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Matrix effects and solute discrimination when injecting dirty samples in capillary columns

Comparative study between classical split and splitless injections☆

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ABSTRACT

Vaporization inside the injector chamber is a very complex process. The presence of non-volatile material may change the volatilization rate of a large number of species. In particular, splitting is far from ideal, as significant differences were observed in the splitting of clean and dirty samples and no discrimination-free chromatographic conditions could be found. The pattern of discrimination was strongly dependent on the glass liner geometry. When the non-volatile content of the sample was increased and packed inserts were used, smaller amounts of the most volatile compounds entered the column, whereas the least volatile compounds seemed not to be affected. The opposite effect was found when using empty injector inserts. Splitless injection was less affected by the presence of non-volatile components in the sample when the distance between the needle and the column entrance was large enough. Incomplete evaporation only occurred with very dirty samples, and this effect was avoided by increasing the injector temperature.

INTRODUCTION

Although current chromatographic research is focused mainly on the development of on-column injectors [1,2] and coupling between HPLC and high-resolution GC systems [3,4], large numbers of samples of very different types are still being analysed using classical injection techniques, *i.e.*, split and splitless injection. One reason is that most extracts are obtained via direct extraction from the original sample and accordingly these extracts are dirty samples that contain a certain amount of non-volatile material that may damage the chromatographic column on entry. On-column injection of dirty samples is now possible [5,6], but the precolumn has to be replaced after a short period of time, which means interrupting normal work and causing additional problems such as recalibration of the system. Classical splitless injection is still used because of the ability to inject dirty samples [7].

Split injection is also still used, not only to analyse concentrated mixtures but also for the analysis of dilute samples containing very volatile compounds. These types of samples are sometimes dirty and difficult to analyse via splitless injection owing to the impossibility of achieving good recondensation effects without cryofocus-

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ing system. This type of sample is very common in food and environmental research.

Heavy material in the sample may cause two main effects: it may change the vaporization rate of the sample and it may even cause incomplete evaporation. Non-volatile material is not vaporized and tends to remain in droplets [8]. These droplets may retain certain solutes from the sample which are released with some delay compared with a clean sample, or they may even be completely retained [8,9]. In split injection, the presence of long-lived droplets may drastically change the pressure wave generated by the almost instantaneous vaporization of a clean sample [10]; this is known to influence the true splitting ratio or, even worse, it may cause a large part of the sample to be lost directly through the split outlet. In splitless injection, the most intense effect is thought to be incomplete evaporation.

The main aim of this work was to evaluate the real ability of both vaporization techniques to analyse dirty samples in the field of flavour research. It is still common to extract volatile compounds with a low-boiling solvent in order to avoid thermal degradation of the solutes, concentrate the extract and analyse it using split injection. However, some microextractive techniques, which allow the concentration step to be eliminated, have been developed [11–14] and some of them allow the extract to be injected via splitless injection.

In this study, "dirtiness" was obtained with a dearomatized wine extract. The solutes considered belonged to different flavour groups, namely fatty acid esters and fusel alcohols.

EXPERIMENTAL

Dichloromethane and pentane (HPLC grade) were obtained from Carlo Erba (Milan, Italy), 1,1,2-trichlorotrifluoroethane (HPLC grade) from Aldrich (Milwaukee, WI, USA) and 2-propanol (for residue analysis) from Merck (Darmstadt, Germany).

The solutes used were obtained from Alltech (West Chester, PA, USA), and were of quantitative quality. Calibration solutions were prepared for each solvent. The solutions injected contained 40 mg l^{-1} of volatiles with split and 4 mg l^{-1} with splitless injection. The injection linearity was tested by injecting calibrated solutions between 20 and 200 mg l^{-1} with split and between 1 and 50 mg l^{-1} with splitless injection.

"Dirtiness" was obtained by continuous extraction of 5 l of a previously distilled red wine with 500 ml of dichloromethane. Subsequently this extract was concentrated by solvent evaporation at 45°C to a final volume of 1 ml. Dirty extracts were obtained by adding different volumes of this extract to clean solutions.

The chromatograph was an HP 5890 Series II (Hewlett-Packard) fitted with split/splitless and on-column injectors. In order to avoid discrimination in the syringe an HP 7673 automatic injector was used. Split injections were performed by the cold needle method. Splitless injections were performed by the hot needle method with a preheating time of 5 s. A 10-μl syringe and an injection volume of 1 μl were used.

A Supelcowax 10 column (60 m × 0.32 mm I.D.) with a film thickness of 0.50 μm was used. The column temperature was initially held at 40°C for 3 min, then programmed at 3°C min^{-1} to 180°C. Flame ionization detection was used. The carrier gas was hydrogen. The purge flow-rate was 3 ml min^{-1}. Two different injection temperatures were considered, 250 and 350°C.

