ELSEVIER

Journal of Chromatography B, 729 (1999) 199–210

JOURNAL OF CHROMATOGRAPHY B

Evaluation of the programmed temperature vaporiser for large-volume injection of biological samples in gas chromatography

M.W.J van Hout, R.A. de Zeeuw, J.P. Franke, G.J. de Jong*

Department of Analytical Chemistry and Toxicology, University Centre for Pharmacy, A. Deusinglaan 1, 9713 AV Groningen, The Netherlands

Received 28 January 1999; received in revised form 1 April 1999; accepted 1 April 1999

Abstract

The use of a programmed temperature vaporiser (PTV) with a packed liner was evaluated for the injection of large volumes (up to 100 μl) of plasma extracts in a gas chromatograph. Solvent purity, which is essential when large volumes are injected into the GC system, was determined. Special attention was paid to the purity of the solvents used for the solid-phase extraction (SPE) procedure. For this SPE method, ethyl acetate was used as the extraction and reconstitution solvent, and thus the purity of the ethyl acetate was critical, especially when a non-selective GC detector was applied. The liquid capacity and inertness of different packed liners were investigated. The liner packed with ATAS ''A'' (modified Chromosorb-based material with special treatment) was found to be the most suitable for the analysis of the tested drugs. Good linearity in response for variations in volume and concentration was observed. A comparison was made between the applicability of flame ionisation detection (FID) and mass-selective detection (MSD). When 50-μl volumes of plasma extracts were injected with the PTV, the detection limits for secobarbital, lidocaine, phenobarbital and diazepam were about 50-times lower than when 1-μl volumes were injected. The detection limits of the tested compounds in plasma for injection of 50–100 μl plasma extract are 5–10 ng/ml for GC–FID whereas plasma concentrations of 250 pg/ml can be detected using the selected ion monitoring (SIM) mode of a MSD. For non-selective GC–FID, the background from a 50-μl injection was substantially larger than with 1-μl injection as a result of co-injected plasma matrix components and solvent impurities. These background effects were less with GC–MSD in the total ion current mode and virtually absent with GC–MSD in the SIM mode.

Keywords: Programmed temperature vaporiser; Diazepam; Lidocaine; Phenobarbital; Secobarbital

1. Introduction

Increasing knowledge of the working mechanism of biologically active substances has led to the development of potent drugs. Hence, lower dosages can be administered to produce a therapeutic effect and, consequently, drug concentrations in biological samples often are much lower than before. For the determination of these lower levels in biological samples, analytical techniques with much higher sensitivity are needed. A way to increase the sensitivity is to increase the amount of sample injected into the analytical system.

In gas chromatography (GC) several techniques are available to perform large volume injections (LVIs) [1]. On-column injection with the use of

*Corresponding author. Tel.: +31-50-3633-337; fax: +31-50-3637-582.
E-mail address: g.j.de.jong@farm.rug.nl (G.J. de Jong)

0378-4347/99/$ – see front matter
PII: S0378-4347(99)00159-0

so-called retention gaps is currently the most common technique [1]. A second possibility for LVI is the loop-type interface [2], originally designed for the coupling of liquid chromatography (LC) and GC. The main advantage of these techniques is that the complete sample is introduced into the GC column. However, this may also become a disadvantage since all impurities are introduced into the GC system as well. A third option to allow LVI in GC is to use a programmed temperature vaporiser (PTV). Despite good results obtained by Vogt and co-workers [3,4] in the late seventies, only recently has PTV injection been applied as a routine technique for environmental analysis [1].

Besides conventional split/splitless injection, the PTV can be used for several modes of LVI. The coupling of LC and GC using the PTV was reviewed by Grob [5], and recently interesting publications appeared on the same subject [6,7]. The PTV is often applied for this purpose because the packed liner generally has a larger liquid storage capacity than a retention gap. In addition, wettability is not very critical for the liquid retention and packing materials are more water-resistant than retention gaps with a silica backbone. The packing is more easily and rapidly heated than a retention gap [5]. Main reasons to couple LC with GC are that LC provides better resolution than more conventional techniques of sample preparation, and secondly, the possibility of automation through on-line coupling, which reduces or eliminates manual sample preparation work and, therefore, reduces analysis time and improves accuracy and precision [5,7]. The use of a PTV as the interface between LC and GC has been demonstrated for the analysis of olive oil and for environmental analysis [6,7]. The PTV is also used for thermal desorption–pyrolysis of solid geochemical samples (characterisation of oil and kerogens in source rocks) [8], and for on-line solid-phase extraction-thermal desorption (SPE-TD) of methyl esters of the C_{10}–C_{26} carboxylic acids, pesticides, chlorobenzenes and chlorophenols in aqueous samples [9–11].

Most applications of LVI are in the analysis of environmental aqueous samples [1,9–13]. Pesticides were determined in aqueous samples after SPE of samples of 200 ml with concentrations between 0.2 and 5 ng/l by Steen et al. [12], whereas Teske et al. [13] determined triazines like atrazine, propazine, ametryne and simazine in water after in-vial liquid–liquid extraction and direct injection of the extracts with detection limits as low as 0.01 μg/l, and polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) at the ppt-level [13]. Another application of the PTV is the residue analysis of 385 pesticides down to the 0.01 ppm concentration level in plant foodstuff [14].

The purpose of the present work is to investigate the possibilities of the PTV coupled to GC for the analysis of plasma extracts to provide lower detection limits for drugs. Special attention was paid to the impact of solvent impurities in view of the larger solvent volumes injected, to the liquid capacity and inertness of the PTV liners, and to the degree of selectivity provided by flame ionisation detection (FID) and mass-selective detection (MSD).

