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SIMPLE AND EFFECTIVE DIRECT COUPLING FOR GAS CHROMATOGRAPHY–MASS SPECTROMETRY ON THE MS 50 MASS SPECTROMETER

FULL SPECTRA UP TO n-$C_{54}H_{110}$

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SUMMARY

A very simple, convenient and inexpensive interface is described which permits the insertion of fused-silica capillary tubing directly into the ion source of the MS 50 mass spectrometer. The design preserves the convenience of a vacuum-loaded re-entrant, and the only ferrules used are easily accessible inside the oven of the gas chromatograph. Results with the new interface are superior to those obtained with the commercial interface. The tetrapeptide Val-Gly-Ser-Glu, as its N^{α}-trifluoroacetyl-N,O- permethyl derivative, is used as an example. Under appropriate gas chromatographic conditions, alkanes up to n-$C_{54}H_{110}$ may be eluted and give spectra up to the molecular ion.

INTRODUCTION

Several methods are in current use for transferring the eluate of capillary gas chromatographic (GC) columns into the ion source of mass spectrometers. One such method, the one supplied with our instrument (the Kratos MS 50S), involves the use of make-up gas, a jet-separator and a glass transfer line. While this method works reasonably well for packed columns (for which it was designed, without make-up gas) and for low-molecular-weight work with capillary columns, we show that it is not suitable for capillary-column analysis of materials of high molecular weight. An alternative method of introduction is the open-split interface. Such devices have been used successfully, *e.g.* to transfer alkanes and esters of high molecular weight to a quadrupole mass spectrometer[1]. For more sensitive compounds, the transfer line from the open-split to the mass spectrometer may be made of deactivated glass instead of noble metal[2]. Nevertheless, adsorption can still be a problem[2], and remains so even with a direct coupling (no jet-separator or split) if the GC columns stops short of the ion source and the eluate is transferred via a capillary in noble metal or glass.

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The most satisfactory method of GC–mass spectrometric (MS) coupling from the point of view of quantitative transfer of difficult or low-level samples is a direct one where the GC column terminates actually inside the ion source of the mass spectrometer[3–5]. This configuration may not suit every application: it is not possible to discard derivatization reagents, which soon affect source performance[2], and the effect of vacuum at the column end results in a chromatogram which is different from that obtained with, *e.g.*, a flame-ionization detector (FID) (although this can be advantageous in terms of analysis time[4,6]). Nevertheless, such a direct coupling is the best way of ensuring that what elutes from the column arrives in the ion source. A recent paper[7] describes a combined interface which allows operation in both the open-split and direct-coupling modes. We describe here the modification of our instrument to permit the insertion of fused silica capillary columns directly into the ion source, and some results obtained.

EXPERIMENTAL

The gas chromatograph–mass spectrometer combination consisted of a Carlo Erba Model 4161 gas chromatograph coupled to a Kratos MS 50S mass spectrometer (Kratos, Manchester, Great Britain). As supplied, the instrument was fitted with a jet-separator interface. In a first attempt to improve performance when using capillary columns, we removed the jet separator and connected a fused silica capillary column to the glass tube of the source re-entrant by means of a 1/4–1/16 in. stainless steel union. The column passed through the union and terminated just in front of a glass spiral. The role of the glass spiral was to give a degree of flexibility for when the vacuum-loaded re-entrant moved backwards and forwards when the source was changed. The glass annular leak was left in position inside the re-entrant.

A further attempt to improve the performance of the interface involved fusing an extension in A45 glass to the standard vacuum-loaded re-entrant (without annular leak) as shown in Fig. 1. Where the glass extension passed through the hole in the oven wall, it was drawn down to 1.4 mm O.D. to permit the use inside the oven of a coupling made by silver soldering one half of a 1/16–1/16 in. stainless steel union to a brass coupling taking graph-pack ferrules[8]. The wall thickness of the glass of the coupling was *ca* 0.3 mm and the end was fire-polished. The coupling was connected using a graphitized Vespel ferrule drilled to fit the glass. The coupling is normally free to slide back and forth in a slot lubricated with Coppa-Slip (The Slip Group of Companies, St. Albans, Great Britain) and cut in an aluminium block screwed to the heated tunnel (see below and Fig. 1). When the coupling is to be tightened, however, it is pressed down into the slot to prevent movement. Part of the aluminium is cut away to provide easy spanner access to the nut. A fused silica capillary column (0.34 mm I.D., 0.5 mm O.D., 0.12 μm coating of Sil 5, Chrompack, Middelburg, The Netherlands) was then pushed through the coupling up to the end of the re-entrant with the source removed. After replacing the source and pumping down under rotary-pump vacuum to cause the re-entrant to mate with the source, the column was pushed in a further 1–2 cm so as to enter the source block and the coupling was then tightened on the column side using a graph-pack ferrule. It is easy to remove and replace the source, or to change columns once this length has been set. The column is simply withdrawn a few cm (with the ferrule attached) when it is

