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Short communication

Coupling device for desorption of drugs from solid-phase extraction-pipette tips and on-line gas chromatographic analysis

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

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

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Abstract

Solid-phase extraction-pipette tips were used for micro solid-phase extraction of lidocaine and diazepam. Off-line desorption was done after in-vial collection for reference purposes, whereas with on-line desorption the eluate was directly introduced in the gas chromatograph. With both methods the total eluate (100 μl) was introduced into the GC system, which was equipped with a programmed-temperature vaporiser (PTV) for large volume injection. For on-line desorption a laboratory-made coupling device was developed to connect the pipette tips with the injector of the PTV. The coupling device was applied successfully since no leakage occurred at the connection of the coupling device and the pipette tip. No significant differences in recovery of lidocaine and diazepam and in presence of impurities were observed between chromatograms obtained with either off-line or on-line desorption. Preliminary experiments with standard solutions showed recoveries of about 75% for a concentration level of 1 μg/ml. The system seems particularly suitable for high-throughput analysis.

Keywords: Instrumentation; Interfaces, solid-phase extraction-pipette tips–programmed-temperature vaporiser; Programmed-temperature vaporiser; Injection methods; Lidocaine; Diazepam; Benzodiazepines

1. Introduction

High sample throughput is becoming increasingly important in various areas of bioanalysis. Therefore, it is essential to reduce analysis time including sample pre-treatment. A very popular sample pre-treatment technique is solid-phase extraction (SPE), which was originally developed as an off-line sample clean-up and pre-concentration procedure [1–7]. In order to obtain high sample throughput, there is a growing interest in SPE with at-line and on-line liquid chromatography [8–11] and, more recently, gas chromatography (GC) [12–19]. In addition, it has been claimed that the various steps in on-line SPE can be carried out with greater precision than in off-line SPE, resulting in more reliable data. Another advantage of on-line SPE–GC is that the total eluate can be analysed, which was not possible with off-line SPE. However, with large volume injection (LVI) of extracts of biological samples in GC via a retention gap [13,19] or a programmed-temperature vaporiser (PTV) [20], it is possible to inject nearly the total eluate of off-line extractions. Yet, with LVI, special attention must be given to solvent purity and

*Corresponding author. Tel.: +31-50-3633-342; fax: +31-50-3637-582.

E-mail address: m.w.j.van.hout@farm.rug.nl (M.W.J. van Hout)

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selectivity of the extraction procedure since with the injection of large volumes an equivalent amount of impurities is also injected into the analysing equipment [20].

Another interesting development is the miniaturisation of analytical systems. The first attempts to miniaturise SPE were done using SPE discs instead of conventional SPE cartridges [5,6,9–11,21–29]. Generally, SPE discs contain a smaller bed with smaller particles and a more homogeneous particle size distribution than conventional cartridges. An advantage of SPE discs over SPE cartridges is the possibility to use smaller desorption volumes (50–400 μl vs. 1–6 ml) [21,27]. Another benefit besides the use of less solvents is that if LVI-GC is applied in combination with miniaturised SPE no evaporation and reconstitution of the extracts is required, which eliminates an error-prone step in the extraction procedure, and thus increases reliability and reduces sample preparation time. Moreover, solvent purity is less critical than when LVI is applied in combination with conventional SPE, because less desorption solvent is used.

Further miniaturisation has led to micro-SPE which can be performed using solid-phase extraction-pipette tips (SPE-PTs) [7,30]. Extractions can be carried out more easily and rapidly than with conventional SPE or with SPE discs simply by using a pipettor and pipette tips with extraction material inside the tip [30]. An interesting aspect of extractions with pipette tips is that bi-directional flow and cycling, that is aspirating and dispensing, can be applied [7,30]. However, micro-SPE may imply that smaller sample volumes have to be used which leads to higher concentration detection limits. This loss in sensitivity may be overcome by applying LVI-GC, which may even lead to an increase in sensitivity when compared to conventional injection volumes of 1 to 2 μl [20].

In order to reduce the analysis time and increase the reliability of the results, an on-line coupling of micro-SPE with GC appears to be attractive. The GC system must be equipped with a special injector, e.g., a PTV, so that LVI is possible. In this study a coupling device for the connection of pipette tips and the PTV injector was developed and evaluated for micro-SPE–GC, that is, the extraction was carried out off-line but the desorption and subsequent GC analysis was done on-line.

