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Feasibility of the direct coupling of solid-phase extraction–pipette tips with a programmed-temperature vaporiser for gas chromatographic analysis of drugs in plasma

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Abstract

Solid-phase extraction–pipette tips (SPE–PTs) were used for micro solid-phase extraction of lidocaine and diazepam from plasma. Off-line extraction was followed by on-line desorption. On-line desorption was carried out by direct coupling of the SPE–PTs with the liner of the programmed-temperature vaporiser. This coupling only required shortening of the liner by maximally 16 mm, cutting the SPE–PT, and equipping the remaining part with two O-rings. Due to the heating of the injector the SPE–PTs were heated as well, which resulted in a significant amount of impurities. Pre-heating and pre-washing was performed prior to the extraction to reduce the impurity level. The internal coupling device was applied successfully for the analysis of plasma samples with gas chromatography (GC) and mass-selective detection. Detection limits of 0.75 ng/ml and 2.5 ng/ml were obtained for lidocaine and diazepam, respectively, using 200 μl plasma. Recoveries for both compounds were about 80%. Although it is possible, the internal coupling device was not developed to be used as such. The main goal of this coupling was to show the feasibility of the integration of SPE–PTs with GC and to realize an important step to new automated SPE–GC systems.

Keywords: Programmed-temperature vaporiser; Solid-phase extraction–pipette tips coupling; Lidocaine; Diazepam

1. Introduction

Increasing knowledge of working mechanisms of drugs leads to the coming on the market of more potent drugs. As a result, administered dosages are decreasing. In order to be able to determine these drugs in low concentrations in biological matrices, more sensitive techniques are required. A possibility to increase the sensitivity in a gas chromatographic (GC) system is to increase the injected sample volume. Several techniques are available to perform large-volume injection (LVI) in GC [1]. On-column injection with retention gaps is a common technique [1]. A second option to allow LVI is the loop-type interface [2], which was originally designed for the coupling of liquid chromatography and GC. A third

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possibility for LVI is to use a programmed-temperature vaporiser (PTV). The PTV has been mainly applied for environmental analysis [1,3–6], although its potential for the analysis of biofluids has been explored as well [7]. With LVI, special attention must be given to solvent purity and selectivity of the extraction procedure. Large volumes imply the injection of an equivalent amount of impurities into the analysing equipment [7].

Biological samples cannot be introduced directly into the GC system. Furthermore, the decreasing concentrations of drugs in biological samples require pre-concentration. For these purposes solid-phase extraction (SPE) is very suitable. Originally, SPE was developed as an off-line sample clean-up and pre-concentration procedure [8–12]. In order to obtain high sample throughput, SPE can be coupled at-line and on-line to GC [13–18]. With on-line SPE–(LVI)–GC, the aim is to introduce the total eluate of the SPE system into the GC system, thus increasing the sensitivity of the system. Furthermore, greater precision is obtained than with off-line SPE, since an error-prone step in the extraction procedure is eliminated. However, the critical aspect remains the amount of eluate that the GC system is able to accept. Moreover, the main limitation of the present on-line systems is the long drying step, which typically takes 10–30 min.

Miniaturisation of SPE has led to the development of SPE discs. Generally, SPE discs contain a small bed with small particles and have a homogeneous particle size distribution [12,19–21]. An advantage of SPE discs is the possibility to use smaller solvent volumes during the several steps of the SPE procedure [19,22]. The use of smaller desorption volumes in combination with LVI–GC implies that no evaporation and reconstitution of the extracts is required, which eliminates critical and time-consuming steps in the extraction procedure. Further miniaturisation of SPE has led to the development of solid-phase extraction–pipette tips (SPE–PTs). An interesting aspect of SPE–PTs is that bidirectional flow and cycling, i.e., aspirating and dispensing, can be applied [22–24]. A disadvantage of micro-SPE may be that smaller sample volumes have to be used, which leads to higher concentration detection limits (expressed in plasma concentrations). However, the injection of relatively large volumes into a GC system can considerably increase the sensitivity. A first attempt to perform on-line GC analysis with SPE–PTs was carried out using an exterior coupling device [22]. Extractions were performed off-line, and consecutive desorption and GC analysis were performed on-line. The system was not applied to the analysis of biological samples.

