Journal of Pharmaceutical & Biomedical Analysis
Vol. 4, No. 2, pp. 163–170, 1986
Printed in Great Britain

0731–7085/86 $3.00 + 0.00
Pergamon Journals Ltd.

The conversion of a standard gas chromatograph to a fused silica capillary system: applications in human toxicology and tropical medicine

P. DEMEDTS[1]*, J. VAN DER VERREN[2] and A. HEYNDRICKX[2]

[1] *Institute of Tropical Medicine, Department of Protozoology, Nationalestraat 155, B-2000 Antwerp, Belgium*
[2] *State University of Ghent, Department of Toxicology, Hospitaalstraat 13, B-9000 Ghent, Belgium*

Abstract: A simple and inexpensive modification of the injection port and detector configuration of a standard gas chromatograph, equipped with a nitrogen–phosphorus detector, permits the installation of a fused silica capillary column. Different ways of connecting the column to the injector and its impact on the interchangeability of the columns are discussed. The application of the system to toxicological determinations of clinical and forensic interest has been investigated, using a direct injection technique. The method allows the quantitative determination of different alkaloids and drugs in human biological samples in the low nanogram range, with reliable reproducibility and linear response.

Keywords: *GC-instrument conversion; fused silica capillary system; nitrogen–phosphorus detection; direct sample injection.*

Introduction

Based on many reports illustrating the superior chromatographic capability of open tubular columns, toxicologists have gradually moved into the field of capillary gas chromatography, and started to make use of the great resolving property of this technique in drug analysis.

Practically all groups of drugs which can be analysed on packed columns can also be determined on capillaries, with much better separation in the same or even shorter time, with enhanced sensitivity and sometimes reduced adsorption.

However, it appears that toxicologists have not fully appreciated the advantages of capillary relative to packed columns. Most instruments are designed for use with packed columns, while remarkably few commercial gas chromatographs are designed as capillary instruments. The fact that glass capillary columns are very fragile and difficult to manipulate might explain why the interest in capillary systems for drug identification has waned, and there have been few reports on the conversion of standard gas chromatographs to capillary systems.

*To whom correspondence should be addressed.

In 1979 Dandeneau and Zerenner [1] first described capillary columns made of fused silica. The high flexibility of this new type of column, which is due partly to the column material and partly to the column dimensions, permits easy installation in a gas chromatograph. Connections to the injector and detector are possible without the need for column-end straightening. At the same time, this new column material provides a high degree of inertness, basically due to the low metal oxide content.

The authors believe that, with the introduction of fused silica capillaries, it has become worthwhile to redesign a conventional gas chromatograph, constructed for use with packed columns and equipped with a nitrogen–phosphorus detector (NPD), for use as a capillary system for drug analysis. The conversion of a NPD-equipped instrument is particularly interesting in this field, not only because of its specific and sensitive response towards nitrogen-containing compounds, but also because it permits the use of a direct injection technique and eliminates the need of extra make-up gas at the detector when used in the capillary mode.

Experimental

Equipment

The instrument used was a Perkin–Elmer Sigma 2 gas chromatograph, equipped with a NP-detector (NPD) and coupled to a Hewlett–Packard 3380 S Integrator–Recorder.

The glass insert used in the injector modification (capillary liner) is not commercially available, but was custom-designed at the University's central glassware department. The 6.2 mm × 0.8 mm i.d. glass insert is 16 cm long and is slightly ground out to form a funnel entrance.

To apply the direct injection technique, a 1.0 μl 7001 NWG Hamilton syringe is used (Hamilton, Bonaduz, Switzerland). The silicone rubber Silirub® (100% transparent) was obtained from Soudal Chemicals (Antwerp, Belgium).

All other parts, necessary to adapt the instrument or install the capillary column, including a ¼–1/16 inch reducing union, a make-up tee, standard swagelok connections, graphite ferrules and three-layer septa were obtained from Chrompack (Middelburg, The Netherlands). Fused silica capillary columns of different length, different diameter, differing polarity and thickness of the stationary phase film were also obtained from Chrompack.

Installation of the capillary columns

Connection to the injector. The fused silica column is inserted into the capillary liner up to 5 mm from the point where the sample is to be deposited (point of the needle), taking into account a 2 mm opening between the septum and the liner as the carrier gas entrance (Fig. 1). Two different ways of holding the capillary in its ideal position in the glass insert have been investigated (Fig. 2). The first method is based on the use of a ¼–1/16 inch reducing union with graphite ferrules. At the ¼ inch side, the capillary column is centered, perforating a septum, which is installed inside the reducing union. This provides a secure and convenient connection system for the capillary column.