2. Experimental

2.1. Instrumentation

Gas chromatographic analyses were performed with a Hewlett-Packard HP 5890 series II with FID or a GC–MSD system (HP 5971 series). A HP-5 30 m×0.32 mm capillary column with 0.25 μm film thickness was used for the analyses with FID, whereas analyses with MSD were performed using a HP-5 MS 30 m×0.25 mm column with 0.25 μm film thickness. The PTV injection system was an OPTIC 2 (ATAS International, Veldhoven, The Netherlands), which was equipped with 80 mm×3.4 mm I.D. liners obtained from ATAS International. The liners were packed with either ATAS ''A'' packing (a modified Chromosorb-based material with special treatment, ATAS International), silanised glass wool (research grade, Serva, Feinbiochemica, Heidelberg, Germany), or disposable capillaries for thin-layer chromatography (TLC) (nine capillaries of 10 μl and two of 2 μl, cut at a length of 2 cm).

Plasma extractions were performed using Bond Elut Certify cartridges (Varian, Harbor City, CA, USA), column type LRC of 10 ml with 130 mg sorbent. A Visiprep system (Supelco, Bellefonte, PA, USA) was used to apply vacuum during the extraction.

2.2. Chemicals

Acetonitrile and methanol (Lab Scan, Dublin, Ireland) were of HPLC quality. Acetone, hexane, acetic acid glacial 100% (v/v), ammonia solution 25%, and KH_2PO_4 were all of analytical-reagent grade quality (Merck, Darmstadt, Germany). Ethyl acetate (Reinst and Suprasolv – for organic residue analysis) was obtained from Merck (Darmstadt, Germany). Ethyl acetate Ultra resi-analysed (for organic residue analysis) was purchased from Mallinckrodt Baker (Deventer, The Netherlands). Water used during SPE was ultra pure (Elgastat maxima, Salm en Kipp, Breukelen, The Netherlands). Secobarbital, phenobarbital (both BP quality, Siegfried, Zofingen, Switzerland), lidocaine (Eur. Ph., Holland Pharmaceutical Supply, Alphen $^{A}/_{D}$ Rijn, The Netherlands), and diazepam (Centrafarm, Etten-Leur, The Netherlands) were used as test compounds (Fig. 1) and dissolved in ethyl acetate (for organic residue analysis, Mallinckrodt Baker). Stock solutions of 1 mg/ml were stored in the dark at 4°C. Stock solutions were mixed and then diluted with ethyl acetate (for organic residue analysis, Mallinckrodt Baker). The compounds of the reference RI-mixture [15] were dissolved in ethyl acetate–methanol (1:1) (1 mg/ml).

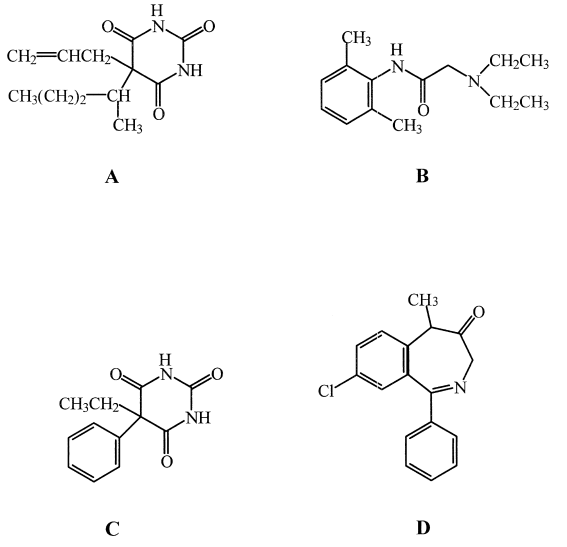

Fig. 1. Structures of the test compounds: (A) secobarbital, (B) lidocaine, (C) phenobarbital, (D) diazepam.

2.3. Methods

The carrier gas for GC–FID and GC–MSD was helium. The same temperature program was used for both methods. The starting temperature was 40°C, and after 3 min the temperature was raised at 20°C/min to 215°C, followed by an increase at 5°C/min to 230°C and a final increase at 25°C/min to 290°C. This final temperature was maintained for 5–10 min. The detector temperature was 300°C. A column flow of 1.35 ml/min was used during analysis with GC–FID and 0.48 ml/min with GC–MSD. The injector was set at 40°C and 10 s after the evaporation of the solvent (delay time) the temperature was raised with 5°C/s to 290°C. The end time was set at a time equal to the total run time of one analysis. Other PTV settings are presented in Table 1.

During analysis performed with GC–MSD in the total ion current (TIC) mode an m/z range of 50–300 was monitored. Using the selected ion monitoring (SIM) mode, the monitored m/z values were 86.0, 167.0, 204.0 and 256.0, which corresponded to the most intense fragment of lidocaine, secobarbital, phenobarbital and diazepam, respectively.

SPE was performed as described previously [16] with some minor modifications. The SPE column was activated with 2 ml methanol (2 ml/min), followed by conditioning of the SPE column with 2 ml of 0.1 *M* K_2HPO_4 buffer, pH 6 (2 ml/min). Subsequently, 1 ml plasma, diluted with 4 ml K_2HPO_4 buffer, was brought on the column during approximately 1 min. Then the SPE column was washed with 1 ml water and 0.5 ml of 1 *M* acetic acid (1.5 ml/min). The column was dried under

Table 1
PTV settings (1 p.s.i.=6894.76 Pa)

