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DIRECT COUPLING OF HIGH-RESOLUTION OPEN-HOLE GLASS TUBULAR COLUMNS TO A MASS SPECTROMETER FOR BIOCHEMICAL APPLICATIONS

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SUMMARY

In order to use the combination high-resolution gas chromatograph–mass spectrometer at high sensitivity for complex compounds of biological origin, direct coupling of the column to the ion source appeared to be the best solution. An AEI MS-12 mass spectrometer has been modified to accept the total effluent of open-hole glass capillary columns. An all-glass interface, containing a restriction, was designed for the coupling. Results show high sensitivity, an excellent usability for different types of compounds and no loss of resolution in total ion current *vs.* flame ionization detector chromatograms.

INTRODUCTION

Many methods for the coupling of a gas chromatograph and a mass spectrometer have been described. Most of the combinations involve packed columns linked through a molecular separator. Review articles[1–4] show that this field has been explored *in extenso*.

The advantage of capillary columns in terms of separation power is well known in gas chromatography (GC)[5]. The increasing use of open-hole glass tubular columns for biochemical applications has created the need for development of a linkage between this type of column and the mass spectrometer.

The simplest approach is a capillary restriction through which part of the effluent leaks into the ion source[6,7]. Low efficiency is a major drawback. The use of metal capillaries is limited to low-molecular-weight hydrocarbons. Another development is coupling via a molecular separator. The best results have been obtained with a (single-stage) jet separator. Novotny[8] discussed the problems involved in this type of linkage. Applications in the biochemical field are given by Völlmin[9] and Maume and Luyten[10]. Luyten[11] found structure-dependent losses of compounds occurring at sub-nanogram levels. He indicated the metal jet separator to be responsible for adsorption and thermal degradation. Other separators are not suitable for capillary columns; they either have a large dead volume and low efficiency or a memory effect resulting in loss of separation.

Schulze and Kaiser[12] reported on coupling through a glass tube of the same diameter as the capillary column. Cold spots in this system restricted use to low-molecular-weight compounds, as demonstrated by their examples. Recently, Henderson and Steel[13] described a system that can handle up to 20 ml/min of carrier gas without the use of a separator. They used a valve and heated stainless-steel tube as coupling device, restricting the application to thermostable compounds and low-sensitivity applications. The examples show poor GC resolution and low sensitivity in the total ion current (TIC) chromatograms.

A high-pumping-speed adaptation of an AEI MS-12 mass spectrometer (AEI Scientific Apparatus, Manchester, Great Britain) was reported by Hogg[14]. This modification involved extensive rebuilding of the mass spectrometer.

This paper reports on the adaptation made to an AEI MS-12 mass spectrometer for direct coupling of capillary columns with little changes to the mass spectrometer. An all-glass heated coupling device with a restriction is used.

