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Comparison of methods used for pre-concentrating small volumes of organic volatile solutions

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Abstract

Eight pre-concentration techniques were compared for their capacity to retain volatile and semi-volatile solutes during evaporation of solvent (dichloromethane). The 2-ml test-samples containing 0.2 ppm or 2 ppm (v/v) of volatile and semi-volatile solutes were concentrated to a final volume of 1 ml, 200 μl and 50 μl, respectively. When pre-concentrating to 50 μl, the highest recoveries for both the diluted (0.2 ppm) and concentrated (2 ppm) solutions were found by passive evaporation in a test tube at 22 °C. The pre-concentration time from 2 ml to 50 μl by this method was 19–20 h. Heating the test tube to 47 °C yielded lower recoveries in dilute samples, but the recoveries of concentrated samples were only slightly lower than the recoveries obtained by passive evaporation. The evaporation time was decreased to 1–2 h. The recoveries and the reproducibility of these methods were superior to the other pre-concentration methods tested. Loss of solute was apparently mainly caused by the fast vapour streams created when speeding up the process of evaporation by heating or by introducing a gas stream into the tube. This increased co-evaporation and thereby solute loss. The capacity of the methods to trap the escaping vapours and create a reflux determined the capacity of the methods to recover the solutes. The experiments demonstrated that more solute is lost during the pre-concentration of dilute samples compared to more concentrated solutions.

Keywords: Pre-concentration; Co-evaporation; Recovery; Volatile compounds

1. Introduction

Volatiles are commonly isolated from plant material by a number of headspace trapping or extraction techniques [1,2]. Some of the methods include transfer of the volatiles to an organic solvent, which often needs to be pre-concentrated prior to analysis due to the low concentration level of solutes in the sample. Pre-concentration of the solution may be carried out by solvent evaporation inside or outside the chromatographic system.

Numerous concentration methods have been developed for the pre-concentration of samples inside the chromatographic system, varying in applicability and ease of use. The most promising methods in this respect are large-volume injection (LVI) techniques, which allows injection of up to 1 ml of sample, without sacrificing separation, calibration, and linearity [3–6]. The best-developed LVI techniques are cool-on-column (COC) [3–5] and programmed-temperature vaporisation (PTV) injection [5,6].

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These methods are commonly used in water analysis for trace concentration of pesticides and other contaminants [7,8], but have also been used for the analysis of dynamic headspace samples with a low content of aroma compounds [9]. However, LVI techniques, such as COC and PTV, and related GC methods must be performed with not readily available materials and/or relatively expensive equipment, and finally generally require considerable experience. Consequently, pre-concentration of samples outside the chromatographic system is more widely used, and includes methods such as rotary evaporator distillation [3,10], distillation with a micro-Kuderna–Danish concentrator equipped with Vigreux or Snyder columns [10–12], distillation with Vigreux columns [13–15], evaporation in a modified Pasteur pipette [16] or under a gentle stream of nitrogen [3,12,16–21]. Another, more sophisticated approach is that of the ''Dynamic Film Concentrator'' [22,23], which concentrates the solution on a bed of small particles by a process analogous to solute focusing using the solvent effect in the GC inlet.

Even though pre-concentration outside the chromatographic system is widely used, there have only been a few studies evaluating the impact of this step of the analysis on the analytical quality of the final results. It is therefore difficult to extract from the literature which method(s) are most suitable for pre-concentration of organic volatile solutions, such as headspace samples extracted from polymer traps. The pre-concentration techniques compared earlier, discriminated among the volatile compounds of the solution [12], i.e. compounds with lower boiling points show lower recovery percentages than compounds with higher boiling points. In a study by Ferreira et al. [12] the recoveries of volatiles ranged from close to 0% to nearly 100% depending on the pre-concentration method applied and the concentration of the solutes in the sample. Thus, this early step may be a source of significant errors in an attempt to analyse the volatiles in the sample. Weurman [24] pointed out the importance of the pre-concentration step back in 1969: ''It must be remembered that errors and faults made in odour research during the early stages of isolation and concentration can never be corrected at any later stage of compound identification''.