For split injection, the carrier gas flow-rate was 1 ml min^{-1}, the split flow-rate was 40 ml min^{-1} and the pre-set splitting ratio was 1:40. Injections were carried out using two different glass liners: a Jennings cup type packed with Chromosorb and an empty cylindrical type. The distance between the column entrance and the tip of the syringe was 4.26 cm.

For splitless injection, the carrier gas flow-rate was 2.5 ml min^{-1}, the split flow-rate was 26 ml min^{-1} and the splitless time was 3 min. An empty cylindrical liner was used. The distance between the column entrance and the tip of the syringe was 4.26 cm unless specified otherwise.

Chromatographic signals were registered with an NEC computer using Maxima 820 from Waters Software. To obtain the true splitting ratio and the mass transfer efficiency, chromatographic peak areas from the different experiments

were compared with those obtained from on-column injection of clean samples.

RESULTS

Split injection

Clean samples. The results are given in Table I. Slight deviations from the preset split ratio were observed while working with the two different inserts, even with clean solutions. However, the linearity and precision of injection were very good in both instances. With the empty cylindrical insert the splitting ratio was not the same for all the compounds, being slightly lower for the least volatile substances. In contrast, with the Jennings cup packed insert, the splitting ratio was constant for all the compounds tested.

Dirty samples. When non-volatile material is added to the solutions, the splitting ratio changes and the pattern of that change is strongly dependent on the geometry of the system. When a packed insert is used, the splitting ratio tends to decrease, but this effect is more pronounced for the most volatile solutes, as can be clearly seen in Fig. 1 and Table II. Thus, when the sample contains non-volatile material, smaller amounts of the most volatile compounds and about the same amount of the least volatile compounds enter the column. However, if an empty insert is used, the splitting ratio tends to increase, and in this instance the least volatile compounds are most subject to this effect. The results are shown in Fig. 2. Surprisingly, the linearity and precision of injection were not affected by the presence of non-volatile material, as can be seen in Table II. Almost the same results were obtained using pentane as the solvent, showing that the solvent does not exert a significative influence.

From an analytical point of view, these effects make quantification erroneous, even using an internal standard. In Figs. 3 and 4, the relative error in the quantification was obtained on the basis of a calibration performed with 1-hexanol as internal standard. To avoid such errors, at least two different internal standards should be used or a standard addition calibration should be

TABLE I

TRUE SPLITTING RATIO IN THE INJECTION OF CLEAN AND DIRTY SAMPLES

Compound	True splitting ratio[a]											
	Clean samples				Dirty samples (in dichloromethane)							
	Dichloromethane		Pentane		E.I.	P.I.	Change (%)[b]		R.S.D. (%)[c]		r[d]	
	E.I.	P.I.	E.I.	P.I.			E.I.	P.I.	E.I.	P.I.	E.I.	P.I.
1-Propanol	29.9	28.2	32.9	31.4	28.2	38.9	−5.6	39.0	3.1	2.8	0.9991	0.9992
Ethyl butyrate	28.8	27.9	31.7	30.8	27.3	38.3	−5.3	36.7	2.1	3.3	0.9996	0.9989
1-Butanol	29.1	27.3	32.6	30.7	26.7	38.4	−8.2	40.8	1.6	2.2	0.9998	0.9995
1-Pentanol	32.2	30.1	34.5	32.3	27.0	37.7	−16.0	25.1	1.9	1.7	0.9992	0.9996
Ethyl hexanoate	34.7	30.9	35.8	33.5	29.7	39.9	−14.4	22.6	3.2	2.9	0.9990	0.9990
1-Hexanol	35.7	28.7	36.9	32.0	28.1	38.0	−21.3	32.4	2.2	2.5	0.9997	0.9999
Ethyl octanoate	34.4	28.4	37.1	31.8	28.5	35.6	−17.2	25.1	1.7	1.2	0.9993	0.9994
Ethyl decanoate	37.2	31.6	38.9	32.4	29.1	37.5	−21.8	18.5	2.5	1.8	0.9992	0.9999
Phenylethyl acetate	33.9	32.1	36.0	32.1	28.3	27.3	−16.5	−15.0	1.9	2.4	0.9989	0.9991
Ethyl laurate	35.6	29.1	37.8	30.6	29.0	31.8	−18.5	9.3	2.6	3.1	0.9994	0.9995
2-Phenylethanol	34.0	28.7	36.3	30.5	30.0	30.2	−11.8	5.2	2.1	1.8	0.9997	0.9997

[a] Results are averages of five injections. E.I. = Empty insert; P.I. = packed insert.
[b] Relative increment of the true splitting ratio when the sample contains 28% of the dirty extract.
[c] Average of six injections.
[d] Linear regression coefficient.