2. Experimental

2.1. Apparatus and chromatographic conditions

Gas chromatographic analyses were performed with a Hewlett-Packard HP 5890 series II instrument with flame ionisation detection (FID). The capillary column was a HP-5 30 m×0.32 mm with 0.25 μm film thickness. Helium was used as carrier gas. The following temperature program was used for GC. The starting temperature was 40°C and after 3 min the temperature was raised at 20°C/min to 215°C, followed by a raise of 5°C/min to 230°C and a final raise of 25°C/min to 290°C. This final temperature was maintained for 5 to 10 min. The detector temperature was set at 300°C, and a column flow of 1.1 ml/min was used during analysis.

The PTV injection system was an OPTIC 2 (ATAS International, Veldhoven, The Netherlands), which was equipped with a 80 mm×3.4 mm I.D. liner obtained from ATAS International. The liner was packed with ATAS ''A'' packing (a modified Chromosorb-based material with special treatment). The injector was set at 40°C in the vent mode 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 raised with 5°C/s to 290°C. This final temperature was maintained during the analysis. The splitless mode was applied for 2.50 min and, subsequently, the valve was switched to the split mode. The used split flow was 57.4 ml/min, whereas the purge flow and pressure were 2.32 ml/min and 2.5 p.s.i., respectively (1 p.s.i.=6894.76 Pa). A transfer pressure of 14.0 p.s.i. was applied for 2.75 min. During the analysis the initial and final pressures were maintained at 8.0 p.s.i.

2.2. Chemicals

Methanol (Lab Scan, Dublin, Ireland) was of HPLC quality. KH_2PO_4 was of analytical-reagent 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 Pharma-

ceutical 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. Methods

Micro-SPE was performed using pipette tips (SPEC·PLUS·PT) with a C_{18}-AR stationary phase (Ansys Diagnostics, Lake Forest, CA, USA). The SPE procedure was carried out by connecting a 10-ml gas-tight plastic syringe (Omnifix syringe, B. Braun, Melsungen, Germany) (Fig. 1A) to the pipette tip (B). The SPE disc (C) in the pipette tip was activated with ca. 200 μl methanol followed by conditioning of the disc two times with ca. 100 μl of 0.1 *M* K_2HPO_4 buffer (pH 8.0). Subsequently, 200 μl phosphate buffer spiked with 1 μg/ml lidocaine and diazepam was extracted on the disc. Then the disc was washed with ca. 100 μl water and, subsequently, dried by pushing air through the disc (10×10 ml). For the desorption of the analytes from the disc 100 μl ethyl acetate were used. The desorption occurred in-vial or on-line. The solvents and samples were drawn into the pipette tip until the fluid had gone through the disc and then the fluid was completely pushed back out of the pipette tip (bi-directional flow). The fluids did not enter the plastic syringe, thus the syringe could be used for subsequent extractions.

On-line desorption was possible by connecting the pipette tip to the PTV injector via a laboratory-made coupling device as depicted in Fig. 1. The coupling device (D) was made by replacing the glass from a gas-tight GC syringe by a piece of PTFE (E). All other parts (F–I) of the coupling device are also present in conventional GC syringes with a removable needle (I). The dimensions of the PTFE piece were 5.5 mm×9 mm O.D.×2.0 mm I.D. (upper half) and 6 mm×6 mm O.D.×0.5 mm I.D. (lower half, inside the screw thread).

3. Results and discussion

The use of pipette tips offered the possibility of bi-directional flow and cycling (aspirating and dispensing) [7,30]. Visualisation of these phenomena was described by Blevins and Hall [30]. A plastic syringe was used in our approach instead of a conventional pipettor since the solvents and samples could be better drawn into and pushed back out of the pipette tip with the plastic syringe. However, bi-directional flow should not be applied with air, since a rapid flow of air in upward direction may dislodge the disc. Therefore, upon drying the disc with air, only dispensing is possible. Generally, aspirating and dispensing solvents was carried out gently to make sure that not too much air went through the disc with the exception of the final part of the dispension in the elution step. Ethyl acetate was pushed back completely followed by air to remove as much ethyl acetate as possible from the disc.

During the development of the coupling device for on-line desorption the dimensions of the PTFE piece were chosen so that it ensured to fit just right in place of the removed glass and that approximately half of the tip of the pipette tip could be inserted tightly into the coupling device. When desorption was carried out on-line, the needle of the coupling device was completely inserted into the PTV injector. Subsequently, ethyl acetate was drawn into the pipette tip, and then the pipette tip was immediately placed on top of the coupling device and the ethyl acetate was pushed back through the disc directly into the PTV injector. The coupling device and pipette tip were removed at the same time, so that no leakage of carrier gas and solvent vapour occurred through the needle of the coupling device.