Characteristic for all methods that pre-concentrate samples outside the chromatographic system is that during the concentration process, volatile and semi-volatile compounds tend to co-evaporate with the solvent, leading to solute losses, and hence makes a quantitative measurement difficult. However, recondensation on the glass walls during solvent evaporation and thereby minimising losses of volatile solutes by co-evaporation, depends on the design of the pre-concentration system [3,12].

The purpose of the present study was to compare several commonly used pre-concentration techniques in order to find the best method for evaporating excess solvent with a minimum loss of volatile and semi-volatile solutes.

2. Experimental

2.1. Reagents

Standard solutions of 0.2 ppm and 2 ppm (v/v), respectively, were prepared in dichloromethane. Dichloromethane was of analytical-reagent grade from Merck and was distilled twice before use. The standard volatile compounds were selected to obtain a variety of polarity and volatility and included: 3-pentanone (b.p. 102–103 °C), *n*-decane (b.p. 173–174 °C), hexanal (b.p. 129–131 °C), limonene (b.p. 176–177 °C), *n*-dodecane (b.p. 214–216 °C), 1-pentanol (b.p. 138–139 °C), *n*-hexyl acetate (b.p. 167–169 °C), 1-hexanol (b.p. 156–157 °C), nonanal (b.p. 190–192 °C), 2-isopropyl-3-methoxypyrazine (=2-isopropyl-3-MP) (b.p. not available), 1-octanol (b.p. 194–195 °C), *n*-octadecane (b.p. 317 °C) (all from Aldrich), dipropyl disulfide (b.p. 195 °C) (Merck), and β-caryophyllene (b.p. 262–264 °C) (Sigma). The standards were of GC analytical-reagent grade purchased from Aldrich (Steinheim, Germany), Merck (Darmstadt, Germany), and Sigma (Deisenhofen, Germany).

Internal standard solutions of 4-methyl-1-pentanol (Aldrich) in dichloromethane were prepared in concentrations of 80 and 800 ppm, respectively, and used to quantify the volatile compounds in the standard solutions.

2.2. Pre-concentration systems

The micro Vigreux column (Quickfit, 130 mm×10 mm I.D., 14/23) was obtained from Aldrich and mounted to a 10-ml test tube. The spinning-band column assembly was obtained from Aldrich (Ace microscale, fixed head, spinning-band column assembly) and mounted to a 5-ml reaction vial. Micro Snyder columns with three pear shaped glass balls [Snyder (3)] and one with four narrowings along the tube [Snyder (0)] were obtained from Supelco [Snyder (3): 170 mm×6 mm I.D., 19/22, Cat. No. 6-4720; Snyder (0): 137 mm×8 mm I.D., Cat. No. 6-4721, Supelco Inc., Bellefonte, PA, USA] and were mounted to 5-ml reaction vials. The other equipment consisted of Pasteur pipettes (150 mm×5 mm I.D., Aldrich) converted to closed tubes by heating at the point where it narrowed and removing the narrow tip of the pipette (Table 1).

Test tubes with a conical formed bottom (10 ml, 115 mm×17 mm I.D., 14/23) were obtained from Bie and Berntsen (Rødovre, Denmark). Reaction vials (5 ml, 19/22) were obtained from Aldrich.

2.3. Capillary gas chromatography

A Hewlett-Packard 5890 Series II gas chromatograph (Hewlett-Packard, Avondale, PA, USA) rebuilt with an injection port, CIS-301 from Gerstel (Mülheim an der Ruhr, Germany). The glass insert volume was 140 μl. The injector was fitted with an automatic injector (HP 7673) and a flame ionisation detector (FID) was used. Analytical separations were performed on a Chrompack CP-Wax 58CB column

Table 1
Description of the pre-concentration techniques tested in the present study