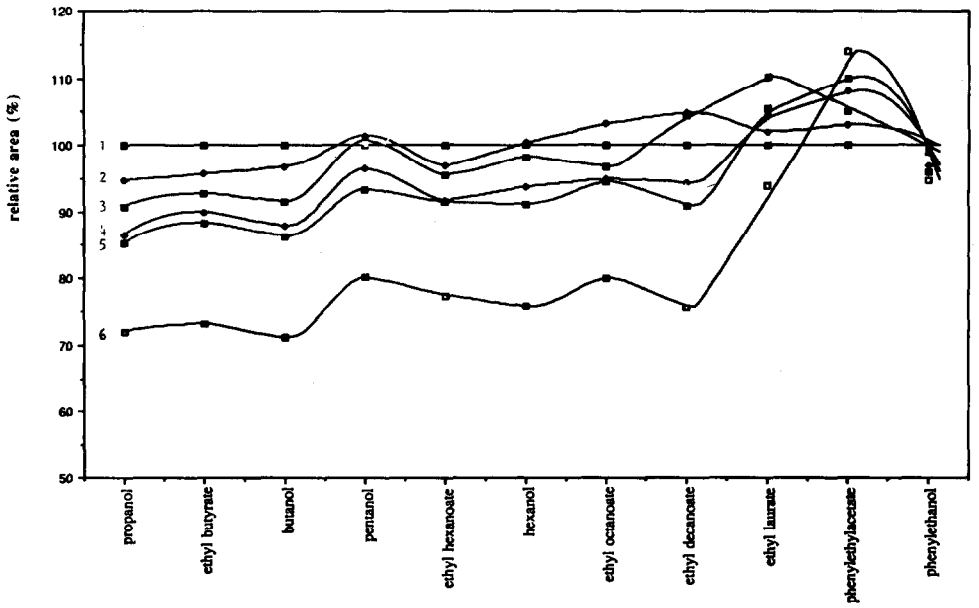

Fig. 1. Effect of non-volatile material. Relative areas of the chromatographic peaks for clean and dirty solutions. Split injection; packed insert; injector temperature, 250°C. Individual points are connected with lines to show the differences, but there is no physical measuring to these lines. For the nature of the contaminants, see Experimental. Percentages of the contaminants are: 1 = clean sample; 2 = with 2%; 3 = with 4%; 4 = with 8%; 5 = with 14%; 6 = with 28%.

performed. It is important to point out that the deviation of the splitting ratio seems to depend on the boiling point of the solute more than on its chemical characteristics, as can be seen in Figs. 5 and 6.

Temperature effect. With both kinds of inserts

TABLE II

ANALYTICAL CHARACTERISTICS OF THE SPLIT INJECTION OF DICHLOROMETHANE DIRTY EXTRACTS

Compound	True splitting ratio (dirty samples)		Change (%)[a]		R.S.D. (%)[b]		r[c]	
	E.I.	P.I.	E.I.	P.I.	E.I.	P.I.	E.I.	P.I.
1-Propanol	28.2	38.9	−5.6	39.0	3.1	2.8	0.9991	0.9992
Ethyl butyrate	27.3	38.3	−5.3	36.7	2.1	3.3	0.9996	0.9989
1-Butanol	26.7	38.4	−8.2	40.8	1.6	2.2	0.9998	0.9995
1-Pentanol	27.0	37.7	−16.0	25.1	1.9	1.7	0.9992	0.9996
Ethyl hexanoate	29.7	39.9	−14.4	22.6	3.2	2.9	0.9990	0.9990
1-Hexanol	28.1	38.0	−21.3	32.4	2.2	2.5	0.9997	0.9999
Ethyl octanoate	28.5	35.6	−17.2	25.1	1.7	1.2	0.9993	0.9994
Ethyl decanoate	29.1	37.5	−21.8	18.5	2.5	1.8	0.9992	0.9999
Phenylethyl acetate	28.3	27.3	−16.5	−15.0	1.9	2.4	0.9989	0.9991
Ethyl laurate	29.0	31.8	−18.5	9.3	2.6	3.1	0.9994	0.9995
2-Phenylethanol	30.0	30.2	−11.8	5.2	2.1	1.8	0.9997	0.9997

[a] Relative increment of the true splitting ratio when the sample contains 28% of the dirty extract.
[b] Average of six injections.
[c] Linear regression coefficient.