In order to be able to inject the extract on-line into the GC system, substantial pressure must be applied during the push-back since a high back-pressure is present when the pipette tip and coupling device are attached to the PTV injector. The back-pressure can be lowered slightly by decreasing the purge pressure of the PTV injector. However, lowering the purge pressure leads to an increase of vent time, and thus to an increase of analysis time. The back-pressure is mainly caused by the small inner diameter of the needle (0.1 mm) of the coupling device. Despite the back-pressure no leakage occurred when desorption was carried out on-line. Due to the back-pressure the disc could not be dried completely. With SPE-PTs, bi-directional flow is applied which means that the desorption solvent that flows through the disc first,

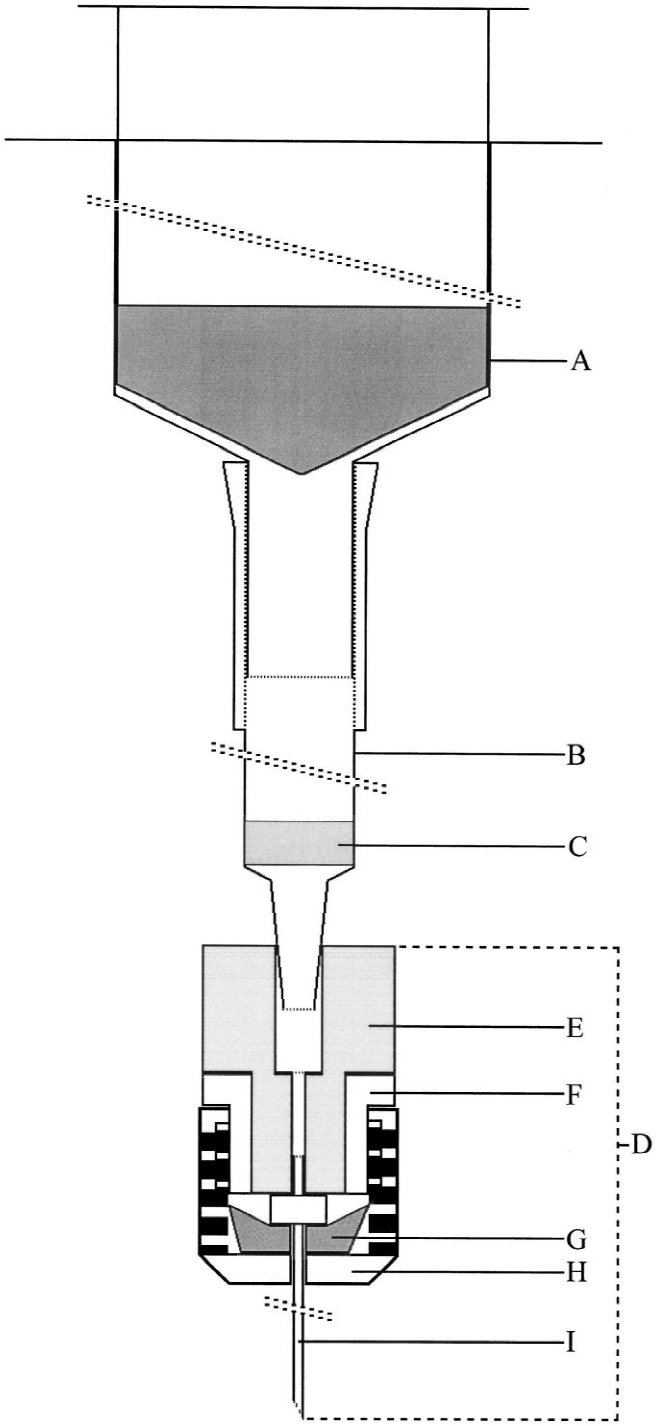

Fig. 1. Scheme of the coupling device of the solid-phase extraction-pipette tip and the PTV injector. Parts: A=plastic syringe, B=SPEC·PLUS·PT pipette tip, C=SPE disc, D=coupling device, E=PTFE piece, F=standard metal screw thread, G=vulcanised rubber, H=standard needle nut, I=needle.

leaves the pipette tip last (''first in, last out-principle''). Hence, the ethyl acetate that remains in the disc may contain a part of the analytes. Thus, if the disc can not be dried completely with on-line desorption, this may cause a decrease in the recovery of the analytes. The decrease in recovery due to the ethyl acetate that remained in the disc was found to be 5%. Thus, due to mixing of the desorption solvent inside the pipette tip the loss caused by the ''first in, last out principle'' is only small. In order to be able to quantify reliably the analytes present in the extracts an internal standard can be added to the ethyl acetate to correct for the loss of analytes that remain in the disc during on-line desorption. Using uni-directional flow during desorption, that is only dispensing with desorption solvent, might prevent the possible loss of analytes with the ''first in, last out principle''. However, this has the disadvantages that the pipettor and pipette has to be disconnected and that desorption solvent can be present in the upper part of the pipette tip which has to be reconnected prior to injection and, so, can contaminate the pipettor.