The second method involves the use of a one-component silicone rubber, Silirub® (100% transparent). A thin film of this product is applied to the outer wall of the first 10 cm section of the capillary column, before it is inserted into the special glass liner. In addition, one drop of the silicone rubber is brushed over the liner–capillary column interface to obtain a perfect sealing. By means of a ¼ inch swagelok connection and a

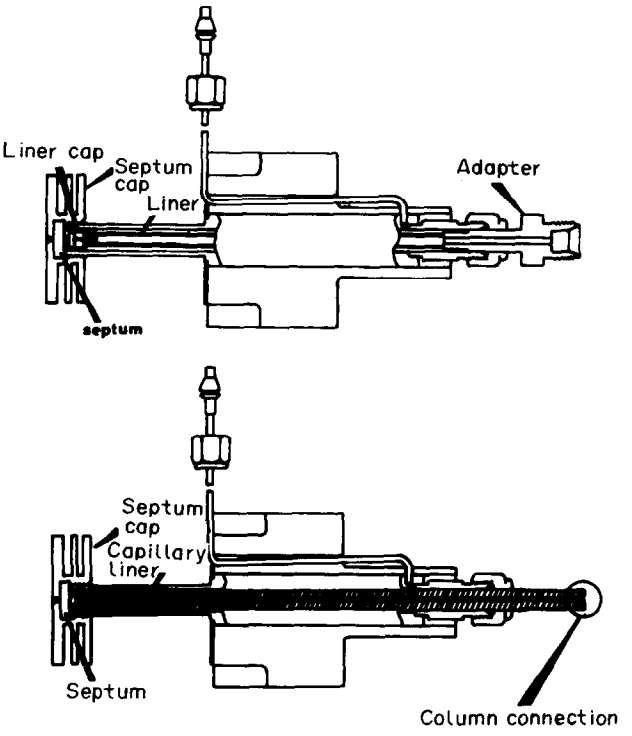


Figure 1
Modification of the injection port configuration. Liner cap, glass liner and adaptor used with packed columns have been replaced by a special small i.d. glass insert (capillary liner), coupled to the injector by means of a ¼ inch swagelok connection.

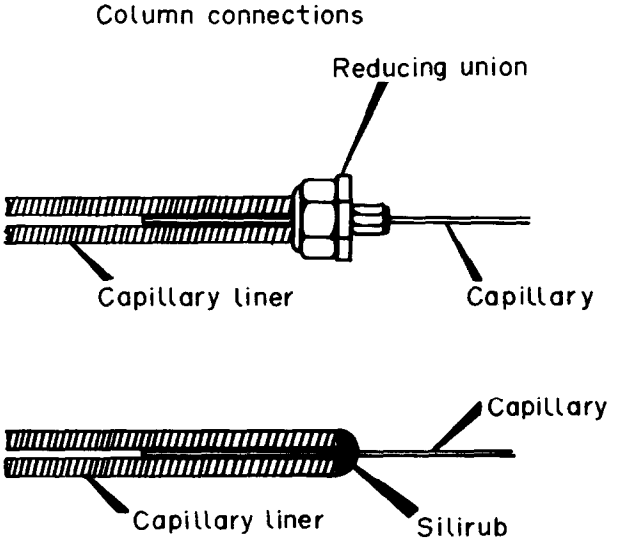


Figure 2
Reducing union coupling and silirub connection, used to hold the capillary column in its ideal position in the special glass insert.

graphite ferrule the capillary liner is then coupled to the injector. When the silicone rubber technique is used, carrier gas pressure and heating should not be applied earlier than 2 h after the connection has been accomplished. Purified helium is used as the carrier gas at pressures ranging from 0.5 to 0.8 bar.

Connection to the detector. Using a commercially available make-up tee (Chrompack), the capillary column is easily connected to the detector block. It is even possible to insert the column-end into the jet-tip of the detector and to position it very precisely, only 1.5 mm from the rubidium bead of the NPD (Fig. 3).