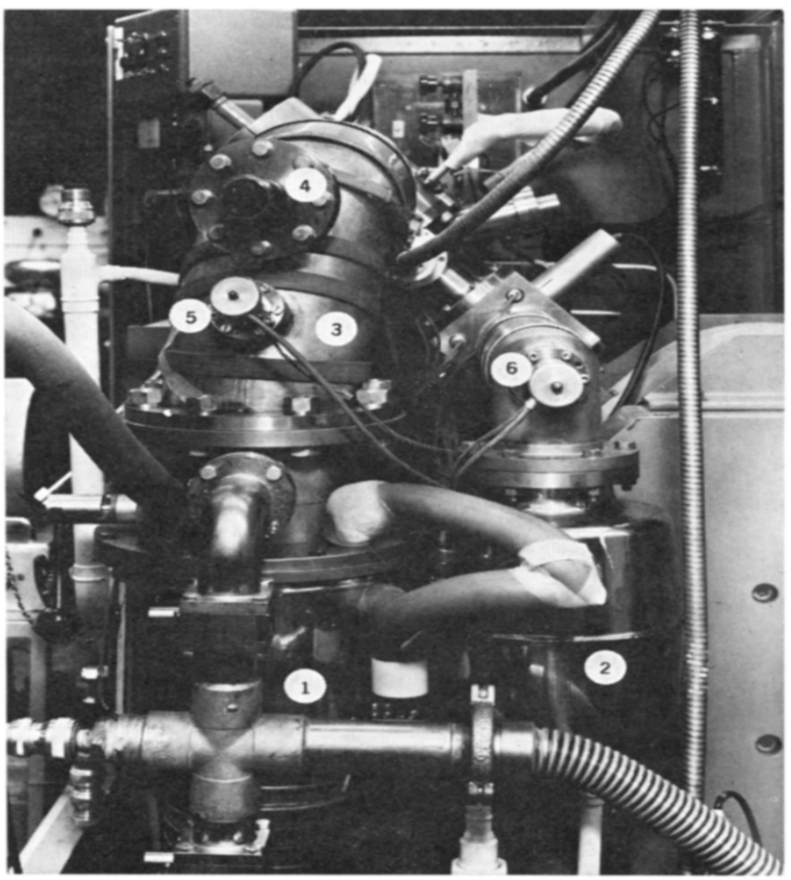

Fig. 1. Photograph of an AEI MS-12 tube unit with modified source pumping system. 1 = High-capacity source diffusion pump; 2 = original analyser diffusion pump; 3 = elbow; 4 = viewing glass; 5 and 6 = ionization gauges.

EXPERIMENTAL

Pumping system

In order to increase the pumping capacity in the ion source, a 10-cm oil diffusion "minimum impedance" pumping system (Model TM-4; TM Vacuum Products, Riverton, N.J., U.S.A.) was installed (Fig. 1). The baffled and trapped pumping speed is 400 l/sec, reduced to approximately 200 l/sec at the source. To obtain this high speed, which will also be necessary for future chemical ionization work, the source housing had to be remodelled (Fig. 2). The pumping port was enlarged to a 9 × 7 cm rectangle and a duct was welded on to this, terminating in a 15-cm-I.D. circular shape 13 cm from the centre line of the source. To the circular end of the duct, a 15-cm-I.D. elbow was welded, ending in a standard ASA flange. The centre line of the pumping tower is 30 cm from the centre line of the source. In the elbow (Fig. 1) two additional flanges were welded for a 4-cm viewing glass and the original AEI ionization gauge, which also provides excellent illumination of the source.

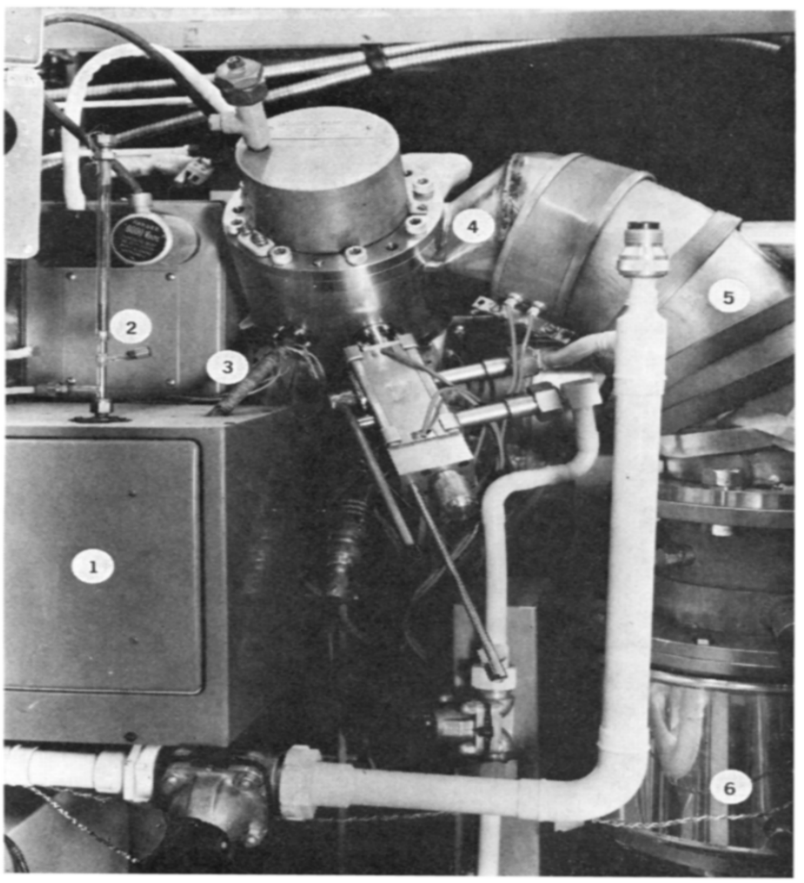

Fig. 2. Photograph of ion source and GC inlet system. 1 = GC oven unit; 2 = falling needle injector; 3 = heated coupling device; 4 = rectangular-to-circular duct; 5 = elbow; 6 = source diffusion pump.

