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

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## 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 50-100µl 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 so-called retention

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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 inj ection 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 AD or a GC-MSD system (HP 5971 series). A HP-5 30 m x 0.32 mm capillary column with 0.25 µm film thickness was used for the analyses with AD, whereas analyses with MSD were performed using a HP-5 MS 30 m x 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 x 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).

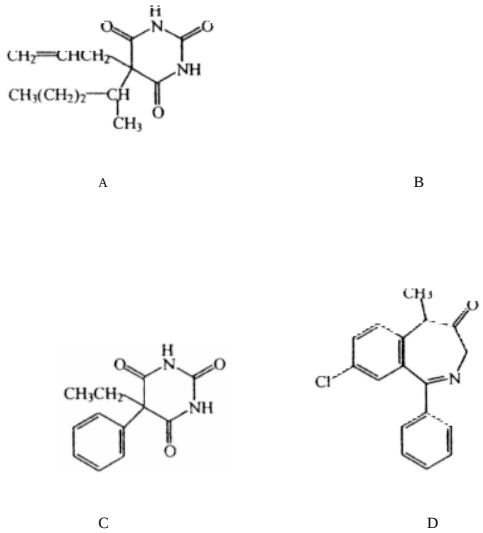


Fig. 1. Structures of the test compounds. (A) secobarbital, ( B ) lidocaine. (C) phenobarbital, (D) diazepam.

### 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 10s 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 vacuum for 4 min,

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 |