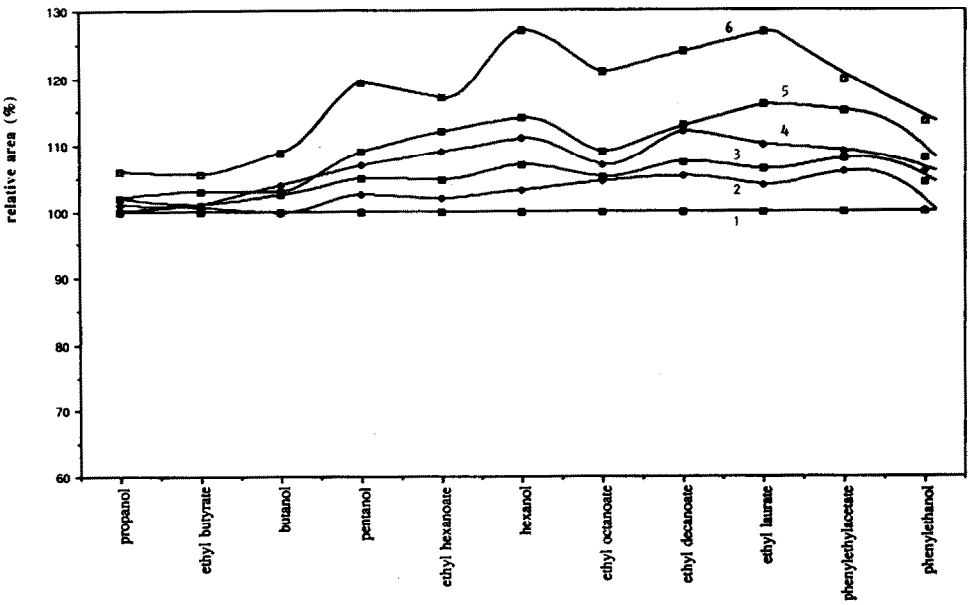

Fig. 2. Effect of non-volatile material. Relative areas of the chromatographic peaks for clean and dirty solutions. Split injection; empty insert; injector temperature, 250°C. Individual points are connected with lines to show the differences, but there is no physical measuring to these lines. For the nature of the contaminants, see Experimental. Percentages of the contaminants are: 1 = clean sample; 2 = with 2%; 3 = with 4%; 4 = with 8%; 5 = with 14%; 6 = with 28%.

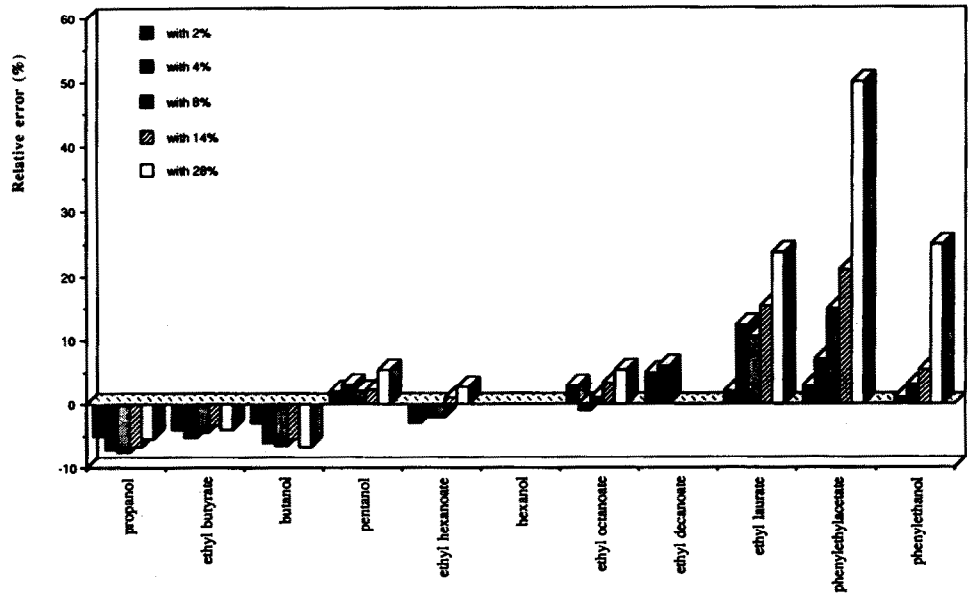

Fig. 3. Effect of non-volatile material on quantification. Relative errors in the quantification of dirty solutions with calibration based on a clean sample and with the use of an internal standard (1-hexanol). Split injection; packed insert; injector temperature, 250°C.

