ELSEVIER

Journal of Chromatography A, 699 (1995) 395–402

JOURNAL OF CHROMATOGRAPHY A

Short communication

Masking as a mechanism for evaporative loss of trace analyte, especially after solid-phase extraction

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First received 19 September 1994; revised manuscript received 23 December 1994; accepted 12 January 1995

Abstract

Using a Pasteur pipette plugged with silanized glass wool and packed with C_{18}–silica particles, we attempted to remove K_2CO_3 from an aqueous acetonitrile solution. In spite of extensive washing of the column with water after the sample was applied, elution with acetonitrile followed by evaporation gave a visible, white residue. It was found that the residue was derived from both the sample and the packing, including particles from the latter. Substitution of a plastic column/polyethylene frit for the Pasteur pipette/glass wool gave a more consistent residue, apparently because this improved the retention of particles. Subsequent experiments were conducted in the plastic hardware. The amount of the residue was observed to vary as much as 19-fold when C_{18}–silica particles were tested from different manufacturers, and the residue could be reduced in amount as much as 9-fold when a column was prepared in the laboratory vs. the use of a comparable, pre-packed column. The water itself contributed some of the residue: even the "purest" water routinely available left a visible residue when 1.0 ml was appropriately evaporated (e.g. on Saran Wrap in a microwave oven). The recovery of an arbitrary trace analyte and internal standard (pentafluorobenzylated nucleobases at the low pg level) was 32% less when they were evaporated in acetonitrile that had been passed through an acetonitrile and water-washed cartridge containing C_{18}–Si vs. evaporation in untreated acetonitrile. Collectively these results reveal that an evaporation can risk some loss of an analyte from masking by even subtle solvent contaminants. This tends to counteract the common strategy of redissolving a trace analyte by using a good solvent for it.

1. Introduction

Solid-phase extraction (SPE), e.g., using an alkyl-bonded silica packing, is widely employed for sample cleanup in chemical analysis [1]. A typical overall sequence of steps is: (1) trap the analyte on the packing in a weak solvent (weak in dissolving power for the analyte), (2) wash with one or more medium-strength solvents, (3) elute with a strong solvent, (4) evaporate and (5) redissolve prior to continuing with the analytical procedure.

Losses of analyte are encountered frequently in SPE ranging from mild to severe [1,2]. Depending on the nature of the analyte, one or more of three mechanisms usually are demonstrated or considered to be responsible: elution of some analyte during the application or wash-

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0021-9673/95/$09.50
SSDI 0021-9673(95)00052-6

ing stage; volatization of analyte during the subsequent evaporation step; and irreversible adsorption of analyte on the solid surfaces (e.g. packing, frits, evaporation vessel) encountered in the procedure. Here we learn about a fourth mechanism: masking of evaporated analyte by solvent impurities.

2. Experimental

2.1. Reagents and materials

Potassium chloride, potassium carbonate and acetonitrile were purchased from American and Jackson (American Scientific Products, Boston, MA, USA). Distilled–deionized water was purified with an Organopure deionizer (Barnstead, Boston, MA, USA). This water was used unless indicated otherwise. HPLC-grade water and Ultrex II water were also purchased from J.T. Baker (Phillipsburg, NJ, USA). Also water was tested that was purified in the laboratory by distillation/ion exchange/carbon/distillation using Barnstead equipment. All glassware, except for the evaporation vials, was gas-phase silanized. SPE columns were prepared in two ways. A 5.75-in. (1 in. = 2.54 cm) borosilicate Pasteur pipette (Fisher Scientific, Pittsburgh, PA, USA) was plugged with silanized glass wool and loaded dry with 200 mg of packing, or a Supelclean column (Supelco, Bellefonte, PA, USA) consisting of a polypropylene tube fitted with a polyethylene frit (20 μm average porosity) was similarly packed. Also, various prepacked SPE columns were tested directly as received from several suppliers: J.T. Baker, Supelco, Waters Chromatography (Milford, MA, USA), EM Separations (Gibbstown, NJ, USA) and Varian (Harbor City, CA, USA). Borosilicate vials for the evaporations (1.8 ml, catalog No. 03-339-25A), which weighed about 2 g each, were obtained from Fisher Scientific.