	GC–FID	GC–MSD
Vent flow (ml/min)	150	150
Split flow (ml/min)	57.4	57.4
Purge flow (ml/min)	2.32	2.32
Purge press (p.s.i.)	8.0	4.0
Transfer press (p.s.i.)	14.0	4.0
Transfer time (min:s)	2:45	2:45
Initial press (p.s.i.)	8.0	2.0
Final press (p.s.i.)	8.0	2.0
Vent mode	Auto	Auto
Split open time (min:s)	2:30	2:30
Threshold	20	20

vacuum for 4 min, after which 50 μl of methanol were passed through to remove remaining traces of water. The column was dried under vacuum for 1 min. The tips of the Visiprep system were dried and tubes were inserted for the collection of the eluate. The acidic fraction was eluted with 1 ml ethyl acetate–acetone (1:1) (0.8 ml/min), followed by the elution of the alkaline fraction with 0.5 ml acetonitrile–ammonia (98:2) (0.5 ml/min). The fractions were evaporated until almost dry and reconstituted in 100 μl ethyl acetate (for organic residue analysis, Mallinckrodt Baker). Finally, 50–100 μl of these extracts were injected into the GC system.

3. Results and discussion

The basic set-up of a PTV injector strongly resembles a conventional split/splitless injector. The main difference is that a (packed) liner system is applied which is temperature-controlled. Injections up to about 150 μl can occur at once, whereas larger volumes must be injected at a controlled rate. In the injection mode the temperature of the liner is set at 30–40°C below the boiling point of the used solvent. A high vent flow ensures selective evaporation of solvent via the split line, whereas less volatile solutes are retained in the liner. After evaporation of almost all the solvent, rapid transfer of the latter components to the column is performed, using the splitless mode, by rapidly heating the liner, or, optionally, by a high transfer pressure. During the transfer of the components, the GC column is maintained at a low temperature (same starting temperature as the injector), thus leading to a refocusing of components at the front of the column. Further analysis is performed with normal temperature-programmed GC.

3.1. Purity of solvents and chemicals

Since much larger solvent volumes are injected into the PTV–GC system, the impact of solvent impurities was checked. In the plasma extraction procedure, ethyl acetate is being used as extraction and reconstitution solvent [16]. Various qualities of ethyl acetate were tested, and some analytical results are presented in Fig. 2. Fig. 2A shows that a brand

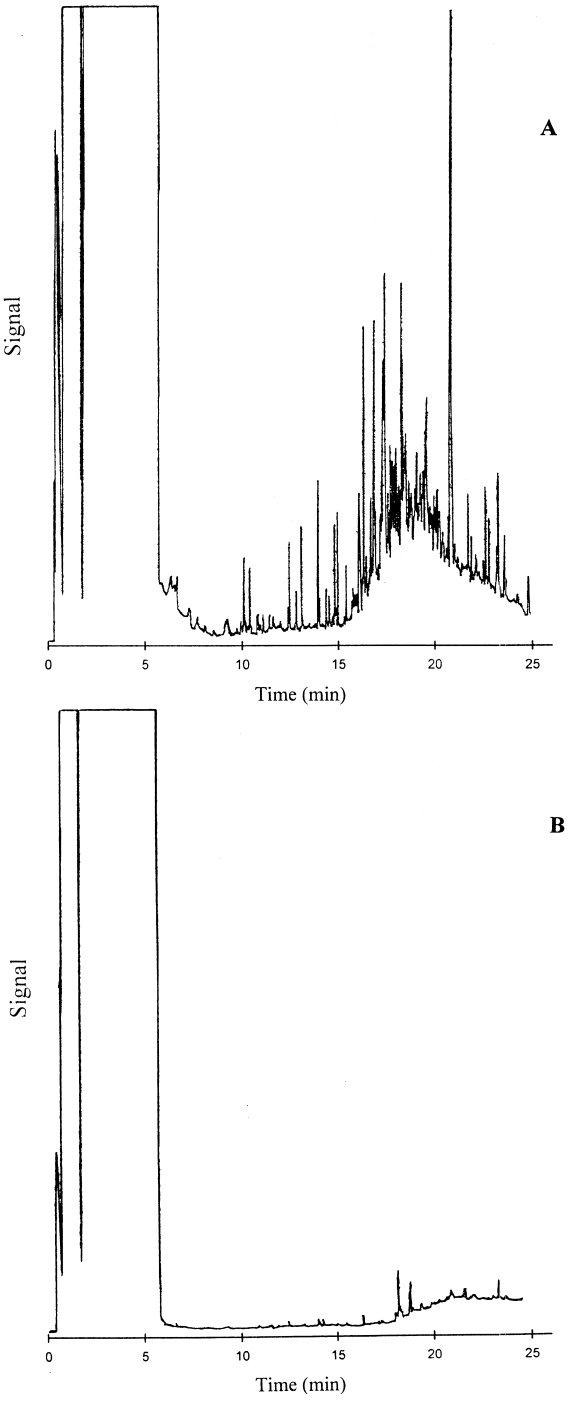

Fig. 2. GC–FID chromatogram of injection of 100 μl ethyl acetate: (A) Reinst (Merck), (B) for organic residue analysis (Baker).

which was found acceptable for 1-μl injections contained far too many impurities when 100 μl was injected into the PTV–GC system. The best results were obtained when using 100-μl injections with the quality ''for organic residue analysis'' (Mallinckrodt Baker) as shown in Fig. 2B. Therefore, this latter quality was used in further experiments. The purity of methanol, acetonitrile, ammonia and KH_2PO_4 were found to be acceptable in that the quantities used in the present procedure did not introduce major impurities. Attempts to purify ethyl acetate by a C_{18}-LC column or a C_{18} cartridge were not successful.

