Journal of Chromatography, 196 (1980) 489–493
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 12,892

Note

Syringe and column adsorption of tertiary amines in gas chromatography

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(First received March 7th, 1980; revised manuscript received April 15th, 1980)

A modified solvent flush injection technique has been used previously[1] to minimize adsorption losses in the electron-capture gas chromatography (GC) of tertiary amines. A large excess of desipramine (DMI) was injected simultaneously as an adsorption suppressor without disturbing the quantitation of picogram amounts of the pentafluorobenzyl ethers of pentazocine and ketobemidone.

Further experiments with this method of inactivation show that it has an effect on adsorption losses in the injection syringe as well as on the recovery from the gas chromatograph.

EXPERIMENTAL

Reagents

Mirex® was purchased from Chemical Manufacture Laboratory (Salinas, CA, U.S.A.). The PFB ethers of pentazocine and ketobemidone were synthesized in milligram amounts according to Brötell *et al.*[1]. The test compounds were dissolved in *n*-heptane and diluted to 15 ng/ml of PFB-ketobemidone, 22 ng/ml of Mirex® and 25 ng/ml of PFB-pentazocine.

Desipramine {10,11-dihydro-5-[3-(methylamino)propyl]-5-*H*-dibenz[*b*,*f*]-azepine} was obtained as the hydrochloride from Ciba-Geigy Läkemedel (Västra Frölunda, Sweden), dissolved in water, rendered alkaline and extracted with *n*-heptane. After checking the purity of the solution by GC with flame-ionization detection it was diluted with *n*-heptane to 2.0 mg/ml.

Instrumentation

A Varian 1400 gas chromatograph with an electron-capture detector (^{63}Ni, d.c. mode) was used. The silanized glass column (150 cm × 2 mm I.D.) was filled with 5% OV-17 on Gas-Chrom Q (80–100 mesh). No glass-wool plug was used in the column inlet. After conditioning at 300°C, it was operated isothermally at 250°C with a nitrogen flow-rate of 25 ml/min. The injector port temperature was 250°C and the detector temperature 300°C.

Injections

All injections were made with Hamilton 701-N syringes (5-cm needle) with the

0021-9673/80/0000–0000/$02.25

needle intruding into the column but not into the column packing. Unless otherwise stated, 5.0 μl of the test solution were injected.

Solvent flush injections were carried out using two *n*-heptane plugs (2 μl), usually containing 2.0 mg/ml of DMI base, on both sides of the 5-μl sample plug.

RESULTS AND DISCUSSION

Syringe adsorption

The results obtained after injection of the PFB derivatives of pentazocine and ketobemidone using a syringe equilibrated with the test solution are shown in Table I. Rinsing the syringe with methanol and drying it resulted in nearly complete disappearance of the tertiary amine peaks with the next injection. The time of contact between the syringe and the test solution prior to this injection was about 10 sec. This shows that tertiary amines at low concentrations are rapidly adsorbed to the glass surface of the injection syringe[2]. Silanization of the glass barrel produced only slightly better results, which is in conformity with the findings of Walle and Ehrsson[3].

TABLE I

DIFFERENCE IN PEAK HEIGHTS FOR THE PFB DERIVATIVES AFTER INJECTING 5.0 μl OF A TEST SOLUTION WITH A SATURATED SYRINGE *VS.* A METHANOL-RINSED SYRINGE

The amounts injected were 146 pg of PFB-ketobemidone and 248 pg of PFB-pentazocine. C.V. = Coefficient of variation.

Syringe	*Peak height (mm)*	
	PFB-ketobemidone	*PFB-pentazocine*
Saturated syringe (mean values, $n = 5$)	18 (C.V. = 6.6%)	50 (C.V. = 7.0%)
Methanol-rinsed syringe (1st injection)	4	3

The adsorption losses can be prevented by adding an excess of DMI to the *n*-heptane plugs used in the modified solvent flush injection technique[1]. With a concentration of DMI of >1 mg/ml, constituting a 10^5-fold excess of adsorption suppressor (Fig. 1), the syringe can be rinsed with methanol between injections to avoid cross-contamination of different samples.