2.2. Equipment

A Mettler M3 microbalance was used (Mettler-Toledo AG, Greifensee, Switzerland). The gas chromatography–electron-capture mass spectrometry (GC–EC–MS) system, and conditions utilized, were described before [3,4].

2.3. Solid-phase extraction

The SPE column (packed with Octadecyl C_{18}–Si from J.T. Baker unless indicated otherwise) was conditioned with 1.0 ml of acetonitrile followed by 1.0 ml of water. A 6-mg amount of potassium chloride or potassium carbonate were dissolved in 50 μl acetonitrile plus 1.0 ml of water in a vial. The salt solution was transferred to the SPE column with the aid of a Pasteur pipette along with 1.0 ml of water taken through the vial. After immediate elution of the SPE column aided by 1 p.s.i. (1 p.s.i. = 6894.76 Pa) of high-purity grade nitrogen (Med-Tech, Medford, MA, USA), 2.0 ml of water taken through the vial were transferred to the SPE column followed again by nitrogen. The column was treated five times with water (2.0 ml each) and nitrogen, and then once with 2.0 ml of acetonitrile–water (1:9, v/v). This was followed by nitrogen until the bulk liquid was eluted, and then a higher flow of nitrogen was applied (for about 1 min) until all droplets were dispelled. The SPE column was eluted with 1.0 ml of acetonitrile into a tared 1.8-ml vial that had been dried by heating overnight in an oven at 105°C, followed by cooling in a desiccator (NaOH pellets) for 25 min. The eluent collected in the vial was removed by evaporation in the same oven overnight, and the vial was weighed as before. The vial also was examined visually, or with a hand-held lens (10×), for any residue. Throughout, each vial was handled in a metal tray and with metal forceps.

2.4. Recovery experiment

A Supelclean column containing 200 mg of C_{18}–silica was conditioned with 1.0 ml acetonitrile and 1.0 ml of water, and then subjected to the procedure (water washing, 10% acetonitrile washing, acetonitrile elution) described above, except no sample was added. The collected acetonitrile was spiked with 1 μl of a solution consisting of 1.10 pg/μl of N1,N3-bis(pen-

tafluorobenzyl) - N7 - [2 - (pentafluorobenzyloxy)-ethyl]xanthine, and 1.01 pg/μl of N1,N3-bis(pentafluorobenzyl)-N7- {2-(pentafluorobenzyloxy)-[$^2H_4$]ethyl}xanthine, in acetonitrile. The preparation of these derivatives of xanthine has been described before [3]. A 1-μl volume of the latter solution also was spiked into a similar vial containing 1.0 ml of acetonitrile dispensed directly from a bottle. The solutions in these vials were evaporated under nitrogen and 25 μl of toluene were added. After vortexing the samples for 1 min, 1.0-μl aliquots were injected into a GC–EC–MS system. Also the starting solution containing these two xanthine derivatives was injected for comparison purposes to define the recovery.

3. Results and discussion

We are developing a method to measure O^6-methylguanine, a DNA adduct, by derivatization–gas chromatography [3]. In the derivatization procedure that we are using, one of the steps involves a reaction with pentafluorobenzyl bromide in the presence of K_2CO_3 and acetonitrile. We decided to purify the derivatized analyte (e.g. 10 pg level) by adding water at the end of the reaction, and subjecting the resulting solution to SPE on a short column containing C_{18}-bonded silica. The column was a Pasteur pipette plugged with silanized glass-wool. After the sample was applied, we washed the cartridge thoroughly (50 column bed volumes) with water, and also some water–acetonitrile, to remove the potassium carbonate and residual pentafluorobenzyl bromide. We then eluted the sample with acetonitrile. To our dismay, evaporation of the collected acetonitrile gave a visible, white residue. Visibly, the residue was insoluble in the intended redissolving solvent (ethyl acetate) and also was not completely soluble in water. The same residue, visibly, was obtained when both the analyte and pentafluorobenzyl bromide were omitted from the procedure. We decided to study the event in more detail, since it complicated our method.