Pre-concentration techniques	Sample container	Evaporation		Description
		Temperature	Time[a]	
Passive evaporation at 22 °C	10-ml test tube	22 °C	19–20 h (50 μl)	The solvent was allowed to evaporate without any heating or gas purging.
Nitrogen flow	10-ml test tube	22 °C	2–3 h (50 μl)	A gentle flow of N_2 (10 ml min^{-1}) purges the space above the liquid phase. The tip of the pipette with N_2 flow protrudes 40 mm centrally into the test tube.
Evaporation at 47 °C	10-ml test tube	47 °C	1–2 h (50 μl)	The test tube was engulfed in water to the 2-ml level of the test tube.
Pasteur pipette	Pasteur pipette (closed in the narrowed end)	47 °C	2.5–3.5 h (50 μl)	Basic set-up as the method evaporation at 47 °C.
Snyder (3)	5-ml reaction vial	47 °C	3–4 h (200 μl)	The vial was engulfed in water to the level of the lower glass ball.
Snyder (0)	5-ml reaction vial	47 °C	2–3 h (200 μl)	The vial was engulfed in water, 2 mm below the top of the vial.
Vigreux	10-ml test tube	47 °C	3–4 h (200 μl)	The test tube was engulfed in water, 3 mm below the top of the test tube.
Spinning-band	5-ml reaction vial	47 °C	2–3 h (200 μl)	The vial was engulfed in water to the 2-ml level of the vial.

[a] The time to evaporate to the lowest level as indicated in parenthesis.

(50 m×0.25 mm I.D., DF=0.2 μm liquid phase, Middleburg, The Netherlands).

2.4. Chromatographic conditions

The injection volume was 1 μl in splitless mode with a purge time of 0.75 min. Ultra pure grade helium was used as the carrier gas at a flow of 1 ml min^{-1}. Injection and FID-detector temperature were both 220 °C. The oven temperature was maintained at 30 °C for 1.5 min, programmed to 80 °C at 2 °C min^{-1}, from 80 to 230 °C at 10 °C min^{-1}, followed by constant temperature for 10 min.

Quantification with an internal standard (80 and 800 ppm 4-methyl-1-pentanol for 0.2 ppm and 2 ppm standard solutions, respectively) was based on FID peak areas. The response factor was set to 1 for all compounds.

2.5. General procedure for pre-concentration

A description of the eight pre-concentration techniques tested in this study is presented in Table 1. A volume of 2 ml of the standard solution, with a concentration of 0.2 ppm and 2 ppm (v/v), respectively, was used. The standard solutions were placed in either a 5-ml reaction vial, a 10-ml test tube or a modified Pasteur pipette (Table 1). The thermostat-regulated water bath was set to 47 °C, which was just below the boiling point of the solution. The 2-ml samples were evaporated to 1 ml, 200 μl or 50 μl. After evaporation, the reaction vial/test tube was immediately introduced into a water ice-bath [16] in order to stop evaporation and 5 μl of internal standard solution was added.

2.6. Statistical analyses

The data from the experiments were analysed using the Statistical Analysis System (SAS Institute, Cary, NC). Mixed model analysis of variance (ANOVA) was used [25]. All main and interaction effects related to compound and replications were considered random. Ls means was used to assess the significant differences. All experiments were performed in triplicate.

3. Results

3.1. Pre-concentration to 50 μl (recoveries and influence of sample concentration)

Passive evaporation at 22 °C and evaporation in a test tube at 47 °C were clearly the most effective methods when it comes to general pre-concentration of samples to 50 μl (Fig. 1). These methods showed the highest recoveries at both solute concentration levels (0.2 ppm and 2 ppm). Furthermore, the low C.V.'s for both the passive evaporation method (7.6 for 0.2 ppm and 3.2 for 2 ppm) and for evaporation in test tube at 47 °C (5.2 for 0.2 ppm and 4.2 for 2 ppm) show that these methods had the best reproducibility of those tested here. An important finding was that in dilute samples (0.2 ppm) the recoveries of the passive evaporation method was higher than when evaporation took place in the test tube at 47 °C. Otherwise no significant differences were observed between these two methods (Fig. 1a,b). For both methods, the losses of solute were significantly higher in dilute samples (0.2 ppm) compared to the 10 times more concentrated samples (2 ppm).