3.2. Liquid capacity of the liners

A liner must have a relatively large liquid capacity (V_{max}) to allow injection of 50–100 μl sample at once (no speed-controlled injection). The liquid capacity of a liner can be easily determined by removing the column from the injector without turning off the carrier gas. Then, 200 μl solvent is injected rapidly and the injector outlet is checked for solvent droplets. The amount of solvent injected is reduced until no droplets are observed which reflects V_{max}. In Table 2 the measured V_{max} for ethyl acetate is given for several liners. All packings were of similar dimensions (height 2.5 cm, 1.8 cm from the column side).

The glass wool and the ATAS ''A'' liner appeared to be best suitable for the injection of large volumes ethyl acetate (>50 μl), and the ATAS ''A'' liner can even be used for samples larger than 100 μl. Mol et al. [17] found a V_{max} of 115 μl for a liner packed with glass wool instead of 65 μl. This difference is probably due to the major problem with glass wool, that is, inserting the packing into the liner in a reproducible way [17,18].

Table 2
Liquid capacity of liners

Liner packing	V_{max} (μl)
ATAS-1[a]	150
Glass wool[b]	65
Glass capillaries[c]	40

[a] Total amount of 80–85 mg ATAS ''A'' packing in fritted liner.

[b] Total amount of 144 mg glass wool was inserted into a open liner.

[c] Nine TLC capillaries of 10 μl and two TLC capillaries of 2 μl were cut at a length of 2 cm, and inserted into a fritted liner. A small plug of glass wool was placed under and above the capillaries.

3.3. Inertness of the liner packings

3.3.1. Comparison of packings

It was necessary to investigate the inertness of liner packing materials for biological samples since a high recovery of the analytes must be obtained during consecutive sample injections. Several ATAS ''A'' liners (Nos. 1–5), a glass wool packed liner (No. 6), and a liner packed with open capillaries (No. 7) were tested. Cutting glass capillaries can create active places at the cutting site, and glass wool is known to have a limited inertness [17]. The ATAS ''A'' liner was originally designed for the analysis of pesticides and mineral oils. The inertness of the ATAS ''A'' liner can be influenced by a high temperature since the packing has a T_{max} of 325°C. Higher temperatures will degrade the packing, which will have a negative effect on the inertness of the liner. Analysis of high boiling compounds (>325°C) is therefore not possible.

Prior to testing the inertness, liners 1 and 2 were used for many injections of standard solution and plasma extracts. The colour of liner 1 had changed from white to completely brown, and the upper half of the packing of liner 2 had changed to brown with the lower half still white. Liners 3–7 were not used before. Injection of 1 μl of 250 μg/ml test compounds (secobarbital, lidocaine, phenobarbital and diazepam) into a liner with a glass frit without packing produced the reference chromatogram, that is, since no active packing was present, the response of the compounds was set at 100%. Injections of 1 μl of 250 μg/ml or 100 μl 2.5 μg/ml test compounds into liners 3, 4 and 5 produced the same responses, thus the ATAS ''A'' liner can be considered to be inert if the liner packing is not previously used for analysis.

In Table 3 the responses are tabulated for liners 1, 2, 6 and 7, as compared to liners 3, 4 and 5. Liners 1, 2, 6 and 7 showed adsorption activity for phenobarbital, liners 1 and 6 being the most active. Injection of 1 μg phenobarbital into liner 1 produced even no peak. For secobarbital, liner 1 is very active whereas

Table 3
Recovery (%) of 250 ng of compounds compared with average response of liner 3, 4 and 5 (set at 100%)

Liner[a]	Recovery (%)			
	Secobarbital	Lidocaine	Phenobarbital	Diazepam
1	3	86	0	87
2	96	92	59	96
3, 4, 5	100	100	100	100
6	81	99	17	100
7	88	97	49	100

[a] Liners: 1–5=ATAS ''A'' (No. 1 completely brown, No. 2 upper half brown, Nos. 3–5 white), 6=silanised glass wool, 7=glass capillaries.

liners 2, 6 and 7 are much less active. For lidocaine, liners 1 and 2 showed limited activity and the same was observed for diazepam. From these results, it appears that adsorption losses are more pronounced in the order diazepam/lidocaine<secobarbital< phenobarbital. The change in colour seems a good parameter to indicate adsorption activity of the ATAS ''A'' liner for barbiturates whereas it has hardly any effect on the response of lidocaine and diazepam.

Glass wool packed liners were shown to adsorb fatty acids from 42 to 100% [17]. In this work glass wool also showed adsorption activity for the weakly acidic compounds secobarbital and phenobarbital, but the material appears to be better suitable for weakly basic drugs. Thus, a glass wool liner can be used for some of the drugs investigated in this work whereas the glass wool liner used by Mol et al. [17] was unsuitable for the analysis of all tested compounds. Biedermann et al. [19] pointed out that the deactivation of vaporising chambers can also play an important role. Deactivating of the glass surface was performed before the packing material was inserted into the liner.

In order to investigate if there are other classes of drugs for which the ATAS ''A'' packing is not inert, a reference mixture [15] was analysed using liners 1, 2 and 5. The results are presented in Fig. 3. The percentages given below are recoveries with the response of the compound during analysis with liner 5 set at 100%. No effect on the response was found for amphetamine, ephedrine, diphenhydramine, tripelenamine and trimipramine. Liner 1 showed a small decrease in recovery for benzocaine (90%), methaqualone (96%), nordazepam (95%), prazepam (85%) and haloperidol (80%). Liner 2 was not active for these substances. The same was observed with liner 2 for strychnine, but liner 1 was active for this compound (46%). The effect of liner activity for methylphenidate (liner 2 and 1, 82 and 5%, respectively), codeine (90 and 28%) and papaverine (38 and 5%) was comparable with that for phenobarbital. Thus, the ATAS ''A'' liner can become less inert to substances due to injection of plasma extracts and/or standard solution. The results confirm the suggestion that a beginning brown colouration can be used as an indication that the liner is starting to loose its inertness and that it needs replacement. From the results with both the test compounds as well as the compounds of the RI-mixture, it can be concluded that barbiturates and opium alkaloids are particularly prone to recovery losses. On the other hand, even a completely brown ATAS ''A'' liner has little effects on the recoveries of benzodiazepines.