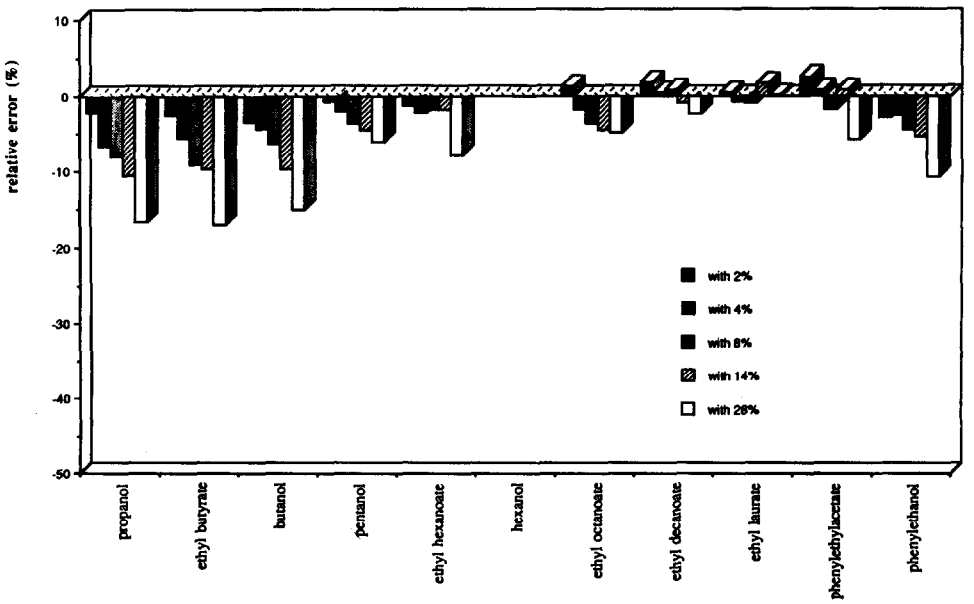

Fig. 4. Effect of non-volatile material on quantification. Relative errors in the quantification of dirty solutions with calibration based on a clean sample and with the use of an internal standard (1-hexanol). Split injection; empty insert; injector temperature, 250°C.

the temperature decreases slightly, but it does not avoid the effects of the addition of non-volatile material. Figs. 7 and 8 show the effect of an increase of 100°C in the injector temperature.

Splitless injection

Splitless injection was tested with different solvents: 2-propanol (b.p. 82°C), 1,1,2-trichlorotrifluoroethane (b.p. 56°C) and dichloromethane (b.p. 44°C). The solvent behaviour was similar in all instances, working with both clean and dirty solutions. In all instances, a very good mass transfer was achieved and only with a large amount of added non-volatile material were the least volatile solutes transferred with poor efficiency. In Fig. 9, results for the injection of 2-propanol extracts are presented. The results obtained with the other solvents were similar but slightly better. Increasing the injector temperature led to suppression of the deviations.

DISCUSSION

Vaporization of the sample in the injector depends not only on the thermodynamic properties of the solvent such as surface tension, boiling point and heat of evaporation, but also on other factors. The system geometry and injection methods also have a significant effect [15–17]. The amount of solute introduced into the column depends not only on the chosen splitting ratio, but also on the real amount of vaporized solute, on the actual splitting ratio at the moment the solute reaches the split point and on the concentration of vaporized solute at the column entrance. In other words, the extent and rate of vaporization will determine the process. Vaporization should become more difficult as surface tension increases and as non-volatile substances are introduced in the sample, preventing nebulization of the sample near the needle exit. Consequently, if incomplete evaporation occurs, smaller amounts of solutes will enter with samples in dichloromethane as solvent than with samples in pentane, and with dirty than with clean samples. However, this was not the case under the present conditions, where evaporation of the samples seemed to follow the first scenario described by Grob and De Martin [16], *i.e.*, flash evaporation, rather than the second one, *i.e.*, incomplete evaporation due to sample liquid not being nebulized. This may be

