sample is dirty, the solutes are in closer contact with the dirt because the droplets extract the vapour phase and fix the material of higher boiling solutes to the insert wall.

Systematic errors in quantitative analysis

The differences in the solute transfer of clean and dirty mixtures result in systematic errors in quantitative analyses as may be shown for the two most commonly used quantitation procedures, using the data of Fig. 1.

n-Docosane (C_{22}) is analysed by the external standard method. The sample contains some involatile by-products, represented by the 5% of DC 200. (Usually the analyst has little idea of the dirt concentration in his sample.) The mixture of the external standard, C_{22} in pure hexane, is injected five times. The obtained peak areas are well reproducible, *e.g.*, ± 3% relative standard deviation, from which the analyst would conclude that his system performs well, and that the relative standard deviation of his analytical result will be around 5%. Then he analyses the sample, presumably using a single injection, because he does not want to contaminate the column more than necessary. Probably he never finds out that the result is 40% too low, because the transfer of C_{22} material was that much less efficient for the dirty sample than for the standard solution. Under these circumstances it is no longer important that the relative standard deviation of the results obtained with the dirty sample is considerably higher than that with the clean mixture, because the droplets of dirt are on one occasion directed to the column entrance, on another towards the wall of the glass insert. Even a correct statistical treatment of the results would produce a misleading assessment.

In the second case the internal standard method is used which is independent of the absolute peak areas. A calibration of *n*-decane (C_{10}) as standard and C_{22} in *n*-hexane shows a relative response for C_{22} of 0.922 (± 1.5% relative standard deviation). This correction factor is used to account for the expected discrimination in the analysis of the sample. However, the data of Fig. 1 show that the discrimination in the dirty sample is different. The determined C_{22} concentration will be 30% too low.

An analysis based on the method of internal normalization using calibrated correction factors is affected analogously.

Many analysts believe that their analytical results are more reliable if they repeat the calibration after every few injections to take account of changes due to a dirt deposit in the injector. The data shown in Fig. 4 indicate that the area ratio of the clean mixture used as a correction factor indeed may approach the area ratio of the dirty sample (it remained "only" 15% too low). On the other hand, repeated calibration would not have helped at all in the case of the experiments with DC 200. The peak areas obtained with the clean mixture remained much different from those

of the dirty sample, whether the injector insert was clean or dirty. Thus, the dirt was more effective in the formation of droplets than as a layer on the insert wall.

CONCLUSIONS

In splitless injection the solute transfer from the injector into the column is a more significant problem than is generally recognised. Even if very high carrier gas flow-rates are used, the solute transfer is often poor due to the presence of involatile by-products (dirt), a concentration of 0.01% of dirt giving a noticeable, 0.1%, strong effect. Hence most samples in trace analysis must be considered as being dirty.

Many papers have reported on reproducibility studies using clean mixtures of standards. Such studies are of limited usefulness. The statistical treatment of reproducibility (precision) is often abused when estimating the accuracy of results obtained with splitless injection, although it is well known that accuracy cannot be estimated by precision. We obtained the impression that the random errors (determined by the reproducibility tests) are often small compared with the systematic errors. At least in those cases the indication of relative standard deviations is useless or even misleading. It is difficult to estimate systematic errors, which probably remain undetected in most cases. The reliability of results can only be estimated by checking the method. An analytical result is as accurate as the extent of the care taken in the quantitation procedure to exclude systematic errors.

The results of some analyses may be improved by an increase of the injector temperature. This temperature must not be optimized (as is commonly done) by the use of clean calibration mixtures because the limiting factor is the evaporation of the solutes from the dirt. A high injector temperature reduces the retention power of the dirt, but it does not generally solve the problem. For many solutes it appears that, once they are adsorbed by the dirt layer, they cannot be recovered for a splitless injection. In Fig. 1 and Table I it is shown that even relatively volatile solutes suffer considerable losses. In any case, the belief that splitless injection can be carried out with relatively low injector temperatures as a result of tests with clean test mixtures does not apply to most real samples.

If dirty samples are injected by the splitless technique, the external standard, the internal standard method or the method of normalization do not assure accurate results. Only the methods of standard addition exclude the systematic errors described above. Their common basis is the use of the sample as a matrix for all determinations and calibrations. The first procedure, with an application range corresponding to that of the external standard method, is based on two injections of each sample. First the sample is injected as such, then with an addition of standards in known amounts which should exceed that of the solute in the sample by a factor of at least two. The calculations may use absolute peak areas, but more accurate results are obtained if another peak of the sample is used as a reference peak. The area ratio of the peak of interest and the reference peak is calculated for both runs. The difference in the area ratios corresponds to the known amount of added standard. It is not necessary to know either the identity or the concentration of the reference, but it is of advantage if it has a similar volatility in the dirt matrix.

The second method is comparable to the internal standard method, but the calibration of the responses of the important solutes compared to the internal stan-

dard occurs in the sample. The sample is spiked with an internal standard and analysed. Then relatively large amounts of standards of the solutes of interest are added and the analysis repeated at an increased attenuation. Provided the samples contain a similar concentration of dirt and the dirt has similar characteristics, series of samples may be analysed using the same internal standard and the same correction factors. This is a convenient procedure, especially when large numbers of samples are to be analysed. Re-injection of the calibration mixture may be advisable.

Studies on sources of errors may be used to argue that quantitation by splitless injection is impossible. We consider this to be a short-sighted simplification, at least until there is a better (and thoroughly investigated) alternative. It should be accepted that it is often unrealistic to believe in a (nearly) complete transfer of the solute material in splitless injection, especially if the solutes are of low volatility. It is probable that many systematic errors occur, if the quantitation procedure for dirty samples is not properly designed. This is the reason why there is an urgent need to know about all sources of error (in any injection techniques). Once these sources of error are determined, methods can usually be found to circumvent the problems.

The criticism of a technique must not obscure its positive aspects. Provided there is no interference between the peaks of interest and those of by-products, extremely dirty samples may be analysed, including samples to which some solvent must be added just to reduce the viscosity to a level which permits uptake by the syringe needle. For most samples the column inlet (retention gap) needs replacement only after a considerable number of injections, and this may be carried out in a negligibly short time compared to that invested in further clean-up.

The splitless injection technique is and remains the method of choice for dirty samples which do not require very high accuracy. The reliability of the results is decreasing with increasing boiling point of the solute. On-column injection tolerates far less dirt, but is more accurate than splitless injection.

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