Since the residue was visible, we assumed that we could quantify it by using a microbalance. First we set up a sensitive technique for weighing. Repeated, immediate re-weighing of an empty vial gave values within a range of 4 μg. However, such weighings day-to-day (with intervening drying of the vial in an oven and then cooling in a desiccator) gave a 3-fold wider range, which was traced to day-to-day changes in the humidity (about 0.5–1 μg increase in mass per 1% increase in the absolute humidity from humidity variations in the range of 70 to 80% at an ambient temperature during weighing of 23–24°C.) Since different vials processed together were affected equally by the humidity (within experimental error), the variation in vial mass due to humidity could be controlled by comparing vials within a given run, or against empty vials to allow data from different runs to be compared. Only the data from some early experiments was obtained using the former approach. All sample and control vials were done as triplicates unless noted otherwise.

We tested samples for SPE consisting of 2 ml of acetonitrile–water (1:39, v/v), and containing "X", where X = nothing, K_2CO_3 or KCl as indicated in Fig. 1. By employing the procedure described in this figure, we found that the mass of the visible white residue from $X = K_2CO_3$ varied significantly (e.g. 48 ± 30 μg as $x \pm$ S.D. for a triplicate of samples; early data not corrected for humidity, but the data are within a given run) among similarly prepared SPE columns. At this stage, as pointed out above, the column consisted of a Pasteur pipette that had

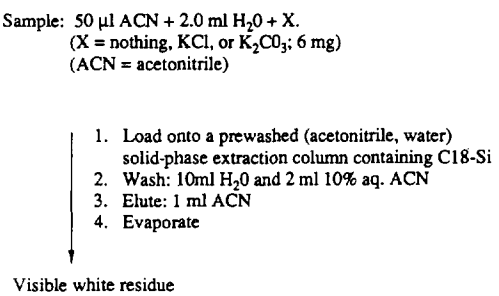

Fig. 1. Scheme for testing alkyl-bonded silica packings in a solid-phase extraction procedure.

been plugged in the laboratory with silanized glass wool and then charged with packing. Using instead a polypropylene cartridge fitted with a 20-μm average porosity polyethylene frit in an SPE tube (Supelclean) gave much more consistent results (42 ± 3.8 μg, data not corrected for humidity). No measurable mass or visible residue was obtained when the procedure was conducted using a Pasteur pipette containing silanized glass wool alone. Thus, the variation in residue mass after evaporation from the packed Pasteur pipette apparently was due to variation in retention of the bonded silica packing by the silanized glass wool plug, depending on how well the plug was formed. We did not pursue this aspect further here, but a better way to plug a Pasteur pipette for SPE, or to avoid plastic in SPE, is needed.