3.3.2. Long-term use

The ATAS ''A'' liner and the glass wool liner have a sufficient liquid capacity to allow injections up to 50 μl. However, the glass wool liner appeared to be less inert for some type of compounds. Therefore, only the inertness of the ATAS ''A'' liners as a function of the number of injections was also investigated. Two new ATAS ''A'' liners were used. For one liner only a standard solution (50 μl of 2.5 μg/ml) was injected 35 times (Fig. 4). The liner appeared to remain inert under these conditions since no loss in response was observed for all test compounds. For the second liner, 50 μl of 2.5 μg/ml standard solution with two injections of plasma extract (one alkaline and one acidic fraction) between subsequent injections of the standard solution were analysed. The response of the test compounds (seco-

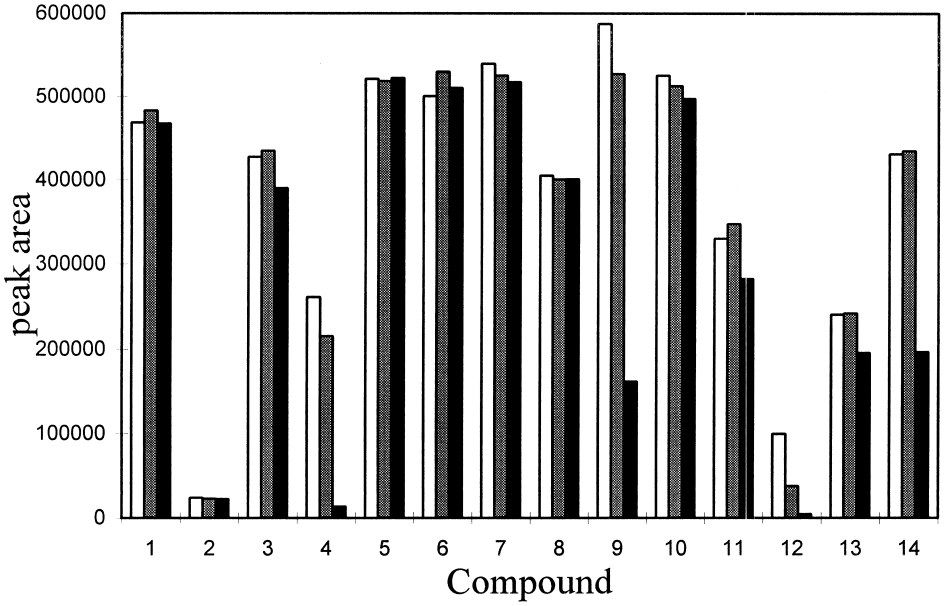

Fig. 3. Effect of packing inertness on response of compounds of RI-mix [15]. ■=Liner 1, ▒=liner 2, □=liner 5. Compounds: 1=amphetamine, 2=ephedrine, 3=benzocaine, 4=methylphenidate, 5=diphenhydramine, 6=tripelenamine, 7=methaqualone, 8=trimipramine, 9=codeine, 10=nordazepam, 11=prazepam, 12=papaverine, 13=haloperidol, 14=strychnine.

barbital, phenobarbital, lidocaine and diazepam) in the standard solution is plotted as a function of the number of plasma injections (Fig. 4).

Since up to 35 injections of standard solution had no influence on the stability of the liner packing, a decrease in response must have been caused by the plasma extracts influencing the inertness of the packing material. Injection of plasma extracts introduces a large decrease in inertness of the packing for phenobarbital starting with a slightly variable response at 14 injections of plasma extracts, and a definite loss in response after 20 injections. For secobarbital, after 20 injections of plasma extracts only a small increase in activity of the ATAS ''A'' packing is observed. Injection of 32 plasma extracts has no effect on the response of diazepam and lidocaine (lidocaine not shown). An increase in activity of the packing was again found to correlate with the colour of the packing material. Inert ATAS ''A'' material is white but this becomes brown with increasing activity. After some 10–15 injections of plasma extracts the colour of the ATAS ''A'' packing started to change from white to brown, and the brown colour became more apparent on continued analysis of plasma extracts. The decrease in inertness and change in colour of the ATAS ''A'' packing when plasma extracts are injected is probably due to the degradation of matrix components that are not desorbed from the liner on heating.

3.3.3. Carry-over

Carry-over was checked by injecting large amounts of the test compounds or RI-mixture (up to 2.5 μg), followed by a second injection in which a blank, i.e., 100 μl ethyl acetate, was introduced. No carry-over was observed for either the test compounds or the components of the RI-mixture when using a ATAS ''A'' liner.