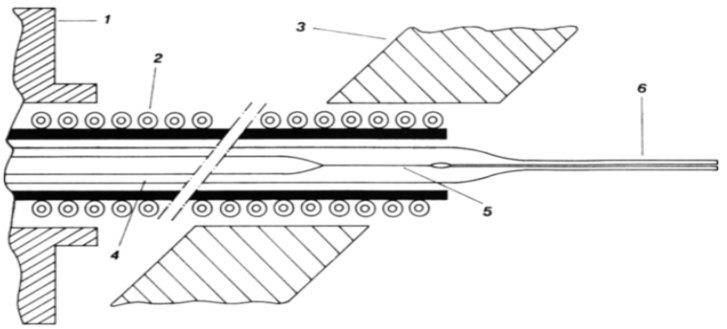

Fig. 3. Schematic diagram of the GC–MS coupling device. 1 = Standard AEI re-entry tube flange; 2 = heater; 3 = oven lid; 4, 5 and 6 = glass coupling device (total length, source to column outlet, 18 cm); 4 = 2 mm I.D. and 4 mm O.D.; 5 = 30-μm restriction, length 10 mm; 6 = 0.25 mm I.D. and 1 mm O.D.

As fore pump a "Trivac D 12-A" (Leybold Heraeus, Cologne, G.F.R.) with a pumping speed of 180 l/min was selected. This pump is used for roughing both the source and analyser diffusion pumps. All the original pressure gauges were re-installed to maintain the vacuum protection system.

Gas chromatographic system

To reduce any dead volume between the gas chromatograph and the mass spectrometer, it is necessary to place a GC oven inside the mass spectrometer frame as near as possible to a re-entry tube. The small oven unit of a Perkin-Elmer Model F-11 chromatograph (Perkin-Elmer, Norwalk, Conn., U.S.A.) was fitted as shown in Fig. 2. A modified re-entry tube (Fig. 3) extends into the oven unit and can be heated independently to about 350°. The restriction dimension is such that adequate

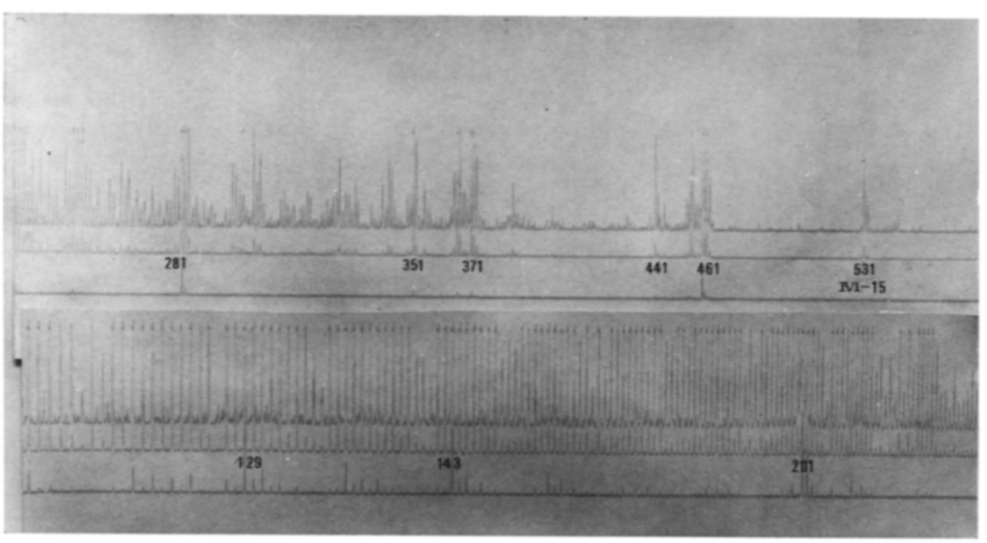

Fig. 4. UV chart recording obtained by GC–MS with on-column injection of 50 ng of 20α-hydroxy-cholesterol trimethylsilyl ether.