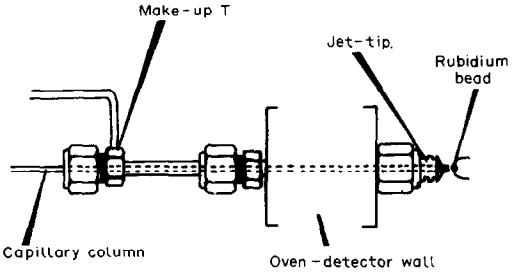


Figure 3
Connection of the fused silica capillary column to the detector and positioning of the column-end just in front of the rubidium bead of the NPD.

Results

Applications in clinical and forensic toxicology and tropical medicine

Most of the authors' efforts have been directed towards the toxicological analysis of narcotics in illicit preparations and extracts of biological samples [2–4]. In particular, morphine and structurally related compounds have been the subject of investigations. Conventionally, the GC determination of morphine was achieved on packed columns. Derivatization was inevitable in order to eliminate peak tailing and strong adsorption effects, which made quantitative analysis unreliable.

On a short fused silica capillary column, however, underivatized morphine and nalorphine can be chromatographed without peak tailing and at the same time a relatively high degree of component separation is achieved (Fig. 4). The fused silica capillary g.c. methodology has been successfully applied as a routine method for qualitative and quantitative analysis of illicit drug samples. The application of the technique has also been extended to the examination of extracts of biological samples.

To illustrate the broad-spectrum of applicability of the technique in the field of drug analysis, Figs 5 and 6 represent the chromatograms of extracts of urine and blood of a patient with late recrudescence in falciparum malaria. Chloroquine levels in blood and urine were determined during treatment to exclude chloroquine malabsorption as the cause of reduced drug response.

Discussion

The internal diameter of the capillary liner is one of the most critical parameters in the conversion to a capillary system. Severson *et al.* [5] used a 2 mm i.d. injection port liner

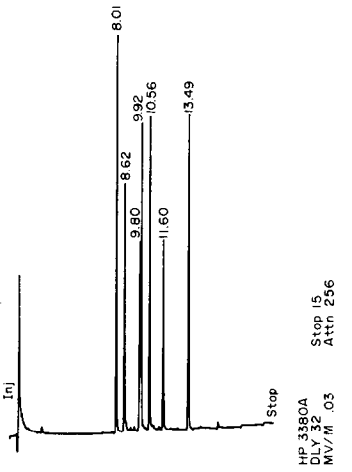


Figure 4
Separation of codeine, morphine, acetylcodeine, 6-monoacetylmorphine, nalorphine, heroin and diacetylnalorphine (10–20 ng of each) on a 10 m CP-Sil 8 fused silica column (retention times in minutes).

to convert two regular GC instruments to all-glass capillary systems with split-mode injection. Fused-silica capillaries, with an o.d. of 0.25–0.40 mm, permit the use of a liner with even smaller i.d. (<1 mm), thus reducing the unswept dead volume, which affects the apparent column performance and peak shape.

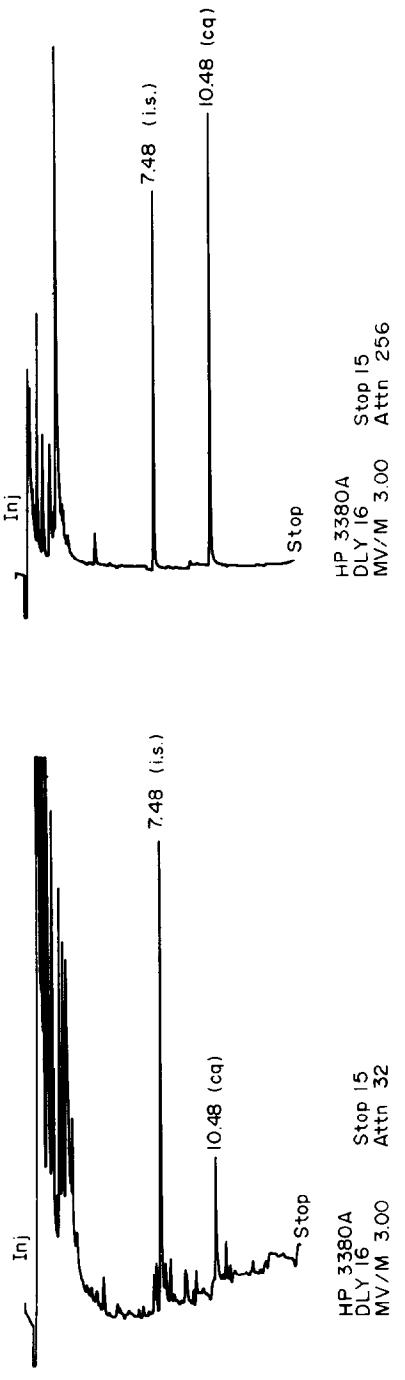
At the same time, a small internal diameter will bring about an increase of gas velocity and thus reduce the contact time of the sample with the glass surface of the liner. This explains why the column-end should be inserted into the liner up to a few mm from the point where the sample will be deposed.