Chromatograms of in-vial and on-line desorption are presented in Fig. 2. No significant differences in peak heights of lidocaine and diazepam were observed between an analysis performed with in-vial desorption (reference purposes) and one in which on-line desorption took place. With in-vial desorption somewhat more impurities were present than with on-line desorption. Optimisation of the extraction procedure with regard to conditioning, activation and washing solvents and volumes may produce cleaner extracts. Besides solvent impurities the presence of impurities in the extracts might also be due to interferences that are being leached from the sorbent material or the sorbent holder. During some extractions white spots appeared inside the pipette tip during the drying step and disappeared when ethyl acetate was drawn into the pipette tip. The white spots seem to produce clusters of peaks in the chromatograms.

Recoveries of lidocaine and diazepam were about 75%. It should be noticed that the SPE procedure has still to be optimised which may result in higher recoveries. After elution of a disc with ethyl acetate, the carry-over from the coupling device was checked by injection of 100 μl ethyl acetate via a pipette tip without SPE disc and was found to be negligible.

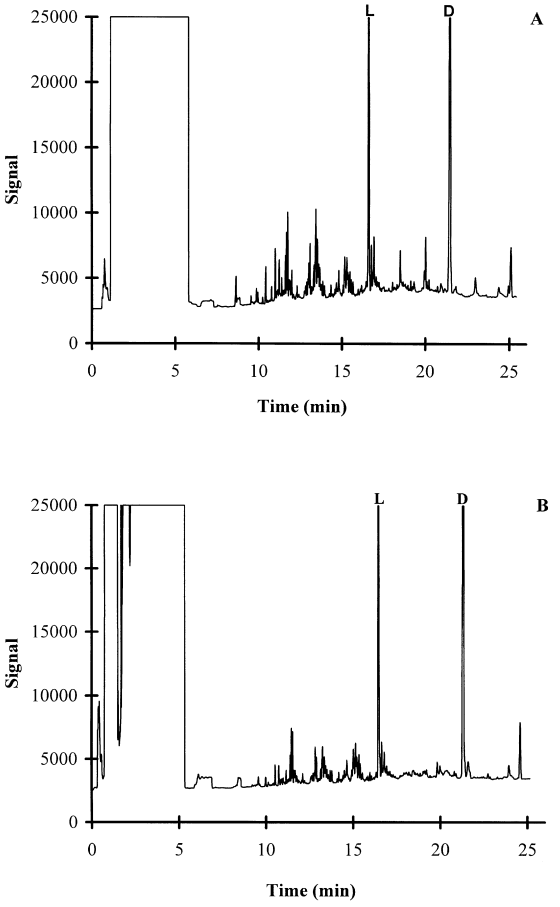

Fig. 2. Chromatograms of extracts of 200 μl phosphate buffer containing 200 ng lidocaine (L) and diazepam (D) after desorption with 100 μl ethyl acetate: (A) in-vial desorption, (B) on-line desorption.

4. Conclusions and perspectives

It is possible to perform an extraction off-line with SPE-PTs and desorb the analytes with on-line GC analysis with a laboratory-made coupling device to connect the pipette tip with the PTV injector. This allows the injection of the complete eluate (100 μl). Since no significant difference between in-vial and on-line desorption and negligible carry-over from the coupling device is observed, it is advantageous to desorb the analytes on-line, because this increases the rapidity and reliability of the extraction procedure.

The coupling device needs further optimisation and evaluation with regard to robustness of the

system. The dimensions of the PTFE piece can be changed so that a more optimal connection between the coupling device and a pipette tip can be obtained. The inner diameter of the needle can be increased which will result in a lower back-pressure when the eluent is pushed back through the coupling device into the PTV injector. However, increase of the inner diameter of the needle will also give an enhanced flow through the needle which might result in leakage of eluate at the connection of the coupling device and the pipette tip.

Preliminary experiments have shown that the total set-up can also be used for plasma samples. The SPE procedure has still to be optimised with regard to solvent and sample volumes and, if available, other SPE stationary phases, which might produce cleaner extracts and higher recoveries. Another possibility to reduce the interference of impurities in the solvents and plasma extracts is the use of more selective detection methods, such as mass spectrometry [20] or nitrogen–phosphorus detection.

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