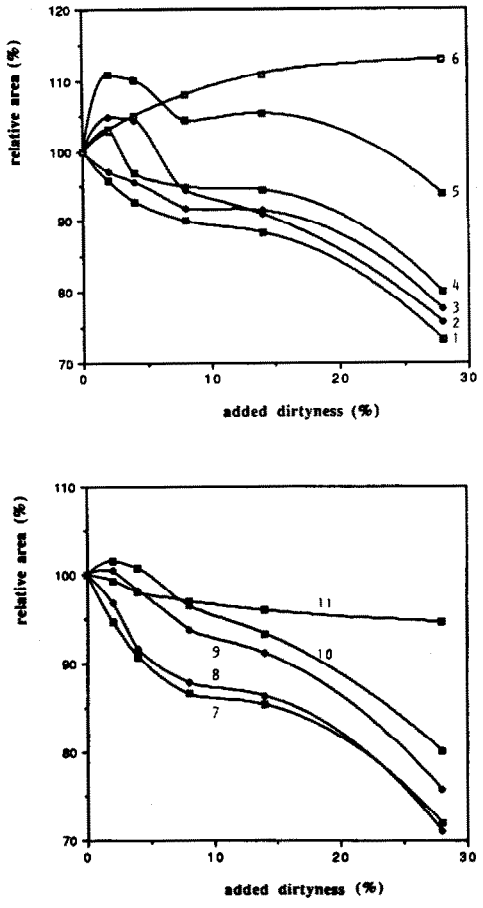

Fig. 5. Effect of non-volatile material. Behaviour of the different solutes. Split injection; packed insert; injector temperature, 250°C. 1 = Ethyl butyrate; 2 = ethyl decanoate; 3 = ethyl hexanoate; 4 = ethyl octanoate; 5 = ethyl laurate; 6 = phenylethyl acetate; 7 = propanol; 8 = butanol; 9 = hexanol; 10 = pentanol; 11 = phenylethanol.

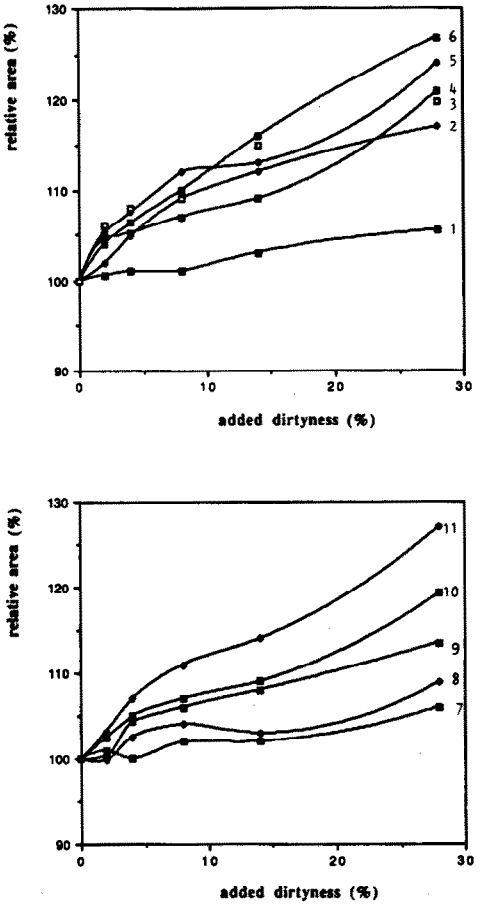

Fig. 6. Effect of non-volatile material. Behaviour of the different solutes. Split injection; empty insert; injector temperature, 250°C. 1 = Ethyl butyrate; 2 = ethyl hexanoate; 3 = phenylethyl acetate; 4 = ethyl octanoate; 5 = ethyl decanoate; 6 = ethyl laurate; 7 = 1-propanol; 8 = 1-butanol; 9 = 1-pentanol; 10 = phenylethanol; 11 = 1-hexanol.

due to the fact that the rapid autosampler injection (<0.02 s) could generate a mechanical spray effect, making vaporization easier. Another, although less probable, cause might be that the liquid droplets from the ejected sample reach the injector bottom and, instead of being lost through the split outlet, bounce and return to the vaporization chamber [17].

Whatever the cause, the fact is that with the injection of dichloromethane samples there is a slightly larger entry of solutes than with pentane samples, probably owing to the larger increase in volume caused by the vaporization of 1 μl of dichloromethane (450 μl) than of pentane (300 μl) and to the more intense pressure wave generated in the former instance.

Some differences are observed in the injection of clean samples, depending on the insert geometry. Better results are obtained with the Jennings cup packed insert which seems to be discrimination free. When injecting dirty samples the resulting effects also depend strongly on the geometry of the insert.

With an empty insert, where incomplete evaporation is more likely, an increase in non-volatile material not only does not reduce the