3.4. Linearity with PTV–GC–FID for standard solutions

Since the ATAS ''A'' liner has a relatively large liquid capacity and is inert for the tested compounds, and no carry-over occurs, the ATAS ''A'' liner is suitable for LVI of bioanalysis. Linearity in response for lidocaine, diazepam, secobarbital and phenobarbital was determined for variation in volume and

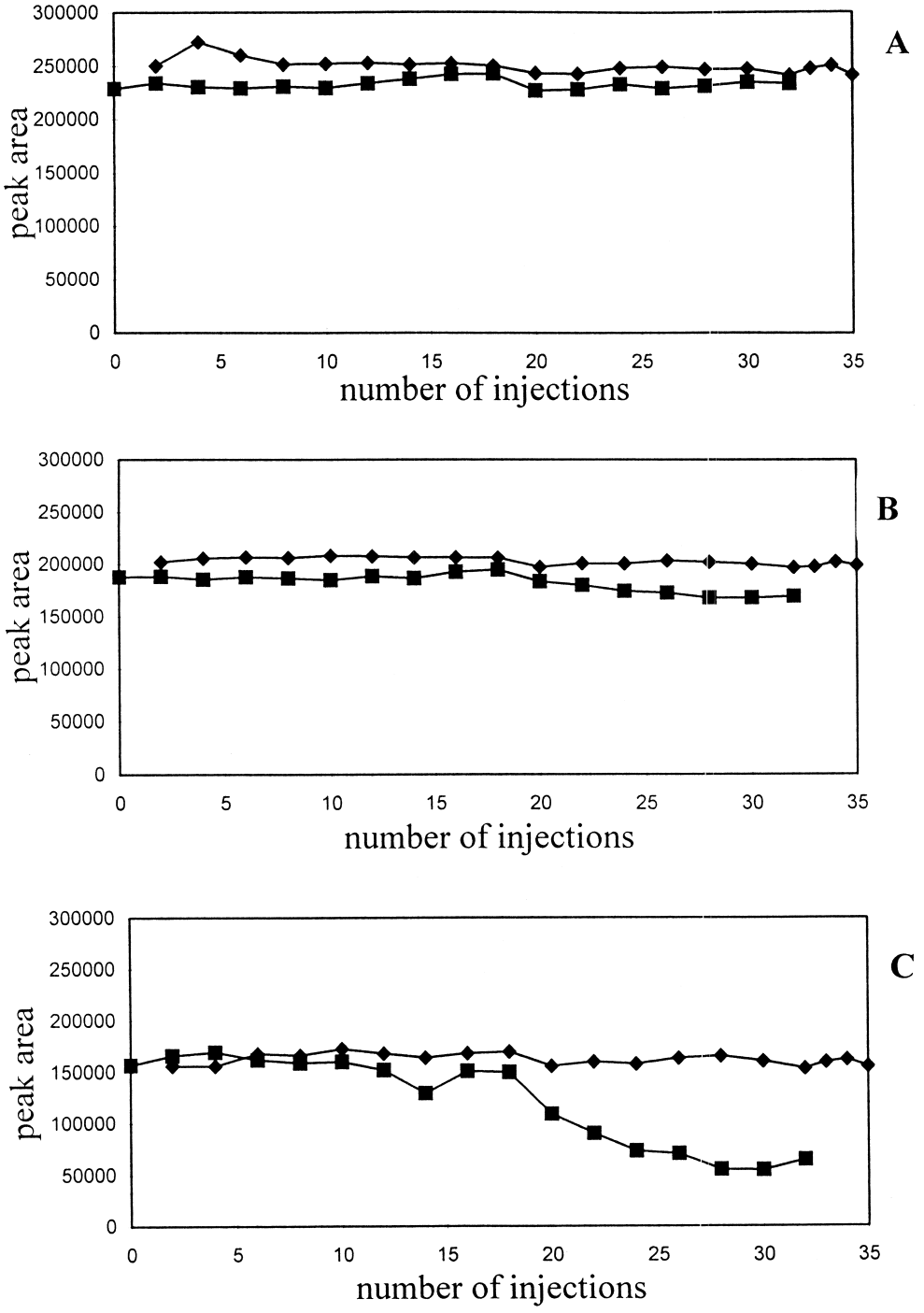

Fig. 4. Stability of ATAS-1 packing. Response of 125 ng test compound in 50 μl standard solution of (A) diazepam, (B) secobarbital, (C) phenobarbital; ♦=response for injections of only the standard solution, ■=response for injections of the standard solution with two plasma extract injections between subsequent injections of the standard solution (numbers of injection correspond with the amount of plasma extract injections).

Table 4
Linearity [coefficient of correlation (*R*)] of secobarbital, lidocaine, phenobarbital and diazepam with variation of volume (20–100 μl of 1 μg/ml) and concentration (5–2000 ng/ml)

	R (volume)	*R* (concentration)
Secobarbital	0.9989	0.9974
Lidocaine	0.9937	0.9965
Phenobarbital	0.9979	0.9923
Diazepam	0.9976	0.9982

concentration. For the determination of the linearity in response when the volume is varied, volumes varying from 20 to 100 μl of a 1.0 μg/ml standard solution were injected on a new ATAS ''A'' liner. It was found that all compounds showed a good linearity (Table 4).

The linearity of response versus concentration was determined by injections of 100 μl standard solution over a concentration range of 5 to 2000 ng/ml. As can be observed in Table 4, a good linearity was obtained. One should note that no internal standard was applied to correct for injection volume and signal drift.

3.5. PTV–GC–FID and PTV–GC–MSD of plasma extracts

Linearity as well as the detection limits for lidocaine, diazepam, secobarbital and phenobarbital in plasma extracts were determined using FID and MSD. With LVI a large amount of matrix components and solvent impurities is injected. Therefore peak identification can become difficult when non-selective detectors are used. Use of a mass-selective detector may help to overcome this problem. With the mass-selective detector analyses were performed in both the TIC and the SIM mode.

Linearity was determined for concentrations ranging from the detection limit up to 100 ng using 1 ml of spiked plasma extract. The results are listed in Table 5. For all detectors linearity was found to be better for the alkaline fraction than the acidic fraction, except for diazepam. For this compound, coefficients of correlation are comparable for both fractions. The acidic fraction analysed with MSD shows a relatively low linearity for phenobarbital. This might be due to an increase in activity of the GC system during analysis (see Section 3.3).