Column adsorption

The effect of DMI on adsorption in the gas chromatograph is demonstrated in Fig. 2. Injections were made with a syringe equilibrated with the test solution and without an addition of DMI (Fig. 2A). The result after a previous injection of 10 μg of DMI on to the column performed with a separate syringe is shown in Fig. 2B, and indicates a marked increase in response for the two tertiary amines.

Various other reagents, such as lecithin, nicotine and heptafluorobutyric anhydride, have also been used to pre-condition the column[4–8] in a similar way.

The duration of column treatment with DMI base is shown in Fig. 3 and indicates that DMI has to be added prior to each injection in order to minimize losses.

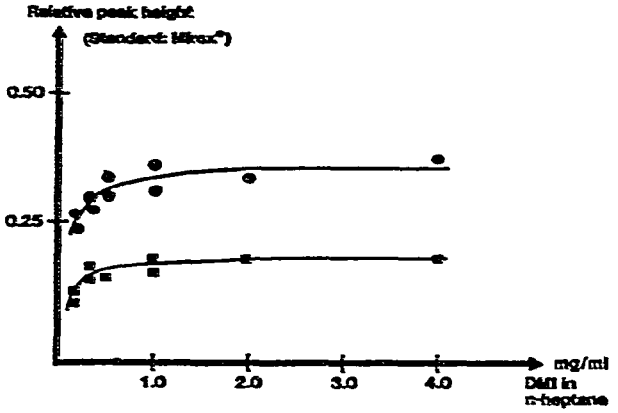

Fig. 1. Influence of DMI concentration on syringe adsorption with the modified solvent flush injection technique. ●, PFB-ketobemidone; ■, PFB-pentazocine.

The column used in this experiment had been in use for 2 months, but with a fresh column the adsorption is less pronounced and the duration of a DMI treatment is longer.

Different reasons for the adsorption of injected substances in a gas chromatograph have been suggested[9–11]. A study by Jansen and Baglan[10] showed that the first part of the column in particular retained injected substances. In the present study "memory peaks" with the same retention times as those of the PFB ethers could be obtained by injecting DMI in *n*-heptane alone, indicating that part of the sample is trapped in the injection port or the first part of the column.

Injection rate

It was found that the GC electron-capture detector responses of the tertiary

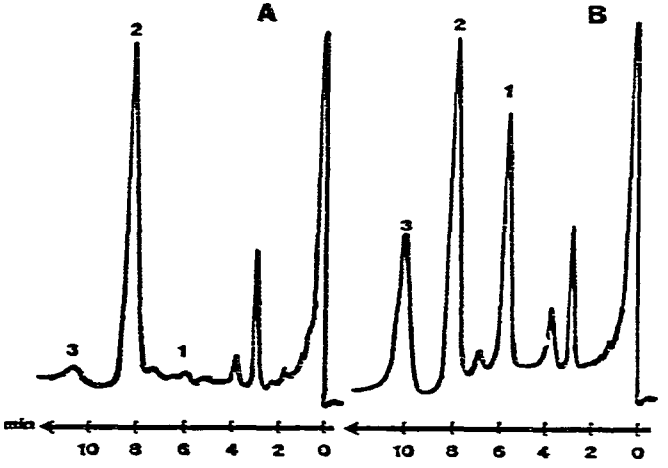

Fig. 2. Effect of DMI on recovery from the gas chromatograph. A, Normal column; B, column treated with 10 μg of DMI from a separate syringe 5 min before injecting sample. (1) PFB-ketobemidone (≈75 pg); (2) Mirex (≈110 pg); (3) PFB-pentazocine (≈125 pg).