In 1981, Mitchum *et al.* [6] reported the use of a room-temperature curing silicone rubber adhesive sealant (Silicoset 151, ICI Ltd) to connect a fused silica capillary column to a glass injector. This type of connection was used at oven temperatures up to 250°C and fulfilled both the requirements of inertness and low dead volume. Another advantage is that the stress on the capillary column is reduced, since this connection possesses greater flexibility than conventional coupling methods. Alternatively, silver chloride can be used as a cement to connect various chromatographic elements, but it is more expensive and requires heating of the surfaces which are to be joined to about 500°C [7].

The authors have routinely used a 'silirub' connection and, just as with the reducing union coupling, it remained leak-free under temperature programming conditions from 200 to 300°C. If columns have to be changed, complete disassembly of the injector unit is unnecessary, since columns connected to a glass insert can be stored after covering the liner opening with a ¼ inch column-end cap.

When, after prolonged analysis of 'raw' extracts of biological samples by the direct injection technique described, cleaning of the capillary liner becomes necessary, the reducing-union coupling permits easy disassembly. With the silirub connection, however, the column-end has to be cut off and a new liner has to be used.

From the first experiments in the present work, it became clear that with the substitution of a fused silica column and a carrier gas flow of 1–2 cm^3 min^{-1}, the NPD did not suffer from a decrease in sensitivity and the use of make-up gas at the detector was completely superfluous. However, the authors continued applying the make-up tee configuration, since it provided a convenient and reliable connection system at the detector side.



Figures 5 and 6
Chromatograms of blood and urine samples obtained from a patient with late recrudescence in falciparum malaria during the second course of chloroquine treatment. Codeine was used as the internal standard (retention times in minutes).

The small column dimensions and low carrier gas flow do, of course, restrict the total amount of sample that can be supplied to the column. Therefore in most cases special injection techniques are used with capillary columns to reduce the amount of sample reaching the column and to avoid overload phenomena, such as leading tail shape and retention deviations.

Nevertheless, the combined use of fused silica capillary columns and a nitrogen–phosphorus detector permits the use of a direct injection technique, without loss in column efficiency. Obviously, a sample size of 0.1–0.2 μl does not upset the equilibrium in the injector during evaporation. As opposed to a FID-detector, the NPD produces a negligible solvent signal, which does not obscure small peaks of the sample having retention times close to the solvent. In addition, good linearity is obtained for most drugs in the 1–50 ng range.

The 'hot needle technique' advocated by Grob [8] could not be applied when using the 1 μl Hamilton 7001 NWG syringe (with plunger in the needle). Therefore a fast, filled-needle injection was chosen and glass inserts with a coned end were used to facilitate the injection procedure (Fig. 1). When compared to the 'large' injections on packed columns (1–2 μl), the 0.1 μl injections on the fused silica columns resulted in higher, more intense signals for a shorter time, and thus improved sensitivity.

Besides its simplicity, the most obvious advantage of a direct injection technique is that all of the sample is passed onto the column so that results are quantitatively exact. Results could possibly still be improved by the use of fused silica needles and a cool on-column injection [9]. This technique is particularly interesting for the analysis of thermally labile compounds. However, since the sample is placed directly on the column, the application of such an injection procedure is limited to the analysis of relatively clean samples.

If separation was not too difficult, the authors prefer the use of a 10 m fused silica column, because the analysis time is directly proportional to the column length. At the same time, on a short column solutes will produce narrower and higher peaks, to give greater sensitivity. Only when raw extracts of biological samples were analysed were columns chosen with a somewhat thicker liquid loading (0.61 μm instead of 0.2 μm), allowing a larger sample size (0.2 μl) to be applied and thus making it possible to measure smaller concentrations.