It was at this point in our work that we subtracted out the contribution of humidity to the results, which brought the value for the residue down to 16 μg (see Table 1). Increasing the volume of water used to wash the SPE cartridge, after loading the K_2CO_3 sample, from 10 to 20 ml (data not shown) did not reduce the amount of the residue. Less residue (8 μg, see Table 1) was obtained, however, with X = KCl. A similar residue (7 μg) was obtained from a sample containing no added salt (X = nothing). We interpret the formation of the visible residue as follows. Apparently the change in eluent on the SPE cartridge from the initial sample to pure water causes the bonded phase on the silica packing to reorient (e.g. collapse) and/or become less wetted. Such variations in alkyl phases bonded to silica as a function of solvent composition and other variables have been studied in several laboratories, including an observation of solvent trapping [5], as has been reviewed [6–8]. This in turn sequesters some of the sample. In the case of K_2CO_3, the high pH causes some hydrolysis of the bonded phase during the washing step with water. The bonded phase later changes back when the column is eluted with acetonitrile, releasing sequestered sample and non-attached bonded phase. Fines also might be released. Less residue is observed from a KCl sample since the pH is closer to neutrality, reducing the degree of bonded phase hydrolysis during the washing step with water. Consistent with this overall mechanism for the formation of the residue, omitting the washing step with 10% acetonitrile between the water-washing and acetonitrile-elution steps (a departure from Fig. 1) increases the mass of the residue by about 2.5-fold. Apparently the 10% acetonitrile wash ordi-

Table 1
Residues derived from evaporation of water (on Saran Wrap in a microwave oven), or from the acetonitrile eluent (evaporation in a vial placed in a 105°C oven) described in Fig. 1

Experiment	Residue		
	Visible intensity	Mass (μg) ($\pm$ S.D.)	Totally soluble in water?
Evaporate 1 ml of Ultrex II water	+	[a]	Yes
Evaporate 1 ml of HPLC-grade water	+ +	[a]	Yes
Fig. 1[b]			
X = nothing	+ + +	7 ± 2	No
X = KCl	+ + +	8 ± 0	No
X = K_2CO_3	+ + + +	16 ± 2[c]	No

[a] The mass is below the range of our microbalance.
[b] A 200-mg amount of Octadecyl-Silica obtained from J.T. Baker was packed in the laboratory in a Supelclean cartridge.
[c] Corresponding data from an equivalent experiment, except that a cartridge was obtained pre-packed from J.T. Baker, is shown in Table 2.

narily causes a partial reorientation of the bonded phase, and thereby partial elution of entrapped sample and contaminants.

Since some visible residue was observed even when X = nothing was tested, as indicated in Table 1, we wondered whether the water in the initial sample (or used in the wash) might be contributing to this residue. Indeed, evaporating 1 ml (arbitrary volume) of water gave a visible residue, the amount of which was too little to detect with the microbalance. (Keep in mind that the evaporation is being done by standing the vial partly filled with solvent in a hot oven. This makes the small residue visible by focusing it at the bottom of the vial.) Careful inspection of the vial is necessary to visualize this residue; use of a hand-held magnifying lens is helpful. The residue typically accumulates part-way around the side wall at the bottom of the vial. Actually we tested three kinds of HPLC-grade water (two prepared in the laboratory and one purchased; see Experimental for details), each of which gave the same visual result. Evaporation of 1 ml of pure acetonitrile gave no residue either visibly or by mass.

Since the water was being evaporated in an oven at 105°C, we needed to rule out the unlikely possibility that the residue was derived from dissolving some borosilicate from the wall of the vial during the evaporation. Evaporating the water in a silanized glass vial gave the same result. Evaporating the water in a Speed-Vac (less time and lower temperature for the evaporation due to the use of a vacuum) gave a visible residue only in some of the vials. Apparently this was due to variation in the final stage of evaporation of the residual water in the orbiting vial: if the residual droplet rotated, it spread out the residue below a visible level. But if the final droplet "hung up" on the vial wall, the resulting residue occupied a smaller zone, becoming visible, more like the evaporation event in the oven where the vial is immobile during the evaporation.

We obtained further evidence that the visible residue from evaporation of 1 ml of HPLC-grade water truly was present in the initial water by evaporating 1 ml of water placed on Saran Wrap that was stretched over the mouth of a dish in a microwave oven: a visible white residue was observed. Thus, some of the residue that we have encountered after SPE and evaporation comes from the water itself.