The purpose of the present work was to investigate the further integration of SPE–PT and the PTV injector. The SPE–PT was inserted into the injector on top of the liner of the PTV to perform on-line desorption, i.e., an internal coupling device. Special attention was paid to the impurity levels introduced by the coupling of SPE–PT and the liner. Also, several aspects regarding the extraction properties of the stationary phase of the SPE–PT were investigated. The system was applied to the analysis of lidocaine and diazepam in plasma. A comparison between the exterior coupling device [22] and the internal coupling device will be made. Both coupling devices, and in particular the internal coupling device, can be considered as an intermediate step to the development of new, miniaturised, and automated SPE–GC systems. Therefore, a complete optimisation and validation of the SPE procedure was not performed.

2. Experimental

2.1. Equipment and chromatographic conditions

SPEC·PLUS·PT pipette tips were obtained from ANSYS Diagnostics (Lake Forest, CA, USA). The extraction disc (4 mg) consisted of C_{18}-AR stationary phase. The pipette tip had an inner diameter of 4.0 mm and an outer diameter of 5.0 mm.

The PTV injection system was an OPTIC 2 (ATAS International, Veldhoven, The Netherlands), equipped with a shortened liner (64 mm×3.4 mm I.D.×5.0 mm O.D.). The liner was packed with ATAS ‘‘A’’ packing (a modified Chromosorb-based material). The behaviour of this packing was previously investigated for the analysis of drugs in plasma, as well as for the settings of the PTV [7]. The injector was set at 50°C and evaporation of the solvent occurred using the ‘‘AUTO vent mode’’ with a vent flow of 150 ml/min. After the evaporation of

the solvent the valve was switched to the splitless mode and after 10 s the temperature was increased at 10°C/s to 250°C. This final temperature was maintained during the analysis. The splitless mode was applied for 1.75 min and, subsequently, the valve was switched to the split mode. The used split flow was about 57 ml/min, whereas the septum purge flow and pressure were 1.2 ml/min and 6.0 p.s.i. (1 p.s.i.=6894.76 Pa), respectively. A transfer pressure of 12.0 p.s.i. was applied for 1.90 min. During the analysis the initial and final pressure were maintained at 8.0 p.s.i.

Gas chromatographic analyses were performed with a Hewlett-Packard HP 5890 series II instrument with a flame ionisation detection (FID) or a GC–mass-selective detection system (HP 5972 series). The capillary column was a HP-5 30 m×0.32 mm with 0.25 μm film thickness for analysis with FID, whereas analyses with mass-selective detection were performed using a HP-5 MS 30 m×0.25 mm with 0.25 μm film thickness. Helium was used as carrier gas. The column flow-rates were 1.1 and 0.5 ml/min for analysis with FID and mass-selective detection, respectively. The following temperature program was used for the GC system. 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 of 5°C/min to 230°C and a final rate of 25°C/min to 290°C. This final temperature was maintained for 3 min. The temperatures of the FID and mass-selective detection systems were set at 300 and 280°C, respectively.

During analysis performed with GC–mass-selective detection in the total ion current (TIC) mode an m/z range of 50–350 was monitored. Using the selected ion monitoring (SIM) mode, an m/z value of 86, being the most intense fragment of lidocaine, was monitored from the start of the run to 16 min. From 16 min to the end of the run the m/z values 256 and 283 were monitored, corresponding to the most intense fragment and the parent ion, respectively, of diazepam.

2.2. Chemicals

Methanol (Lab Scan, Dublin, Ireland) was of HPLC quality. KH_2PO_4 was of analytical-reagent grade quality (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). Lidocaine (Eur. Ph., Holland Pharmaceutical Supply, Alphen A/D Rijn, The Netherlands) and diazepam (Centrafarm, Etten-Leur, The Netherlands) were used as test compounds and dissolved in ethyl acetate (for organic residue analysis, Mallinckrodt Baker) or in phosphate buffer, pH 8.0. Stock solutions of 1 mg/ml were stored in the dark at 4°C.