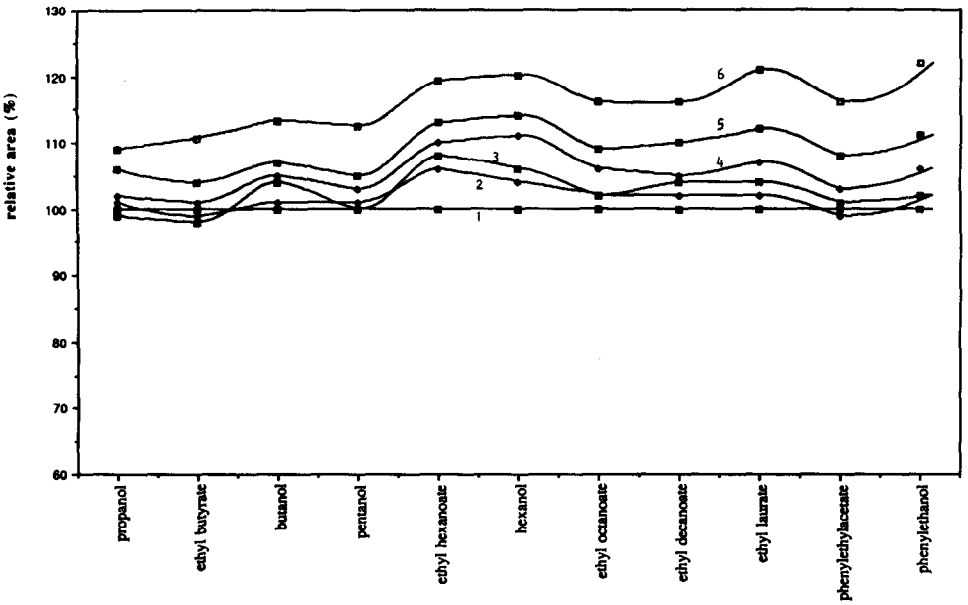

Fig. 7. Effect of increasing the injector temperature. Conditions and presentation as in Fig. 2; injector temperature, 350°C. 1 = Clean sample; 2 = with 2%; 3 = with 4%; 4 = with 8%; 5 = with 14%; 6 = with 28%.

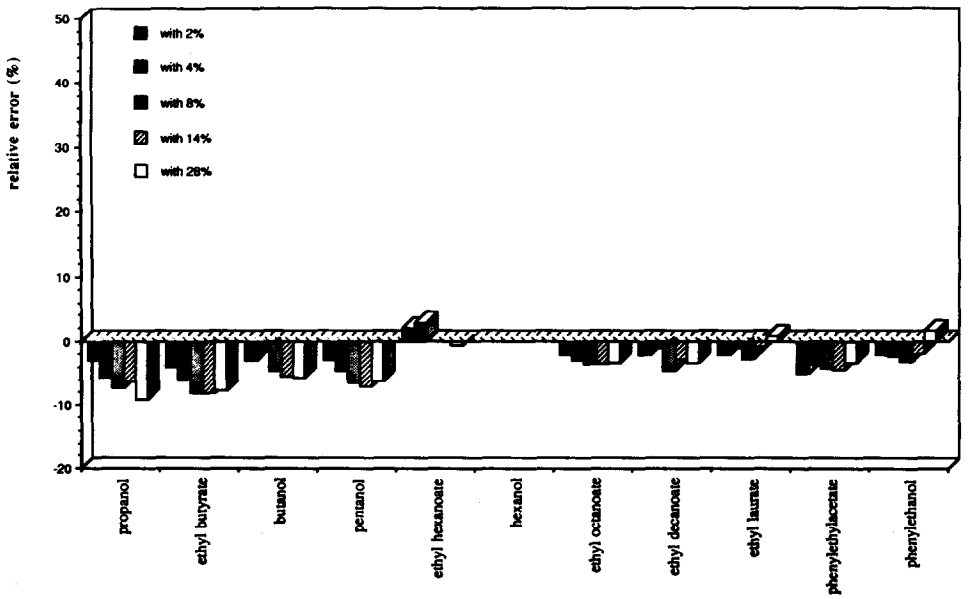

Fig. 8. Effect of increasing the injector temperature. Conditions as in Fig. 4; injector temperature, 350°C.

splitting ratio but also increases the ratio of the less volatile solutes introduced into the column. This could be due to a delay in vaporization caused by the presence of non-volatile substances, thus allowing the drops expelled by the syringe to travel further. For this reason, these droplets can transport the less volatile compounds faster inside the glass insert and they can