The nitrogen flow method (Fig. 1c) showed a significantly lower capacity to recover solutes compared to the methods mentioned above. In the dilute samples, more than 50% of the compounds with low boiling points were lost. Furthermore, this method was more discriminative, as the loss of lower boiling compounds tended to be higher than the compounds with higher boiling points compared to the former methods. The C.V.'s were 18.0 and 12.2 for the dilute and concentrated samples, respectively. Again, relatively more solute was lost in diluted samples than in the concentrated samples.

The losses observed for the Pasteur pipette were comparable to those of the nitrogen flow method. The recoveries were significantly lower than those observed for passive evaporation at 22 °C and evaporation in a test tube at 47 °C. This was the case in both diluted and concentrated samples (Fig. 1d). The C.V.'s were 21.6 for the dilute samples and 11.5 for the 2 ppm samples. As for the former methods, lowering the original solute concentration decreased the recovery percentage of solutes.

Pre-concentration of samples to a final volume of

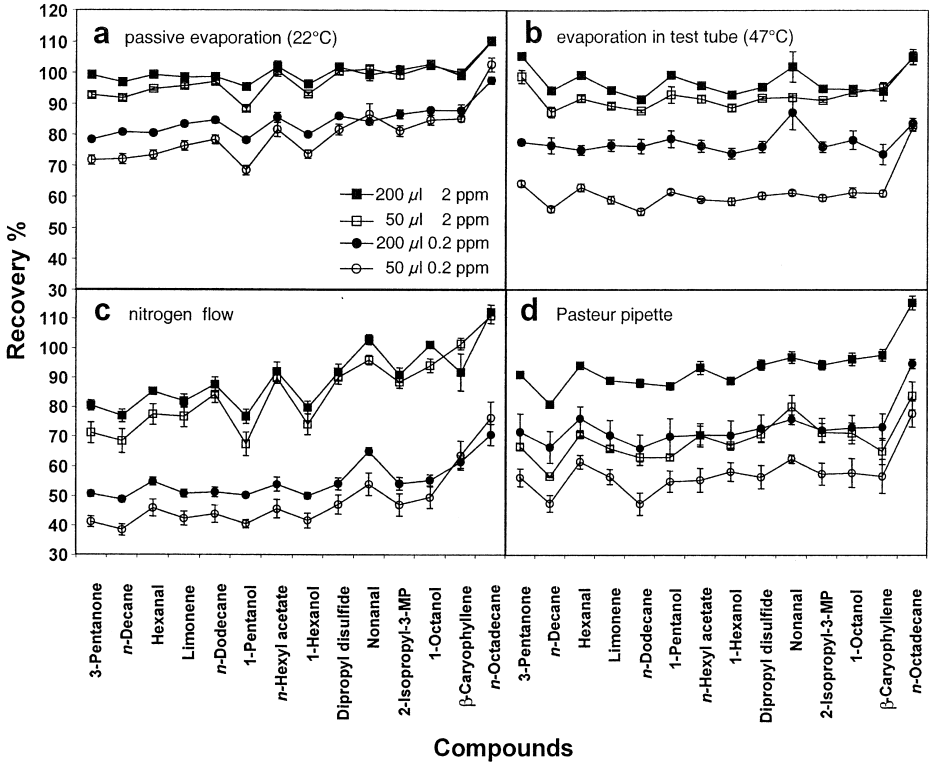

Fig. 1. Solute recovery throughout the sample concentration process: influence of final volume and initial sample concentration for the pre-concentration techniques (a) passive evaporation at 22 °C, (b) evaporation in test tube at 47 °C, (c) nitrogen flow (22 °C), (d) Pasteur pipette (47 °C).

50 μl was only feasible using the four methods described above. Concentration to 50 μl was not possible for the Vigreux and Snyder columns nor the spinning-band technique, because all of the solution had to be evaporated before the total reflux became as low as 50 μl. In practice, it was not possible to predict the degree of reflux following transfer to the ice-bath.

3.2. Pre-concentration to 200 μl (recoveries and influence of sample concentration)

When evaporation was ceased at 200 μl the two test tube methods (22 and 47 °C) showed, not surprisingly, higher or similar recoveries compared to those obtained when pre-concentration was allowed to proceed to the 50-μl level (Fig. 1). These two methods showed the highest mean recoveries of the eight methods tested at the 200-μl level followed by the Pasteur pipette method and spinning band distillation (Table 2). The superiority of the test tube methods was most pronounced for compounds with the lower boiling points, whereas the recoveries of the less volatile compounds such as *n*-octadecane were less influenced by the pre-concentration design when pre-concentrating to 200 μl (Figs. 1 and 2). The best reproducibility was, again, observed for the passive evaporation method (Table 2).