2.3. SPE procedure and coupling to PTV

The SPE procedure was carried out by connecting a 10-ml gas tight plastic syringe (Omnifix syringe, Melsungen, Germany) to the upper end of the pipette tip. Liquid transport through the disc was done by applying bidirectional flow with the exception of the desorption solvent. Air was only applied in the downward direction. Drawing air into the pipette tip through the disc implies the possibility of dislodging the disc.

The SPE–PTs were pre-heated in an oven at 145°C for 2 h. The SPE disc in the pipette tip was pre-washed with five times 300 μl ethyl acetate. The disc was then activated with ca. 200 μl methanol followed by conditioning with two times ca. 100 μl of 0.1 *M* K_2HPO_4 buffer (pH 8.0). Subsequently, 200 μl spiked phosphate buffer or 200 μl spiked plasma diluted with 200 μl blank phosphate buffer were extracted on the disc. The sample was drawn into and pushed out of the tip twice. Then the disc was washed twice with ca. 100 μl water and, subsequently, dried by pushing air through the disc (10×10 ml). The SPE–PT was cut at the tip (4 mm) and the barrel (28 mm). Two O-rings were placed around the remaining part of the pipette tip, one around the upper part and a smaller one around the remainder of the tip. The silicone O-rings had dimensions of 6.0 mm and 1.3 mm, respectively. After opening the injector, the SPE–PT system was inserted into the liner house on top of the shortened (16 mm) liner, and subsequently the injector was closed. For the desorption of the analytes from the disc 250 μl ethyl acetate was used. The ethyl acetate was injected through the septum of the GC injection

port with a conventional GC syringe with a shortened needle, so the ethyl acetate was injected into the pipette tip. The desorption occurs inside the injector. Due to the pressure and gas flow in the injector the ethyl acetate is transferred through the disc to the liner packing. The rest of the analysis is performed like conventional LVI–GC with a PTV injector. The total system of the shortened liner and the cut pipette tip with the O-rings will be further mentioned as internal coupling device. The set-up of the internal coupling device is depicted in Fig. 1. For each extraction a new SPE–PT was used; the O-rings were used multiple times.

After the pipette tip was inserted into the injector, ethyl acetate as eluent could be injected on top of the disc. To ensure that the injection needle did not perforate the disc, the needle of a conventional GC syringe was shortened to 23 mm, positioning the tip of the needle about 1 mm above the disc of the pipette tip. If standard solutions were analysed, the fluids should not be injected on top of a SPE disc. Therefore, a cut pipette tip without the disc assembly was inserted into the injector, which mimicked the system with a disc inside the pipette tip.

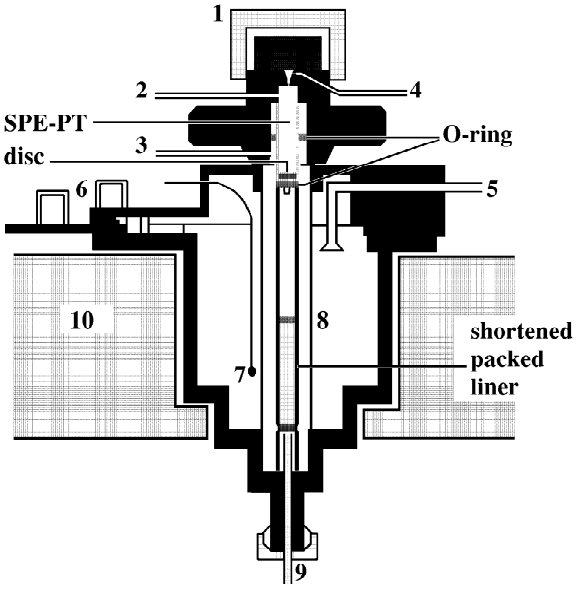

Fig. 1. Internal coupling device: integration of SPE–PT and the liner of the PTV. Parts of a conventional PTV injector: (1) septum, (2) carrier gas, (3) split flow, (4) septum purge, (5) cooling pipe, (6) power supply, (7) thermocouple, (8) heating unit, (9) capillary column, (10) oven wall.