flow-rates occur, keeping the column end near atmospheric pressure (for minimal bleeding and to avoid gas discharges). The capillary column is connected with a PTFE shrinkable tube, at one end to the re-entry tube and at the other end to a "falling needle injector"[15]. The carrier gas (helium) is pressure regulated. Prior to operation the coupling device was silanized with dimethyldichlorosilane.

Operating data

The glass capillary column (48 m × 0.25 mm I.D.) was pretreated and coated with OV-101, according to the procedure given by Rutten and Luyten[10]. The column was operated isothermally at 230°; the inlet pressure was 1 atm. The coupling device was kept at 250°. Under these conditions the flow-rate of carrier gas through the column was 1 ml/min, causing an ion source pressure of 4×10^{-4} N/m² (not corrected

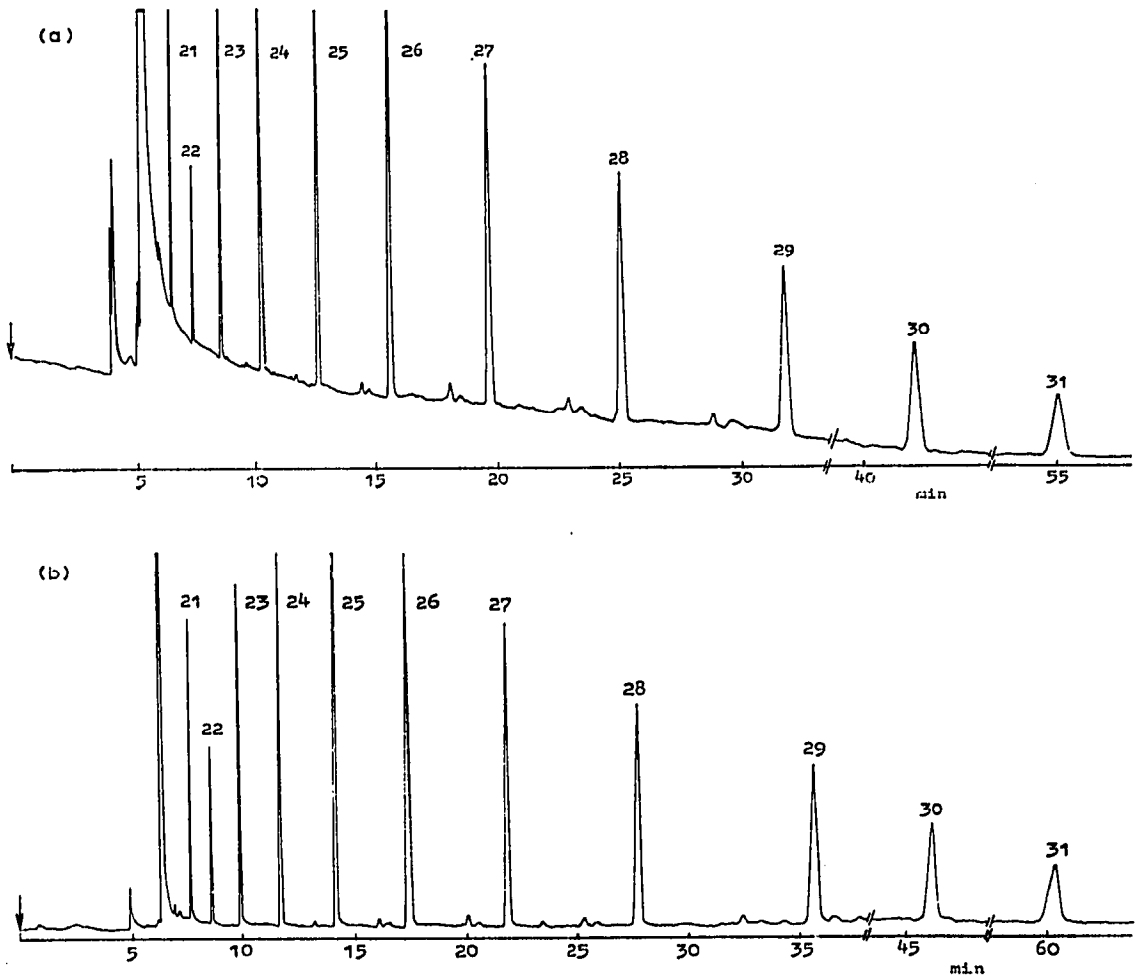

Fig. 5. (a) FID chromatogram of *n*-alkanes C_{21}–C_{31}. 30 ng per peak for C_{23} and higher homologues. (b) TIC chromatogram of the same mixture and amounts as in (a).

for helium). The chromatograms were recorded at 4 kV accelerating voltage, 20 eV electron ionization energy and 100 μA trap current. The mass spectrum was taken at 4 kV, 70 eV and 500 μA, multiplier voltage 3 kV, magnetic scan-rate 2 sec/decade and resolution 1000. The source temperature in all experiments was 250°. For recording of the flame ionization detector (FID) chromatograms the column was disconnected from the restriction and attached to a FID.

RESULTS AND DISCUSSION

The GC–MS combination described above has been satisfactory in use in our laboratory since November 1972. It is extensively applied to steroids, pesticides, amino acids and peptides. Generally, good spectra can be produced from 1 to 10 ng per compound injected on the GC column. Fig. 4 shows the mass spectrum as obtained from 50 ng of 20α-hydroxycholesterol trimethylsilyl ether.