To the authors' knowledge, no reports have been published on the application of a direct injection technique in combination with capillary gas chromatography and nitrogen–phosphorus detection. Baily *et al.* [10] applied a solid sample injection and capillary gas chromatography on glass columns fitted with a NP-detector for the determination of Nomifensine in plasma, after derivatization. Christophersen and Rasmussen [11] used a split-injection and FID-detection for the GLC analysis of some narcotics, including morphine and heroin, on a 20 m SE-30 glass capillary column, after flash heater-derivatization. They successfully applied the technique to the analysis of illicit heroin samples. Pitts *et al.* [12] determined phencyclidine, ketamine and some other structurally related compounds on a 25-m OV-101 glass WCOT column, fitted with a NPD. Also in 1980, VandenHeuvel and Zweig [13] published an excellent and detailed review on the analysis of drugs and related compounds by capillary column GC. However, in none of the references was the use of a direct injection technique mentioned.

Caddy *et al.* [14] has described several applications of capillary gas chromatography for the identification of drugs. These included the separation of some underivatized narcotic

analgesics on a 12-m quartz capillary column, using a nitrogen–phosphorus detector and splitless injection. However, the morphine peak still displayed marked tailing, probably due to adsorption. Better peak symmetry was obtained in experiments performed on fused silica columns, which possess a lower metal oxide content [2] (Fig. 4).

Finally, Alm *et al.* [15] reported the simultaneous GC analysis of drugs of abuse on two fused-silica columns of different polarities, coupled to a split-splitless injector. Provided the instrument is equipped with two electrometers, this procedure can be easily adapted to the described direct injection technique.

Conclusion

It was the aim of this paper to illustrate the advantages of a simple, direct injection technique, applicable with a combination of (fused-silica) capillary columns and nitrogen–phosphorus detection. The prospect of using a short capillary for speed, coupled with nitrogen specific detection for sensitivity, were the starting point of the present investigations.

Provided the electrometer response is not limiting, standard gas chromatographs equipped with NPD can be redesigned at low cost to afford a capillary system with direct injection, capable of performing many determinations in the field of toxicology and drug analysis with improved specificity and sensitivity.

References

[1] R. Dandeneau and E. Zerenner, *J. High Resolut. Chromatogr. Chromatogr. Commun.* **2**, 351–356 (1979).
[2] P. Demedts, M. Van den Heede, J. Van der Verren and A. Heyndrickx, *J. Anal. Toxicol.* **6**, 30–33 (1982).
[3] P. Demedts, M. Van den Heede, J. Van Der Verren and A. Heyndrickx, *Microgram* **XV**, 173–180 (1982).
[4] P. Demedts, M. De Waele, J. Van der Verren and A. Heyndrickx, *J. Anal. Toxicol.* **7**, 113–115 (1983).
[5] R. F. Severson, R. F. Arrendale and O. T. Chortyk, *J. High Resolut. Chromatogr. Chromatogr. Commun.* **3**, 11–15 (1980).
[6] R. K. Mitchum, W. A. Korfmacher and G. F. Moler, *J. High Resolut. Chromatogr. Chromatogr. Commun.* **4**, 180 (1981).
[7] V. Pretorius, *J. High Resolut. Chromatogr. Chromatogr. Commun.* **3**, 23–24 (1980).
[8] K. Grob Jr. and S. Rennhard, *J. High Resolut. Chromatogr. Chromatogr. Commun.* **3**, 627–633 (1980).
[9] R. R. Freeman, K. B. Augenblick and R. J. Phillips, Hewlett-Packard Technical Paper No. 88 (1980).
[10] E. Bailey, M. Fenoughty and L. Richardson, *J. Chromatogr.* **131**, 347–355 (1977).
[11] A. S. Christophersen and K. E. Rasmussen, *J. Chromatogr.* **174**, 454–460 (1979).
[12] F. N. Pitts Jr, L. S. Yago, O. Anilino and A. R. Pitts, *J. Chromatogr.* **193**, 157–159 (1980).
[13] W. J. A. Vandenheuvel and J. S. Zweig, *J. High Resolut. Chromatogr. Chromatogr. Commun.* **3**, 381–394 (1980).
[14] B. Caddy, C. B. M. Kidd and S. C. Leung, *J. Forens. Sci. Soc.* **22**, 3–31 (1982).
[15] S. Alm, S. Jonson, H. Karlsson and E. G. Sundholm, *J. Chromatogr.* **254**, 179–186 (1983).

[*Received for review 16 January 1984*]