The Pasteur pipette method showed relatively high recoveries at the 200-μl level, however, the C.V. of 20.9 indicated that this method has to be refined further in order to improve the reproducibility (Table 2).

The Vigreux column showed relatively low recoveries when pre-concentrated to 200 μl (Table 2) and a C.V. of 23.1 and 18.0 for dilute and concentrated solutions, respectively, were in the poor end of the spectrum. In contrast to the other methods, the

Table 2
Recovery (%) of volatile and semi-volatile solutes during evaporation of dichloromethane from 2 ml to 200 μl or 1 ml, respectively, using different concentration levels of solutes (0.2 ppm or 2 ppm)

Methods	Pre-concentration to 200 μl				Pre-concentration to 1 ml			
	0.2 ppm	C.V.	2 ppm	C.V.	0.2 ppm	C.V.	2 ppm	C.V.
Passive evaporation	84.4 aB[a]	3.4	100.1 aA	2.7	83.2 aB	6.5	100.2 aA	4.5
Test tube at 47 °C	80.6 aB	9.2	98.7 aA	3.8	86.0 aB	4.7	101.6 aA	3.2
Pasteur pipette	75.5 abA	20.9	93.5 abA	5.1	95.5 aB	6.7	102.8 aA	3.9
Spinning-band	71.8 abB	11.8	93.1 abA	11.2	92.2 aA	6.0	99.4 aA	3.3
Vigreux	71.3 abA	23.1	67.8 cA	18.0	87.5 aB	8.0	99.6 aA	3.6
Snyder (3)	60.6 bcB	7.7	74.7 cA	13.3	85.3 aB	3.3	102.7 aA	2.6
Nitrogen flow	55.1 cB	10.5	89.4 abA	9.4	83.2 aB	8.1	103.3 aA	3.6
Snyder (0)	53.8 cB	4.9	81.2 bcA	13.6	93.9 aA	11.6	101.2 aA	13.6

For details on the methods, see Table 1. C.V., coefficient of variance.
[a] Mean separation within columns (small letters) and rows (capital letters) at 200 μl and 1 ml, respectively; by ls means at $P=0.05$.