In Figs. 5a and b the FID and TIC chromatograms of isothermal runs of

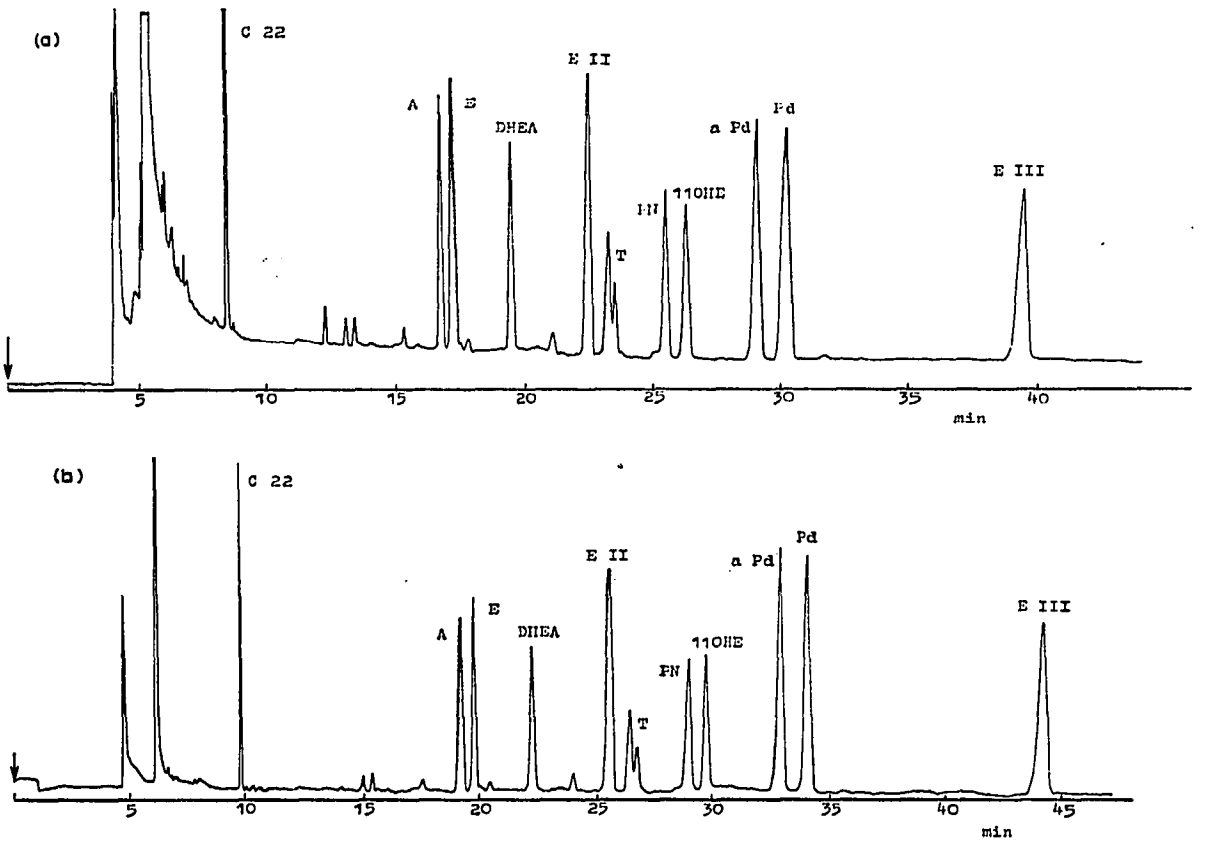

Fig. 6. (a) FID chromatogram of a mixture of methoxime trimethylsilyl derivatives of steroids. A = Androsterone; E = etiocholanolone; DHEA = dehydroepiandrosterone; E II = estradiol; T = testosterone; PN = pregnanolone; 11OHE = 11-hydroxyetiocholanolone; a Pd = *allo*-pregnanediol; Pd = pregnanediol; E III = estriol. A is present in an amount of 15 ng. (b) TIC chromatogram corresponding to the FID chromatogram of (a).

normal alkanes C_{21}–C_{31} are compared. This illustrates the constant yield throughout the entire range of compounds.

An analogous comparison is made in Figs. 6a and b for a mixture of methoxime trimethylsilyl (MO-TMS) derivatives of steroids. These chromatograms demonstrate that there is practically no loss in separation. The number of theoretical plates for MO-TMS androsterone and etiocholanolone is approximately 1900 per metre in both cases.

The fact that the TIC chromatograms always show less tailing of solvent peaks than the corresponding FID chromatograms is a useful, although not yet understood, side effect.

CONCLUSION

Over many years our laboratory has thoroughly tried several types of molecular separators and splitter devices for high-resolution GC–MS for biochemical applications. All these devices could not meet with our demands. The system described in this