Although it was somewhat of a sidetrack at this point, we were curious as to whether any routine water existed that would not give a visible residue when 1 ml was evaporated. We obtained some ultrapure water from J.T. Baker (Ultrex II water, which is purified by sub-boiling distillation at atmospheric pressure in an all-quartz apparatus, and collected in a polyethylene bottle). This water is intended to be highly purified with respect to inorganic but not necessarily organic contamination. Microwave evaporation of 1 ml of this water on Saran Wrap as above still gave a visible residue, although it was the smallest residue (visibly) that we had observed from water (see Table 1). The residue was totally soluble in water. We shared our observation with personnel at J.T. Baker, who suggested that the residue was water-soluble phthalates derived from the plastic container. They sent us some Ultrex II water which they had collected in a PTFE bottle, and which they suggested would be free of such phthalates and thereby not give any visible residue upon evaporation. However a visible residue was observed as before. (The Ultrex II water is reported by Baker to contain less than 10 ppb total inorganics based on analyzing 1 l of the water by inductively coupled plasma emission spectroscopy after evaporation to a small volume. Perhaps some contaminants like silica are lost to adsorption onto the wall during the evaporation, and thereby escape detection in their procedure.)

Returning to the SPE experiments, we compared analogous packings from several suppliers using the scheme shown in Fig. 1 with X = K_2CO_3. The results of these experiments, including some characteristics of the packings, are presented in Table 2. In most cases pre-packed cartridges were tested, each containing an amount of packing ranging from 200 to 500 mg. For the packings that were obtained in bulk, a 200-mg amount was tested packed in the laboratory into a Supelclean column. We normalized all

Table 2
Residues derived from the acetonitrile eluent of an SPE column (see Fig. 1) after applying an aqueous–acetonitrile K_2CO_3 sample to an SPE column (plastic tube and frit)

Supplier and packing	Packing characteristics		Amount of packing (mg)	Mass of residue (μg)	
	Monomeric (m) or polymeric (p)	End capped		Observed ($\pm$ S.D.)	Normalized (to 200 mg)
J.T. Baker					
Octadecyl	p	Yes	500	73 ± 8	29
Light Load Octadecyl	p	No	500	84 ± 19	34
Octyl	p	Yes	500	165 ± 23	66
EM Separations					
RP-18	m	No	200	62 ± 5	62
RP-18[a]	m	No	200	7.3 ± 0.6	7
RP-18e	m	Yes	200	40 ± 8	40
RP-18e[a]	m	Yes	200	8 ± 3	8
RP-Select B[b]	m	No	200	106 ± 9	106
Waters Chromatography					
C_{18} plus	m	Yes	360	236 ± 13	131
t C_{18} plus	m	Yes	400	32 ± 3	16
C_8 125 Å plus	m	Yes	400	42 ± 4	21
Supelco					
LC-18	m	Yes	500	180 ± 6	72
ENVI-18	p	Yes	500	40 ± 2	16
LC-8	m	Yes	500	179 ± 26	72
Varian					
Bondesil C_{18} [a]	p	yes	200	89 ± 8	89
Envirelut	p	yes	200	41 ± 7	41
Bond Elut C_{18} [c]	p	yes	200	132 ± 40	132

[a] Packed in the laboratory; all other packings came pre-packed from the manufacturers.
[b] This is a C_8–Si packing.
[c] Bond Elut C_{18} is a pre-packed form of Bondesil C_{18}.

results to 200 mg as indicated in Table 2, although the actual residue masses are also shown. Consistent with this normalization (within experimental error), testing of both 200- and 500-mg amounts of one of the packings (RP-18, laboratory packed) gave residues of 7 and 13 μg, respectively (only the prior value is shown in the table).