The detection limits were determined at a signal-to-noise ratio of 3 for both FID and MSD. It should, however, be mentioned that when using FID and MSD in the TIC mode, interfering matrix compounds and solvent impurities can make the determination of the detection limit laborious as blank peaks have a negative influence. Blank plasma was extracted and the extracts were spiked with the test compounds and detection limits for plasma extracts were calculated assuming a 100% recovery of the test substances in the SPE procedure. The actual recoveries using this SPE method were found to be 80–100% [16].

Chromatograms of 40–45 ng compounds in the alkaline fraction analysed with FID, TIC and SIM are presented in Fig. 5. Using FID, the detection limit using 1 ml of plasma is 5–10 ng for all compounds in both the acidic and alkaline fraction. The detection limits observed using the TIC mode of the MSD are 4–5 ng. Therefore, a small gain in detection limit can be achieved. This is mainly due to the fact that with TIC a positive identification can be given for the peaks present in a sample since reliable mass spectra are obtained with good correspondence with library spectra. Acidic plasma extracts analysed in the SIM mode give a detection

Table 5
Linearity (coefficient of correlation) of secobarbital, lidocaine, phenobarbital and diazepam in acidic and alkaline SPE fraction of plasma; range: detection limit (see Section 3.4) to 100 ng/ml

	R					
	FID/acidic	FID/alkaline	TIC/acidic	TIC/alkaline	SIM/acidic	SIM/alkaline
Secobarbital	0.9963	0.9981	0.9940	0.9976	0.9997	0.9998
Lidocaine	0.9936	0.9992	0.9986	0.9962	0.9991	0.9997
Phenobarbital	0.9947	0.9996	0.9993	0.9958	0.9719	0.9992
Diazepam	0.9975	0.9962	0.9969	0.9953	0.9991	0.9994

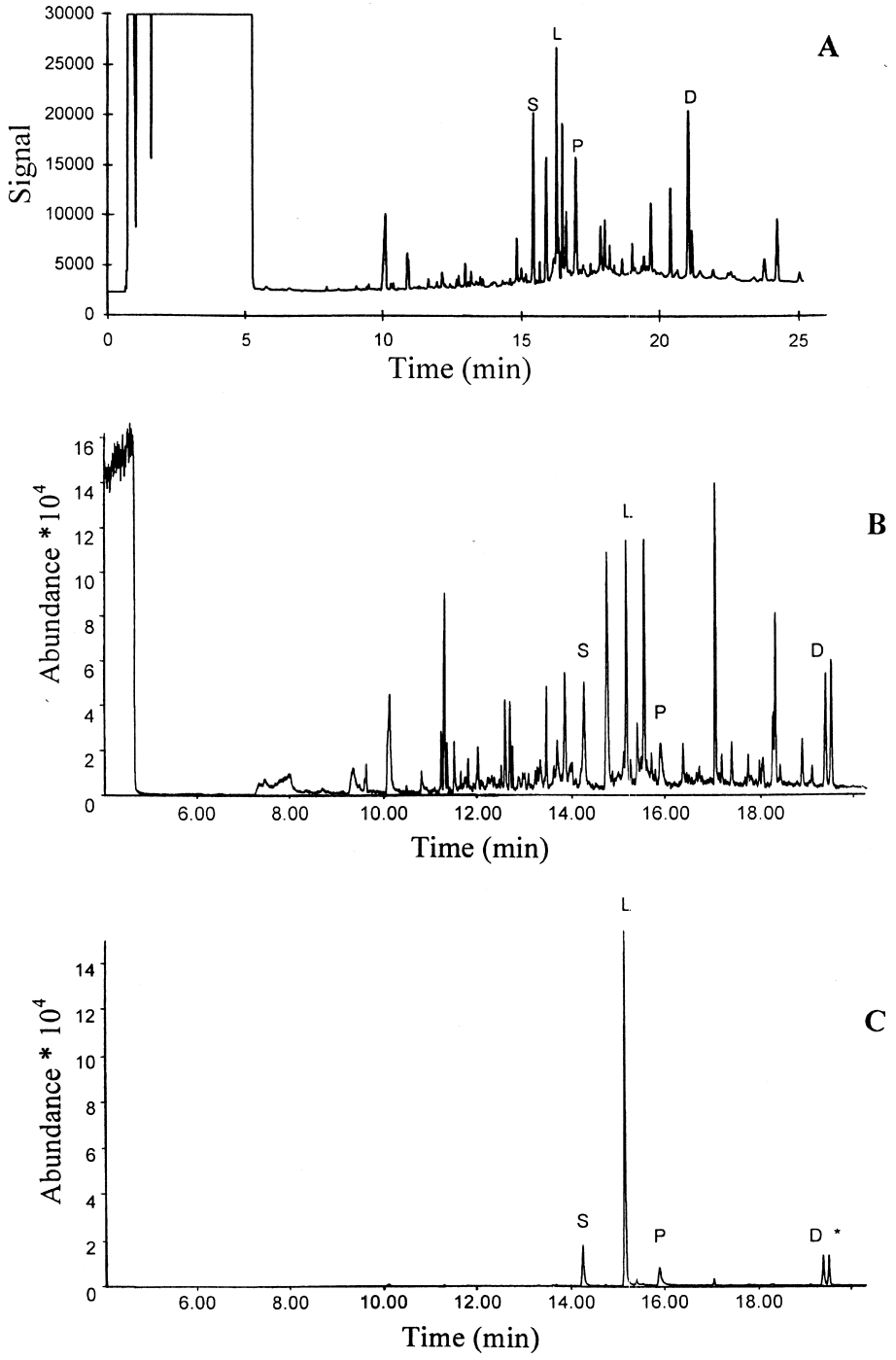

Fig. 5. Chromatograms of 50 μl of the alkaline fraction of plasma extracts analysed with (A) GC–FID (41.7 ng), (B) GC–MSD; TIC mode (45.5 ng), (C) GC–MSD; SIM mode (45.5 ng). The monitored m/z values were 86.0, 167.0, 204.0 and 256.0 for lidocaine (L), secobarbital (S), phenobarbital (P) and diazepam (D), respectively. Note: the peak (*) eluting just after the diazepam peak (D) is caused by an impurity with a m/z value similar to phenobarbital.