3. Results and discussion

3.1. Development of internal coupling device

To be able to perform on-line SPE–PT–GC, solid-phase extraction–pipette tips should be directly attached to the injection system of the PTV. In a previous paper [22], we described an exterior coupling device in which the SPE–PT unit remained outside the injector. Though this device worked well, it had some disadvantages, such as a high back-pressure during desorption and a loss of about 5% of analyte due to bidirectional flow during desorption. Furthermore, the system was not very robust.

Therefore, a further integration of SPE–PT and GC was developed by inserting the pipette tip into the liner house (see Fig. 1). Since the outer diameter of the pipette tip and the liner were similar, no changes had to be made to the injector. Yet, to be able to accommodate the pipette tip into the liner house and install it on top of the liner, the 80-mm liner had to be shortened. If the injector was set at 250°C, a temperature distribution was observed as shown in Fig. 2. This implies that the liner packing is at 250°C, and that there is a temperature drop to both

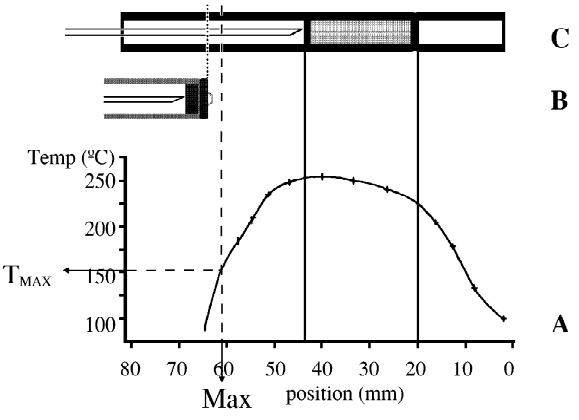

Fig. 2. Temperature distribution (A) over the range of the entire liner of PTV. The maximum temperature of SPE–PTs defines the allowable position of the tip of SPE–PTs (B, dashed line) inside the injector and the shortened liner (C, dotted line).

ends of the liner. This allows insertion of the SPE–PT into the injection system. The plastic holder of the SPE–PT was found to melt at about 160°C. Using 150°C as maximum temperature (t_{max}) to which the plastic of the SPE–PT should be exposed, it can be concluded from Fig. 2 that the SPE–PT can be inserted for maximally 18 mm. Shortening the liner by maximally 16 mm and cutting off 4–5 mm from the tip of the SPE–PT ensures that t_{max} will never be exceeded. Cutting off about 28 mm (±1 mm) from the barrel of the SPE–PT allows accommodation of the pipette tip into the liner house, thus replacing the removed part of the liner. The cutting should ensure that maximum lengths are not exceeded. The shortened liner and the cut PT should have a combined length of 80–85 mm. Slightly shorter or not very even cutting of the PT does not affect the performance of the system.

An O-ring was placed around the barrel of the PT. This ring was necessary to prevent gas to go from the carrier gas line to the split flow line without going through the disc of the pipette tip and the liner packing. A second O-ring was placed around the tip of the PT. This ring was essential to prevent leakage of elution solvent and carrier gas. Without the latter O-ring carrier gas could flow from the carrier gas line through the disc to the split flow line. The O-rings and the combination of SPE–PT and liner, which is slightly longer than a conventional unshortened liner of 80 mm, ensured a gas-tight connection between PT and liner. No fluids came into contact with the O-ring. The total time required for the cutting and installation of the PT is less than 2 min, whereas the extraction time is less than 4 min.

3.2. Effect of pre-heating and pre-washing

Upon heating the liner packing, the temperature at the position of the pipette tip also increased. This heating of the internal coupling device resulted in a significant amount of compounds in the front of the chromatogram (up to 16 min). Thus, (semi-) volatile compounds were released by this heating process. The impurities probably originate from both the disc and the PT-housing. After 2 h pre-heating at 145°C in an oven, less than 1% of the initial amount of impurities was still present. No visible changes of the disc were observed. An important drawback of pre-heating is that the extraction properties changed. Extracting lidocaine and diazepam from buffer after pre-heating the pipette tip resulted in lower recoveries (from about 80 to 40%) with more variation.