Perhaps the major observation is that all of the packings produced a weighable residue from the acetonitrile eluent when subjected to the scheme shown in Fig. 1 with $X = K_2CO_3$. As seen, the amount of this residue ranged from 7 to 132 μg per 200 mg of packing. The pre-packed RP-18 columns from EM Separations gave 9 times (RP-18) and 5 times (RP-18e) more residue than the presumably same packings that they provided to us in bulk, which we packed in the laboratory. Similarly, the prepacked Bond Elut C_{18} from Varian gave 1.5 times more residue than the equivalent Varian packing (Bondesil C_{18}) that we packed in the laboratory. Also, the pre-packed form of Octadecyl from J.T. Baker gave 2 times more residue than when packed in the

laboratory (compare data in Table 2 vs. 1 for $X = K_2CO_3$). We did not study this general observation further. Perhaps the packings undergo physical damage when they are pre-packed by the manufacturers between the frits in the plastic columns.

For the packing where both monomeric and polymeric versions of C_{18}–Si were provided (bonding of monofunctional and polyfunctional alkylsilanes, respectively) from the same manufacturer (Supelco), the polymeric packing gave less residue, although other variables (not under our control) were not necessarily held constant. This is consistent, nevertheless, with Scott's [7] conclusion that polymeric C_{18}–silica packings are less prone to collapse in the transition to 100% water than corresponding monomeric packings. Too few data are available to draw any conclusion about whether the presence or absence of end-capping plays a role.

Since the visible residue derived from a C_{18}–silica packing is not very soluble, we considered it might cause loss of a trace analyte by masking it upon evaporation. We evaporated 1.0 ml of acetonitrile in triplicate containing 1.10 pg of a stable analyte of current interest in our laboratory, N1,N3-bis(pentafluorobenzyl)-N7-[2-(pentafluorobenzyloxy)ethyl]xanthine [2], along with a similar amount of a corresponding stable isotope internal standard. Similarly we evaporated 1.0 ml of analyte-spiked acetonitrile, where the latter solvent before spiking had been collected from a C_{18}–silica column after subjecting the column to the procedure shown in Fig. 1 with X = nothing. Indeed, redissolving (toluene) followed by GC–EC–MS showed that 32% less analyte (and internal standard) was recovered in the latter sample. (The absolute recoveries were 83 ± 10 and 56 ± 11% in the acetonitrile and packing-exposed acetonitrile, respectively).

The visible residue derived from evaporation of organic solvent exposed to a cartridge packed with C_{18}–silica was not characterized in this study. Others have found a wide variety of organic compounds in procedural blanks from such cartridges, including alkylsilanols and alkylsiloxanes derived from the packing [9].

Our results from testing the various commercial packings are not intended to assess their general usefulness or relative quality. The packings are produced with a number of considerations in mind, and their relative effectiveness can depend on the application and other criteria besides the one examined here.

4. Conclusions

Two overlapping topics are presented in this paper: evaporation losses and water quality. Our conclusions for each are as follows.

4.1. Water quality

While it is known that no solvent is "100% pure", it still is surprising that a visible residue is obtained even when 1 ml of ultrapure water is evaporated. This implies that some masking loss of analyte may always occur when an aqueous solution (or perhaps any solution) of an analyte is evaporated. Of course, the loss tends to become noticeable only for a trace analyte.

4.2. Evaporation losses

It has always puzzled us as to how a simple, small, soluble, non-volatile compound could undergo an adsorption loss when a solution of it is evaporated in a glass vial, and no visible residue (using conventional evaporation techniques) is apparent that might interfere with redissolving. Here we learn that the mechanism may involve masking by insoluble (with respect to the redissolving solvent) impurities present in the original solvent, especially when the original solvent is aqueous, or when the sample has been eluted from a bonded-phase silica packing. Using the unconventional evaporation techniques described here can make the residue visible. Although it is well-known that trace analytes tend to undergo losses when evaporated, the contribution of subtle solvent impurities to this loss has not been appreciated. This knowledge can guide efforts to minimize this problem.

Acknowledgements

This work was funded by Grant OH02792 from the National Institute for Occupational Safety and Health, Centers for Disease Control, and Grant CN-71 from the American Cancer Society. Contribution No. 610 from the Barnett Institute.

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