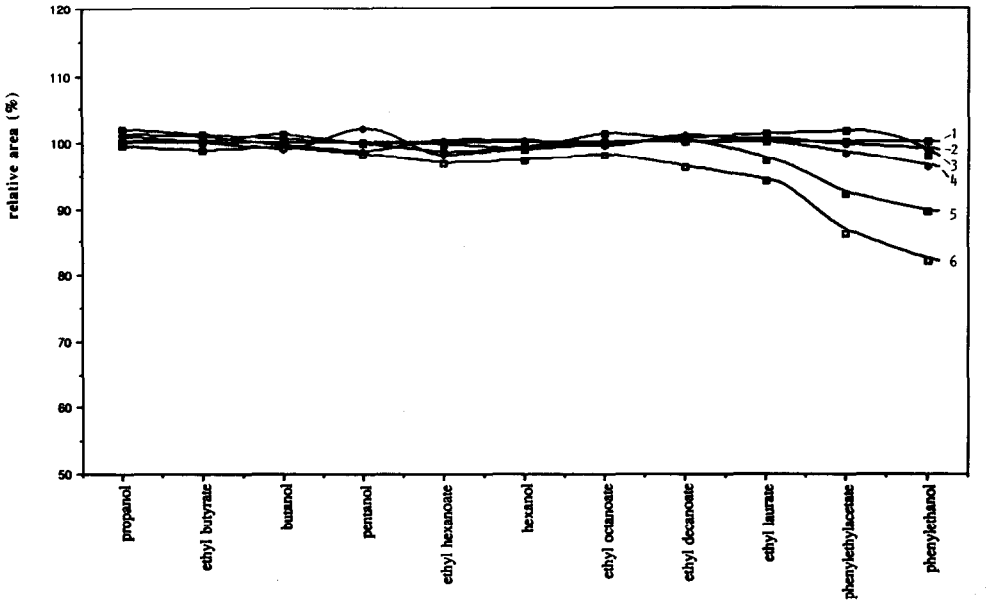

Fig. 9. Effect of non-volatile material. Relative areas of the chromatographic peaks in the dirty solutions compared with clean solutions. Splitless injection; 2-propanol as solvent; injector temperature, 250°C. Presentation as in Figs. 1 and 2. 1 = Clean sample; 2 = with 2%; 3 = with 4%; 4 = with 8%; 5 = with 14%; 6 = with 28%.

form a more concentrated cloud of vapour near the column entrance than in the injection of a clean sample. As a result, a stronger recondensation effect is produced, which is shown by a higher splitting ratio.

This means that droplets are not trapped by the layer of dirtiness covering the internal surfaces of the glass liner, otherwise the least volatile material would enter in a lower proportion. It is thought that droplets rebounce off the wall owing to their boiling material, which acts as a vapour cushion covering them [18]. In this instance, the effect of increasing the injector temperature is small because the vaporization time decreases slowly, according to the concept of Leidenfrost temperature introduced by Wang *et al.* [18]. The fact that the most volatile solutes do not seem to be affected in this kind of insert by the addition of non-volatile material may be due to the compensation of the decrease in the pressure wave by accelerated transport of the solutes in the micelles.

When the level of non-volatile compounds is increased in a Jennings cup packed injector, the entry of the most volatile solutes is reduced, whereas the least volatile solutes are hardly affected. It is thought that vaporization of the most volatile solutes is considerably delayed with respect to a clean injection, whereas this delay is not as significant with the least volatile solutes.

This seems to imply that even in the injection of clean sample there is a difference in the vaporization times between the most volatile solutes, which would vaporize faster, and the least volatile, which would vaporize more slowly. As a result, the delay caused by the presence of non-volatile compounds has a more significant effect on the most volatile compounds. This delay can be manifested in smaller pressure wave and recondensation effects. Accordingly, the most volatile solutes enter to a lesser extent than they would do with a clean injection. Again, the effect of increasing the injector temperature was minimal.

Splitless injection

Under the chosen conditions, the mass transfer efficiency is very high, although a solvent effect could not be achieved. A decrease in mass transfer efficiency should now be due to incomplete evaporation. The flow-rate of the carrier gas inside the liner is slow, and micelles could be

trapped by the layer of dirtiness covering the inner surface of the liner, thus following the third scenario described by Grob and De Martin [16], *i.e.*, liquid splashing on the insert wall, particularly in the case of 2-propanol. The solvent in this instance plays a secondary role because the vaporization rate is lowered for all the solvents in the same proportion, depending more on the amount of non-volatile material contained by the sample than on the boiling point of the solvent. Although above the Leidenfrost temperature the increase in heat transfer with increasing temperature is very small, as the vaporization time is larger compared with that of flash evaporation the effect of increasing the injector temperature is highly significant.

CONCLUSIONS

The vaporization process inside the injector is very complex and strongly dependent on the non-volatile content of the sample. Split injection is strongly affected by this and the true splitting ratio can be significantly altered, following different patterns according to the geometry of the system. In general, with an empty insert, solutes travel faster to the column entrance when the sample is dirty, and those solutes which are not greatly affected by the pressure wave enter in larger amounts in dirty than in clean samples. In contrast, the only effect observed with a packed insert is that the amount of the least volatile solutes entering the column is lower owing to the decrease in the pressure wave. An increase in temperature reduces these effects only slightly. It should be noted that under the conditions used no incomplete vaporization was observed, perhaps owing to the intense nebulization caused by rapid autosampler injection. Splitless injection appears to be less affected by the presence of non-volatile material. Thus, better results can be achieved by using solvents with a good recondensation effect than with the most volatile solvents. From this point of view, microextractions show several advantages over classical extractions for the routine determination of thermally degradable compounds.

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