Cutting of the pipette tip and insertion of the remaining part of the pipette tip into the injector and subsequent injection of ethyl acetate on top of a pre-heated pipette tip resulted in a significant amount of medium- and less-volatile impurities which were observed in the chromatograms as clusters of peaks (Fig. 3A). These impurities interfered with the determination of lidocaine and diazepam and, therefore, had to be removed. Removal of the impurities was done by applying a pre-wash step prior to the actual SPE procedure. After pre-washing the disc with 1.5 ml ethyl acetate using bidirectional flow, over 99% of the impurities were removed (Fig. 3B). The recoveries of lidocaine and diazepam were not effected by this pre-wash step. This means that the properties of the stationary phase are not changed by the washing.

Since the (semi-) volatile impurities did not interfere with the determination of lidocaine and diazepam, no pre-heating was applied prior to use of the SPE–PTs for further experiments. Furthermore, since the pre-washing removed interfering impurities and did not affect the extraction process, this step was applied during further experiments. Each SPE–

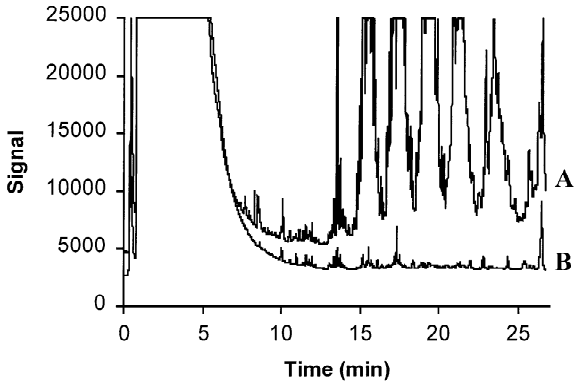

Fig. 3. Effect of pre-washing SPE–PTs on the impurities in the chromatogram: (A) without pre-washing (off-set signal: 2000); (B) 1.5 ml ethyl acetate. Note: for both chromatograms pre-heating is applied.

PT was used for only one extraction, since the heating of the injector during analysis could also affect the properties of the stationary phase.

3.3. Application of internal coupling device

3.3.1. Practical aspects

Injection of ethyl acetate on the disc and consecutive evaporation resulted in some tailing of the peak of the organic solvent. This was caused by the incomplete transfer of ethyl acetate from the disc to the liner packing. Upon heating the injector, the remaining ethyl acetate was evaporated from the disc and transferred to the GC column. The tailing of the solvent peak did not interfere with the analysis of lidocaine and diazepam.

With slow injection of ethyl acetate (100 μl in 7–10 s), only a part of the disc was moistened with ethyl acetate, which resulted in low recoveries. Injecting the organic solvent rapidly, i.e., injection of 100 μl in less than a second, produced higher recoveries of lidocaine and diazepam and smaller variations in recovery. Upon rapid injection of ethyl acetate, a reservoir of solvent was formed on top of the disc and this ensured that the entire disc was moistened by the solvent. Desorption of lidocaine and diazepam with 250 μl ethyl acetate resulted in still higher recoveries (increase from about 60 to 80%) than desorption with 100 μl ethyl acetate. However, the liquid capacity (V_{max}) of the ATAS ''A'' liner is only 150 μl [7]. If V_{max} is exceeded solvent will enter the column. Therefore, the injection of 250 μl ethyl acetate was performed in a three-step injection. First, 100 μl ethyl acetate was rapidly injected on top of the disc. After 2.25 min, a second portion of 75 μl ethyl acetate was injected rapidly, and after 4.25 min the remaining 75 μl ethyl acetate was injected rapidly. The total evaporation time was about 6.25 min. This injection procedure ensured that the next amount of ethyl acetate was never injected after the previous amount was completely evaporated. Too late injection of the next amount of solvent implies injection while the actual GC analysis has already been started. The three-step injection procedure also ensured that V_{max} of the liner was not exceeded at any time.