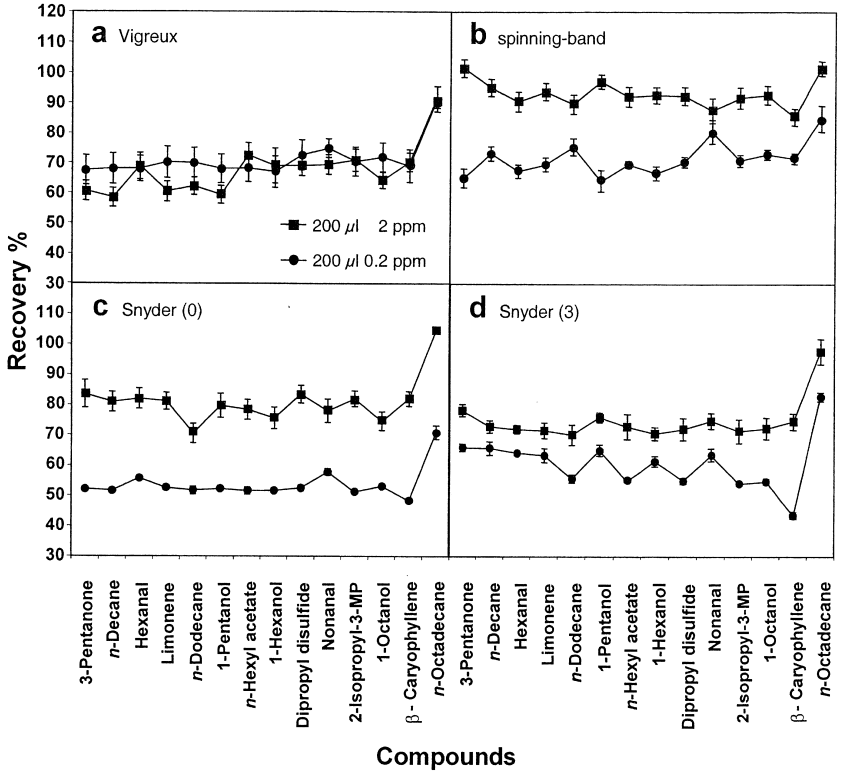

Fig. 2. Solute recovery throughout the sample concentration process: influence of final volume and initial sample concentration for the pre-concentration techniques (a) Vigreux (47 °C), (b) spinning-band (47 °C), (c) Snyder (0) (47 °C), (d) Snyder (3) (47 °C).

Vigreux column showed no significant differences between the recovery percentages of the 0.2 ppm and 2 ppm solutions, respectively (Fig. 2a).

The spinning-band technique performed well with the concentrated samples (2 ppm) but the losses were significant when dilute samples (0.2 ppm) were concentrated to 200 μl (Table 2 and Fig. 2b). The spinning-band distillation gave recoveries in the same range as the Vigreux column during pre-concentration of diluted samples to 200 μl, whereas the spinning-band was clearly more efficient than the Vigreux column during pre-concentration of the concentrated samples to 200 μl (Fig. 2a,b). The Snyder columns showed generally lower recoveries than spinning-band distillation regardless of solute concentration (Fig. 2).

Generally, with pre-concentration to a final volume of 200 μl, losses were kept at acceptable levels using an initial sample concentration of 2 ppm, except for the Vigreux, Snyder (3) and Snyder (0), which all showed losses of >20% compared to the loss detected by pre-concentration to 1 ml (Table 2). As was the case when concentrating to 50 μl, the recovery percentages at 200 μl depended to a great extent on the solute concentration (Table 2).

3.3. Pre-concentration to 1 ml (recoveries and influence of sample concentration)

All methods showed high recoveries of solute when pre-concentration was stopped at 1 ml, with a mean recovery around 90% and 100% for dilute and concentrated samples, respectively (Table 2). Accordingly, no significant differences between the pre-concentration methods were observed during concentration of the standard solutions to 1 ml (Table 2).

Recoveries in the concentrated 1-ml samples were significantly higher than those of the diluted samples, so pre-concentration to 1 ml follows the same recovery pattern with regard to sample concentration as pre-concentration to 50 and 200 μl (Table 2).

3.4. The role of volatility of the solutes

The losses of solute depended only slightly on the boiling point of the solute. *n*-Octadecane was the exception to the rule, but this compound had by far the highest boiling point as described in Experimental. Only the nitrogen flow method was clearly discriminative as noted above (Fig. 1c).

4. Discussion

4.1. Comparison of the pre-concentration methods

The best recoveries when concentrating samples to 50 μl were observed using the passive evaporation method at 22 °C. This method is, however, time consuming and was included in the study as a reference method in order to detect the consequences of speeding up the evaporation by, e.g. heating the solution or by introducing a gas stream into the test tube. The only drawback of the passive evaporation method is that it takes 19–20 h to pre-concentrate 2 ml to 50 μl (Table 1). Speeding up the process to 1–2 h (Table 1), by keeping the solution just below the boiling point in a test tube engulfed in a water bath at 47 °C, yielded lower recoveries in dilute solutions than the passive method but very similar recoveries in the 2-ppm samples. Therefore, more concentrated solutions may be heated in the test tube without excessive losses, whereas solutions with lower, or unknown concentration levels, should initially be concentrated by passive evaporation. Subsequently, theoretical losses by heating may be determined and this may be compensated for by addition of one or more internal standards at the routine analysis.