limit of 0.5 ng for each test compound whereas the alkaline fraction gives a detection limit of 0.25 ng. The gain in sensitivity when compared with FID or TIC can be explained by the enhanced selectivity since detection occurs only at four m/z values. If the complete sample is injected, plasma concentrations as low as 250 pg/ml could be detected in the alkaline fraction. With conventional GC, that is injection of 1 μl plasma extract, also a detection limit of 0.25 ng for the test compounds is found. As a consequence, the corresponding plasma concentration is 50-times higher than with injection of 50 μl, i.e., a concentration of 12.5 ng/ml can be detected.

4. Conclusions

It was demonstrated that the PTV is potentially suitable to inject large volumes of extracts of biological samples in GC. In this way detection limits can be improved considerably. In order to be able to apply LVI in GC it is necessary to select an appropriate liner packing. As for the liner packings tested in this work the ATAS ''A'' packing was found to be the most suitable since this material has a large liquid capacity and is relatively inert. However, the packing can become active after a number of injections of plasma extracts for certain types of compounds. It is therefore recommended to carefully monitor the inertness of the packing material by injection of a few suitable compounds and to monitor the colour of the liner packing.

Injection of large volumes implies that an equivalent amount of impurities is injected. Therefore, it is essential to use very pure solvents and chemicals during the work up-procedure. However, not only solvent impurities are injected. Also matrix components that are co-extracted with the analytes make identification and quantitation difficult when a non-selective detector is used. The use of a selective detector is essential to overcome this problem. Using a mass-selective detector, a 100-fold gain in concentration sensitivity can be achieved if 100 μl of a plasma extract instead of 1 μl is injected.

The present system can be used as a routine technique in research and clinical laboratories. However, further evaluation of the system for various purposes (including other matrices) and different types of compounds is needed. The on-line coupling of SPE and GC for bioanalysis will also be investigated in the near future in our laboratory.

Acknowledgements

Jan Henk Marsman and Ronald Veenhuis (Department of Chemical Engineering, University of Groningen) are gratefully acknowledged for the use of the GC–MSD system and their assistance. This research was supported by the Technology Foundation STW, applied science division of NWO and the technology programme of the Ministry of Economic Affairs.

References

[1] H.G.J. Mol, H.-G.M. Janssen, C.A. Cramers, J.J. Vreuls, U.A.Th. Brinkman, J. Chromatogr. A 703 (1995) 277.
[2] K. Grob, J.-M. Stoll, J. High Resolut. Chromatogr. Commun. 9 (1986) 518.
[3] W. Vogt, K. Jacob, H.W. Obwexer, J. Chromatogr. 174 (1979) 437.
[4] W. Vogt, K. Jacob, A.-B. Ohnesorge, H.W. Obwexer, J. Chromatogr. 186 (1979) 197.
[5] K. Grob, J. Chromatogr. A 703 (1995) 265.
[6] P. Van Zoonen, G.R. van der Hoff, LC·GC Int. 16 (1998) 240.
[7] T. Hyötyläinen, M.-L. Riekkola, J. Chromatogr. A 819 (1998) 13.
[8] M.P.M. van Lieshout, H.-G. Janssen, C.A. Cramers, G.A. van den Bos, J. Chromatogr. A 764 (1997) 73.
[9] J.J. Vreuls, U.A.Th. Brinkman, G.J. de Jong, K. Grob, A. Artho, J. High Resolut. Chromatogr. 14 (1991) 455.
[10] J.J. Vreuls, G.J. de Jong, R.T. Ghijsen, U.A.Th. Brinkman, J. Microcol. Sep. 5 (1993) 317.
[11] A.J.H. Louter, J. Van Doornmalen, J.J. Vreuls, U.A.Th. Brinkman, J. High Resolut. Chromatogr. 19 (1996) 679.
[12] R.J.C.A. Steen, I.L. Freriks, W.P. Cofino, U.A.Th. Brinkman, Anal. Chim. Acta 353 (1997) 153.
[13] J. Teske, J. Efer, W. Engewald, Chromatographia 47 (1998) 35.
[14] H.J. Stan, M. Linkerhagner, J. Chromatogr. A 750 (1996) 369.
[15] R.A. de Zeeuw, J.P. Franke, H.H. Maurer, K. Pfleger, Gas Chromatographic Retention Indices of Toxicologically Relevant Substances on Packed and Capillary Columns with Dimethylsilicone Stationary Phases, 3rd ed, VCH, Weinheim, 1992.

[16] X.-H. Chen, J. Wijsbeek, J.P. Franke, R.A. deZeeuw, J. Forensic Sci. 37 (1992) 61.
[17] H.G.J. Mol, P.J.M. Hendriks, H.-G. Janssen, C.A. Cramers, U.A.Th. Brinkman, J. High Resolut. Chromatogr. 18 (1995) 124.
[18] H.G.J. Mol, H.-G. Janssen, C.A. Cramers, U.A.Th. Brinkman, J. High Resolut. Chromatogr. 18 (1995) 19.
[19] M. Biedermann, K. Grob, M. Wiedmer, J. Chromatogr. A 764 (1997) 65.