Injection of 100 μl ethyl acetate at a pressure of 2.5 p.s.i. instead of 6.0 p.s.i. resulted in a small loss in recovery. The evaporation time was also longer (about 0.3 min) than the three-step injection of 250 μl ethyl acetate at 6.0 p.s.i. Therefore, in the present work desorption was performed using 250 μl ethyl acetate while the pressure was maintained at 6.0 p.s.i.

3.3.2. Analytical data

In previous experiments the use of mass-selective detection proved to be necessary to determine analytes in low concentrations after extraction from buffer and especially from biological samples if LVI–GC was applied [7,22]. With the present system, i.e., the internal coupling device, extraction of lidocaine and diazepam from buffer already showed the necessity of mass-selective detection, since an interfering peak was observed for lidocaine.

The injection of 100 μl of standard solutions using the internal coupling device without SPE disc resulted in good linearity ($R>0.998$, range from detection limit to 250 ng/ml), demonstrating the reliability of the device. The detection limit (LOD), which was determined as S/N 3 or three times the blank peak, was 10 ng/ml for both lidocaine and diazepam using the TIC mode. The LOD was decreased to 0.5 ng/ml for both compounds if the SIM mode was used. For the determination of lidocaine and diazepam the m/z values 86 and 283 were used, respectively. Many silica-based compounds have a fragment with m/z 86.The m/z value of 283 corresponds with compounds from silicone-based materials [25]. These compounds probably originate from the septa used on top of the vials in which the samples were stored and/or from the silicone O-ring of the internal coupling that was used for the connection of the pipette tip and the liner.

The analysis of 200-μl plasma samples was performed using both the TIC and SIM modes. Correlation coefficients (R) and LODs are presented in Table 1. Both scan modes showed good linearity over the entire concentration range. Also for plasma recoveries of about 80% were observed. Use of the SIM mode resulted in a lower LOD as compared with the TIC mode. A similar difference in LOD between TIC and SIM was observed for plasma extracts as compared with standard injections. If the SIM mode was used the background peaks were reduced, but not completely eliminated. This is partly

Table 1
Detection limits (LODs) and correlation coefficients (R) for lidocaine and diazepam in 200 μl plasma with GC–MS analysis using the internal coupling device for the coupling with SPE (desorption with 250 μl ethyl acetate)

	TIC		SIM	
	LOD (ng/ml)	R*	LOD (ng/ml)	R*
Lidocaine	15	0.9990 (n=4)	0.75	0.9999 (n=7)
Diazepam	60	0.9998 (n=3)	2.5	0.9995 (n=6)

*Ranges from LOD to 250.0 ng/ml.

due to the fact that no pre-heating was applied and due to the low m/z value of lidocaine. Moreover, as with standard injections m/z 86 and 283 were present due to the silica-based compounds and the silicone-based compounds, respectively. In Fig. 4, representative chromatograms are depicted for the TIC mode (Fig. 4A and B) and the SIM mode (Fig. 4C and D) of blank and spiked plasma extracts, respectively. In Fig. 4D, the peaks of silicone-based compounds are lower than in Fig. 4C. This can be explained by the fact that the silicone O-ring between SPE–PT and liner was used before. Furthermore, while closing the injector the SPE–PT and liner are pressed together. The tighter these parts are pressed together, the greater the risk of tearing the ring, thus the O-ring can release more compounds.

4. Conclusions and perspectives

The direct coupling of SPE–PTs to the liner of the PTV injector could easily be established. The SPE–PTs, and thus the extracts, can be purified by simple pre-heating and pre-washing. However, the extraction properties of the stationary phase of the SPE–PTs can be altered during the pre-heating, which is only needed for the analysis of relatively volatile compounds. Other compounds present in the extracts originate from the biological matrix and solvent impurities. With LVI the use of selective detection, such as mass-selective detection, is essential to determine drugs at low concentrations in biological samples.