Passive evaporation at 22 °C and evaporation in a test tube at 47 °C were superior to evaporation of solvent under a gentle stream of nitrogen. The nitrogen flow method is one of the most commonly used techniques for pre-concentration of small volume dilute samples obtained from, e.g. the dynamic headspace sampling technique [17–19], because it is relatively fast, easy to handle, and can quickly concentrate samples down to very small volumes. The nitrogen flow method is, however, sensitive to the concentration level of the solutes (Fig. 1c). The clear discrimination among solutes makes it also very problematic to compensate for by inclusion of appropriate internal standards. Compatible observations were reported by Chang et al. [21] who used the nitrogen method combined with heating to 40 °C,

to pre-concentrate polycyclic aromatic hydrocarbons in dichloromethane from 300 ml to final volumes of 50, 30, 5, 3 and 1 ml, respectively. The recoveries were high when pre-concentrating to 3 ml, but below this level, the losses of volatiles increased significantly. Ferreira et al. [20] used the nitrogen flow method to pre-concentrate solutes in hexane solvent at 22.5 °C. They concluded that more than 50% of the solute may be lost when pre-concentrating compounds with boiling points below 150 °C from 2 ml to 10 μl.

The Pasteur pipette method is to some extent similar to evaporation in a test tube at 47 °C. Although this pre-concentration method gave acceptable mean solute recoveries, both in dilute and concentrated samples, the Pasteur pipette method was not quite reliable for dilute samples. A similar design has previously been shown by Dünges [16] to give high solute recoveries although most of the compounds in Dünges study were less volatile than those applied in this study.

The efficiency of the traditional Vigreux and Snyder columns were only satisfactory at pre-concentration to 1 ml. Below 1 ml these results were not reproducible, although Vigreux and Snyder columns have been shown to give excellent recoveries in combination with a micro-Kuderna-Danish concentrator [12]. Also, the fact that it is impossible to pre-concentrate to 50 μl and the high C.V.'s are serious drawback's for these methods.

The spinning-band technique, with a higher rectification capacity than the Vigreux and Snyder pre-concentration techniques used in the present study, gave accordingly higher recoveries, especially at the 200-μl level. But like the Vigreux and Snyder columns the spinning-band was not useful at the 50-μl level, as the amount of evaporated solvent was not sufficient to maintain the reflux. Also, reproducibility has to be improved for this method when concentrating dilute samples to 200 μl or lower.

As noted by Dünges [16] the performance of each method may be influenced by varying physical characteristics of the method. These include: the temperature of the water bath and the reflux column, to which extent the vessel is engulfed in the water bath, the length of the column used to create reflux, the diameter of the reflux column, the shape of the vessel, the flow of nitrogen, etc. Therefore, the performance of the methods described here may be moderated and optimised further.

4.2. On the causes for solute loss during pre-concentration

The results raise a number of questions concerning causes for loss of solute in the different experimental designs. These should be approached prior to an attempt to refine the pre-concentrations methods further.

What causes the lower recoveries of the Pasteur pipette compared to the test-tube method (both at 47 °C)? The main difference between these methods is the ratio between the glass surface in contact with the liquid ($2\ \pi r$) and the liquid surface (πr^2), with r being the internal radius of the tube. For the test tube this ratio was 0.24 and for the Pasteur pipette it was 0.80. A high ratio means (1) relatively more glass surface available to ''trap'' vapours; (2) more liquid exposed to evaporate on a dry surface; and (3) the speed of the vapours able to transport solutes are higher. The first point increases the recovery of solutes, the 2nd and 3rd decreases it since they will promote co-evaporation. We suggest that the results presented here clearly demonstrate that the 2nd and 3rd factors are dominating and that the retention capacity of the glass surface above the liquid is of less significance. The hypothesis that co-evaporation is the dominating factor for solute escape is supported by the following key observations and results.

(a) The very poor recoveries obtained under nitrogen flow compared to passive evaporation are explained by the high speed of solvent vapours caused by the introduction of a gas flow above the surface of the solution (point 3 above). It is well known that the volume of passing solvent vapour and the vapour pressure of the solute are important factors in the co-evaporation process [3]. This difference in gas flow is the only significant difference between the nitrogen method and passive evaporation as the gas flow did not force the liquid surface against the glass wall. Furthermore, the ratio $2\pi r/\pi r^2$ are the same, as the test tubes are identical (point 2 above). Also, reflux occurred in neither of these designs, and the temperatures in the two systems were close to 22 °C. Therefore, differences

in the retention capacity of the glass can be excluded.