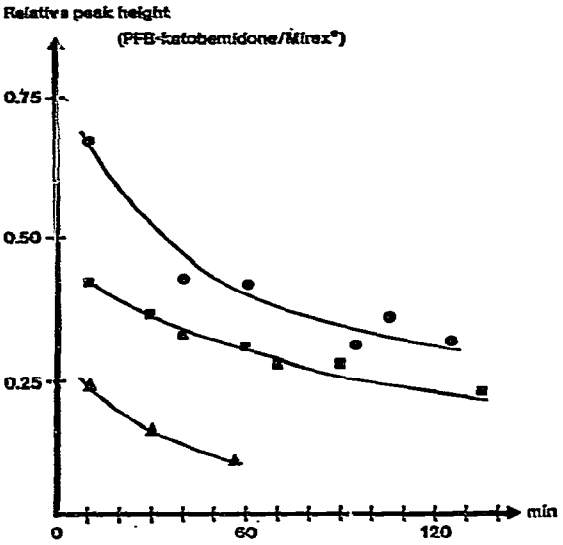

Fig. 3. Duration of column treatment with varying amounts of DMI injected with a separate syringe. Amount of DMI: ●, 10 μg; ■, 4 μg; ▲, 1 μg.

amines were highly dependent on the injection rate, while that of the more inert Mirex was unaffected. This indicates that the effect is related to the chromatographic properties of the substance. The influence of the injection rate on peak height differed slightly for the two amines (Fig. 4, lower curves). For all three components, widening of the peaks was negligible even with an injection time of 10 sec.

However, simultaneous addition of DMI by the injection technique described completely eliminated the influence of the injection rate =Fig. 4, upper curves). It

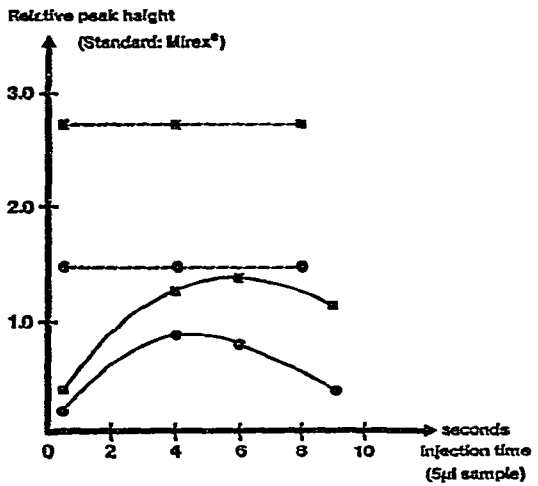

Fig. 4. Influence of injection rate on relative peak heights using two different injection techniques. Broken line, modified solvent flush injection; solid line, conventional injection technique. ■, PFB-ketobemidone; ●, PFB-pentazocine.

should be emphasized that DMI must be injected simultaneously and that a treatment carried out only a few minutes in advance is not effective.

These observations suggest that the low response obtained with rapid, conventional injections are due to adsorption losses caused by "blow-back" of solvent vapour and sample into the metal surfaces of the injection port. In the presence of a large excess of DMI these losses are minimized. The reason for the low responses with very slow conventional injections appears to be that on insertion of the needle into the hot injection port the solvent is boiled away, leaving the tertiary amines adsorbed on the inner surface of the syringe needle unless an excess of adsorption suppressor is present.

It has been observed earlier that the speed of injection can influence peak size. Getzendaner[12] and De Faubert Maunder *et al.*[13] have attributed this to effects within the injector or the first part of the column, while Mendoza[14] suggested that the electron-capture detector may be involved.

The observation by De Faubert Maunder *et al.*[13] that the depth of penetration and angle of the syringe needle are important could not be confirmed, and the use of an injection port extender[15] made no significant difference. Similar results were also obtained with different injector port temperatures ranging from 235 to 290°C.

To rule out possible defects in the instrument as the cause of these effects, other gas chromatographs were also used. Similar results were obtained both with another Varian 1400 and with a Pye Unicam GCV equipped with a pulse-modulated ^{63}Ni electron-capture detector.

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