The system is better applicable than the exterior coupling device [22], since the pipette tips and the liner are directly connected, which makes the system more robust. Furthermore, no carry-over is observed, and no leakage of desorption solvent can occur. The exterior coupling device has the advantage of bidirectional flow during desorption, which allows in principle use of less desorption solvent. However, with this device 5% loss of analyte was observed because of incomplete desorption caused by the high back-pressure.

The necessity of high sample-throughput requires automation. On-line systems for SPE–GC already exist, but the main limitation of these systems is the relatively long drying of the SPE cartridge, which typically takes 10–30 min. The use of SPE–PTs enables decrease of the extraction time and especially the drying step, which now only takes about 30 s. Automation of a system based on SPE–PT and GC can be obtained more easily with the internal coupling device than with the exterior coupling device. A pipette robot based on a 96-well plate design can simultaneously perform the extractions at-line in pre-cut SPE–PTs with O-rings and a metal cap at the top. A recently developed liner exchanger [26] can be used to combine the SPE–PT with the PTV liner, after which the injector is closed automatically via a pneumatic system. Subsequently, the desorption solvent can be injected on top of the disc. For automatic injection the injection height should to be adjusted, or a shorter needle must be used in order to prevent perforation of the disc. In such an approach, high-throughput analysis is performed by means of a combination of miniaturised SPE and GC using at-line extraction and on-line desorption. In the future, an even further integration of SPE and GC might be obtained by performing the extraction in the liner of the GC system.

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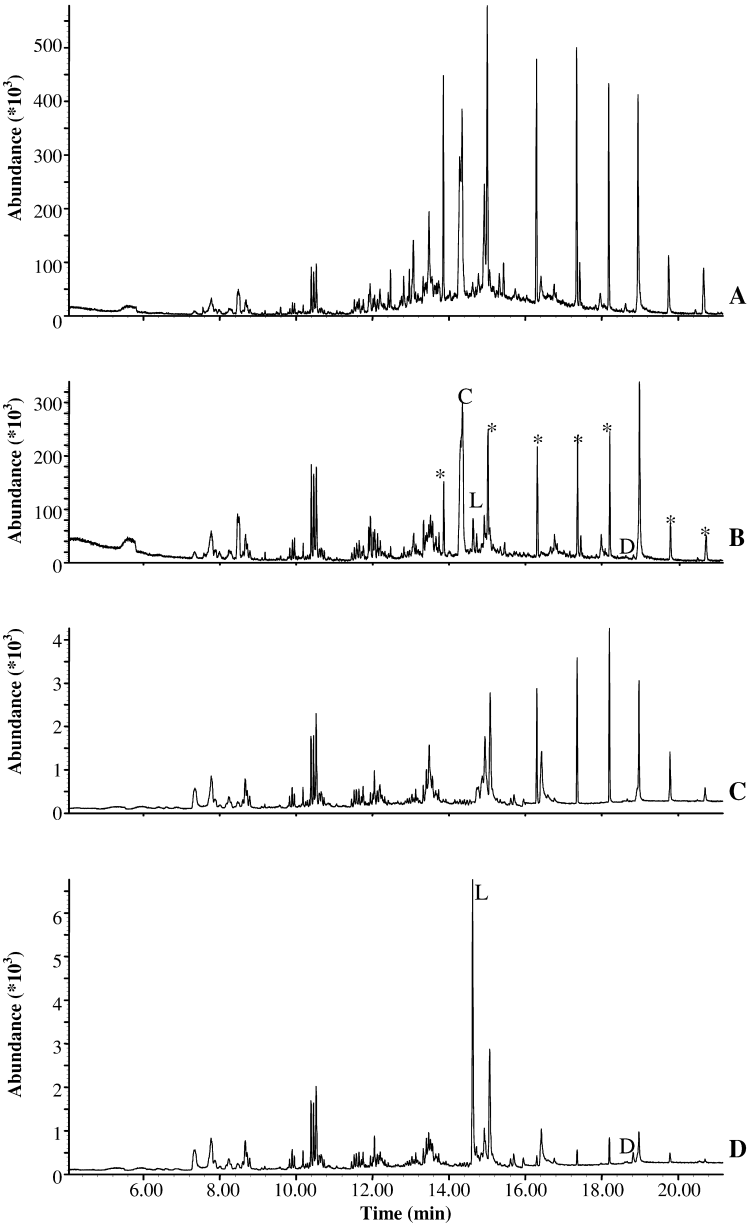