(b) The lower recoveries of diluted samples using the 47 °C test tube method compared to passive evaporation is also explained, at least partly, by the increase in vapour speed caused by the faster evaporation at 47 °C compared to 22 °C. Although the higher temperature of the glass may also have an effect, we could conclude from (a) above that an increase in the speed of evaporating vapour increased the losses by co-evaporation. This loss was moderated by the re-condensed solvent layer and reflux formed ahead of the evaporation site.

(c) The volatility of the solute had relatively little influence on the recoveries (Figs. 1 and 2). This pattern was almost universal and was not influenced by the temperature of the solution. This observation also supports the hypothesis that losses were primarily caused by co-evaporation, which is kinetic rather than thermodynamic.

Theoretically the vapour pressures of the solutes are low as long as they are in the solution so co-evaporation with the solvent is of less importance for the level of solute recovery [3]. The high retention power of the solvent (liquid phase) serves as a ''stationary phase'' for retaining solute material. These statements seem to be confirmed here, at least by the high recoveries in an ''undisturbed'' system (no heating, no external gas, no reflux splashing) as the passive evaporation design. However, the results clearly demonstrate that the loss of solute is initiated when heat or gas flux is introduced. This is demonstrated by the lower recoveries of all the ''non-passive'' pre-concentration methods. The heating speeds up the evaporation considerably, and the expansion when the solvent enters the gas phase produces a rapid gas flow, comparable to that initiated by the nitrogen gas. In the case of the methods where heating is employed, a re-condensation and reflux of a proportion of these vapours take place on the wall of the glass tubes. This solvent layer theoretically retains at least some of the escaping vapours [16]. The efficiency of the column to re-trap the vapours was crucial for the capacity of the method to recover the solutes of the sample. Our experiments demonstrate that this trapping was insufficient for most methods. Although from the higher recoveries of the 47 °C test tube method (with re-condensation) compared to the nitrogen flow method (without re-condensation), it was evident that the re-condensed layer trapped a proportion of the evaporating solutes (Fig. 1b,c). We suggest that only a proportion of the vapours which, in the heated tubes, are travelling up through the tube with considerable speed, get in contact with the condensed trapping layer.

4.3. Influence of initial sample concentration on the recovery of solutes

The significantly higher loss of solutes in 0.2 ppm solutions compared to 2 ppm samples suggests that losses in even more diluted samples may be massive. Previous studies on the effect of initial sample concentration on solute recovery have shown that this point in the analytical process plays a significant role [3,12]. The size of the droplets generated from concentrated solutions is larger than those generated from dilute solutions. As a result, the droplets from the concentrated solutions take longer to evaporate, delaying the release of solutes contained in them, and thereby diminishing the amount of solute lost during this step. Furthermore, after the majority of solvent that forms the droplet has evaporated, the solute coating covering the glass wall makes the retention capacity of this type of stationary phase larger than the glass wall itself and thereby avoiding solute loss, or at least delaying its release. The retention capacity of the solute coating depends on its thickness. Consequently the retention capacity of the coating, formed by concentrated solutions, is larger than that of diluted solutions. The results from the present investigation are in accordance with these considerations, as more solute was lost during pre-concentration of dilute solutions. In fact the recoveries of solutes were found to be more dependent on the initial solute concentration than the final volume (Table 2 and Figs. 1 and 2).

5. Conclusions

Loss of solute was mainly caused by the fast vapour streams created when speeding up the process of evaporation by heating or by introducing a gas stream into the tube. This increased co-evaporation

and thereby solute loss. The capacity of the methods to trap the escaping vapours and create a reflux determined the capacity of the methods to recover the solutes.

If the fast, but less suitable pre-concentration methods are employed on very dilute samples (sub ppm level) the loss of solute may be even more massive than demonstrated here. Therefore, dilute samples and samples of unknown composition and concentration should initially be pre-concentrated by the passive evaporation method or a method with a similar capacity to recover solutes efficiently. Subsequently, the effects of a faster method are determined, and the observed losses may be compensated for by addition of internal standard(s).

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