paper is far superior. It combines the advantages of high-resolution GC, good sensitivity and very low column background. It can be used over a wide temperature range, restricted only by the type of capillary column.

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REFERENCES

1 A. N. Freedman, *Anal. Chim. Acta*, 59 (1972) 19.
2 G. A. Junk, *Int. J. Mass Spectrom. Ion Phys.*, 8 (1972) 1.
3 D. I. Rees, *Talanta*, 16 (1969) 903.
4 J. T. Watson, in L. S. Ettre and H. W. McFadden (Editors), *Ancillary Techniques of Gas Chromatography*, Wiley-Interscience, New York, 1969.
5 M. Novotny and A. Zlatkis, *Chromatogr. Rev.*, 14 (1971) 1.
6 A. E. Banner, R. M. Elliott and W. Kelly, in A. Goldup (Editor), *Gas Chromatography 1964*, Institute of Petroleum, London, 1965, p. 180.
7 G. Nota, G. Marino and A. Malorni, *Chem. Ind. (London)*, (1970) 1294.
8 M. Novotny, *Chromatographia*, 2 (1969) 350.
9 J. A. Völlmin, *Chromatographia*, 3 (1970) 238.
10 B. F. Maume and J. A. Luyten, *J. Chromatogr. Sci.*, 11 (1973) 607.
11 J. A. Luyten, *Ph. D. Thesis*, University of Technology, Eindhoven, 1973, p. 167.
12 P. Schulze and K. H. Kaiser, *Chromatographia*, 4 (1971) 381.
13 W. Henderson and G. Steel, *Anal. Chem.*, 44 (1972) 2302.
14 A. M. Hogg, *Anal. Chem.*, 44 (1972) 227.
15 P. M. J. van den Berg and Th. P. H. Cox, *Chromatographia*, 5 (1972) 301.
16 G. A. F. M. Rutten and J. A. Luyten, *J. Chromatogr.*, 74 (1972) 177.