Fig. 4. GC–MS chromatograms of extracts of 200 μl plasma: (A) blank (TIC mode), (B) 62.5 ng/ml (TIC mode), (C) blank (SIM mode), (D) 5.5 ng/ml (SIM mode). C=Caffeine, L=lidocaine, D=diazepam, *=silicone-based compounds.

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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] J.J. Vreuls, U.A.Th. Brinkman, G.J. de Jong, K. Grob, A. Artho, J. High Resolut. Chromatogr. 14 (1991) 455.
[4] J.J. Vreuls, G.J. de Jong, R.T. Ghijsen, U.A.Th. Brinkman, J. Microcol. Sep. 5 (1993) 317.
[5] A.J.H. Louter, J. van Doornmalen, J.J. Vreuls, U.A.Th. Brinkman, J. High Resolut. Chromatogr. 19 (1996) 679.
[6] R.J.C.A. Steen, I.L. Freriks, W.P. Cofino, U.A.Th. Brinkman, Anal. Chim. Acta 353 (1997) 153.
[7] M.W.J. van Hout, R.A. de Zeeuw, J.P. Franke, G.J. de Jong, J. Chromatogr. B 729 (1999) 199.
[8] M. Dressler, J. Chromatogr. 165 (1979) 167.
[9] A. Lagana, B.M. Petronio, M. Rotatori, J. Chromatogr. 198 (1980) 143.
[10] R.E. Majors, H.G. Barth, C.H. Lochmüller, Anal. Chem. 56 (1984) 300R.
[11] X.H. Chen, J.P. Franke, R.A. de Zeeuw, Forensic Sci. Rev. 4 (1992) 147.
[12] Z.E. Penton, Adv. Chromatogr. 37 (1997) 205.
[13] E.C. Goosens, D. de Jong, G.J. de Jong, U.A.Th. Brinkman, Chromatographia 47 (1998) 313.
[14] A.J.H. Louter, E. Bosma, J.C.A. Schipperen, J.J. Vreuls, U.A.Th. Brinkman, J. Chromatogr. B 689 (1997) 35.
[15] A. Namera, M. Yashiki, Y. Iwasaki, M. Ohtani, T. Kojima, J. Chromatogr. B 716 (1998) 171.
[16] P. Enoch, A. Putzler, D. Rinne, J. Schüter, J. Chromatogr. A 822 (1998) 75.
[17] A.J.H. Louter, C.A. van Beekvelt, P. Cid Montanes, J. Slobodník, J.J. Vreuls, U.A.Th. Brinkman, J. Chromatogr. A 725 (1996) 67.
[18] R. Sasano, T. Hamada, M. Kurano, M. Furuno, J. Chromatogr. A 896 (2000) 41.
[19] D.A. Wells, G.L. Lensmeyer, D.A. Wiebe, J. Chromatogr. Sci. 33 (1995) 386.
[20] E.H.R. van der Wal, E.R. Brouwer, H. Lingeman, U.A.Th. Brinkman, Chromatographia 47 (1994) 239.
[21] K. Hartonen, M.L. Riekkola, J. Chromatogr. B 676 (1996) 45.
[22] M.W.J. van Hout, R.A. de Zeeuw, G.J. de Jong, J. Chromatogr. A 858 (1999) 117.
[23] R.E. Majors, LC·GC Int. May (1998) 8.
[24] D.D. Blevins, D.O. Hall, SPEC News 3–1 (1998) 1.
[25] M. Spiteller, G. Spiteller, Massenspektrensammlung von Lösungsmitteln, Verunreinigungen, Säulenbelegmaterialien und Einfachen Aliphatischen Verbindungen, Springer-Verlag, Vienna, 1973, S70.
[26] ATAS International, Veldhoven, The Netherlands, http://www.ATAS-INT.com