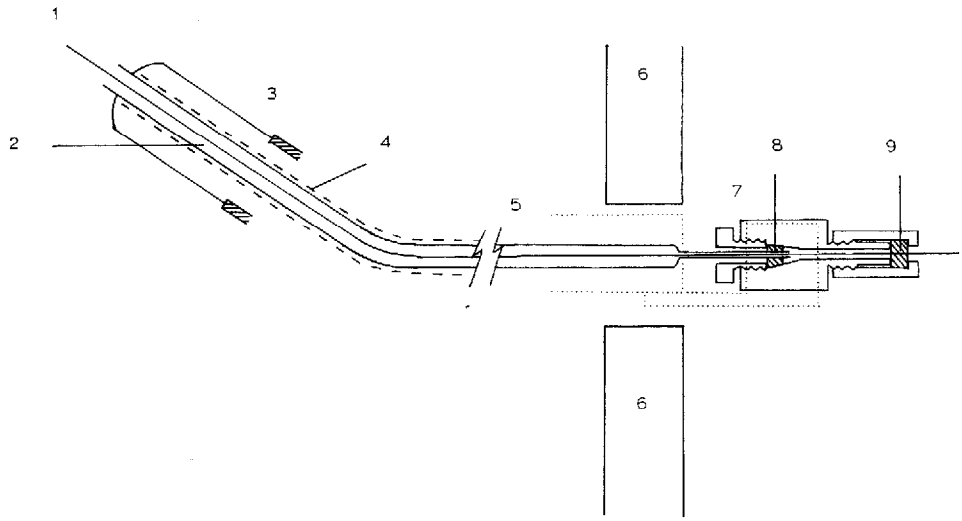

Fig. 1. Schematic diagram of the direct coupling permitting the insertion of fused silica tubing into the ion source. 1 = Column in source block. 2 = Point at which annular leak fitted, where used; the leak consists of a few cm of straight glass rod of a diameter slightly smaller than that of the A45 glass tube; it is prevented from sliding by the bend in the tube. 3 = Glass-to-metal seal of vacuum-loaded re-entrant. 4 = Ribbon heaters. 5 = A45 glass tube running through a heated aluminium tunnel mounted on and insulated from the gas chromatograph wall. 6 = Oven wall. The aluminium tunnel is flush with the inside wall. Gaps are plugged with glass wool. 7 = Metal coupling with hexagonal body mounted in a slot in an aluminium block screwed to the aluminium tunnel; movement of the coupling along the axis of tube 4 is thereby permitted, but the coupling cannot rotate. 8 = Graphitized Vespel ferrule. 9 = Graph-pack ferrule (metal casing not shown).

necessary to remove the source, then pushed home when the source is replaced. When changing columns, the new column may be pushed home the same distance without removing the source.

The assembly is heated ribbons between re-entrant and an aluminium tunnel which had housed the jet separator (see Fig. 1). The tunnel is heated independently of the ribbons by cartridge heaters controlled by the detector heater circuit of the gas chromatograph oven, and is insulated with rock wool. Five thermocouples serve to monitor the temperature at various points. The tunnel, heaters and thermocouples are those supplied with the jet-separator interface.

GC–MS experiments were carried out using the type of column mentioned above in lengths varying from 5 to 9 m. Helium was used as carrier gas at pressures of 0.2–0.5 kg cm^{-2}. Samples were injected in solution (0.5–1 μl) in either hexane (alkanes) or chloroform (peptide derivatives) via the on-column injector with an oven temperature of 120°C. At 1 min after injection the oven temperature was raised at 6°C/min to 300°C. The interface was held at 285°C, higher than the elution temperature of the compounds described.

When testing the new interface with alkanes of high molecular weight (> 700) its temperature was raised to 300°C. After injection of 1 μl of alkane solution (less than 100 ng of a particular component) on to an 8-m column at an inlet pressure of 0.1 kg/cm^2 (above ambient), the oven temperature was raised from 120 to 220°C over

3 min. During this time the carrier gas flow was adjusted to 0.7 ml/min, measured at the source pump outlet, thus causing the column inlet pressure to fall below ambient. The column was then programmed at 4°C/min to 340°C.

The mass spectrometer was operated in low resolution mode, source temperature 200°C, accelerating voltage 8 kV, electron beam 70 eV. The accelerating voltage, filament and ion gauges were turned off whilst the solvent eluted. Mass spectral data was acquired and processed by a DS55 data, system (Kratos). Peptides were transformed to their $N^{\alpha,\varepsilon}$-trifluoroacetyl-N,O-permethyl derivatives as described earlier[9].

A stock solution of derivatives was used throughout the work described here.

RESULTS AND DISCUSSION

The all-glass jet-separator interface supplied with the instrument was not satisfactory for capillary-column analyses of materials of high molecular weight. In particular, alkanes above n-$C_{40}H_{82}$ and oligopeptides as their $N^{\alpha,\varepsilon}$-trifluoroacetyl-N,O-permethyl derivatives showed marked tailing and the mass spectra of the peptide derivatives exhibited excessive fragmentation (*e.g.* Fig. 2).. Some improvement was obtained at our first attempt at direct coupling by simple removal of the jet-separator. When freshly deactivated by the injection of silylating agents (Silyl-8, hexamethyldisilazane, etc.) the modified interface showed a useful performance, but the mass spectra of certain oligopeptides still showed signs of excessive fragmentation. In addition,

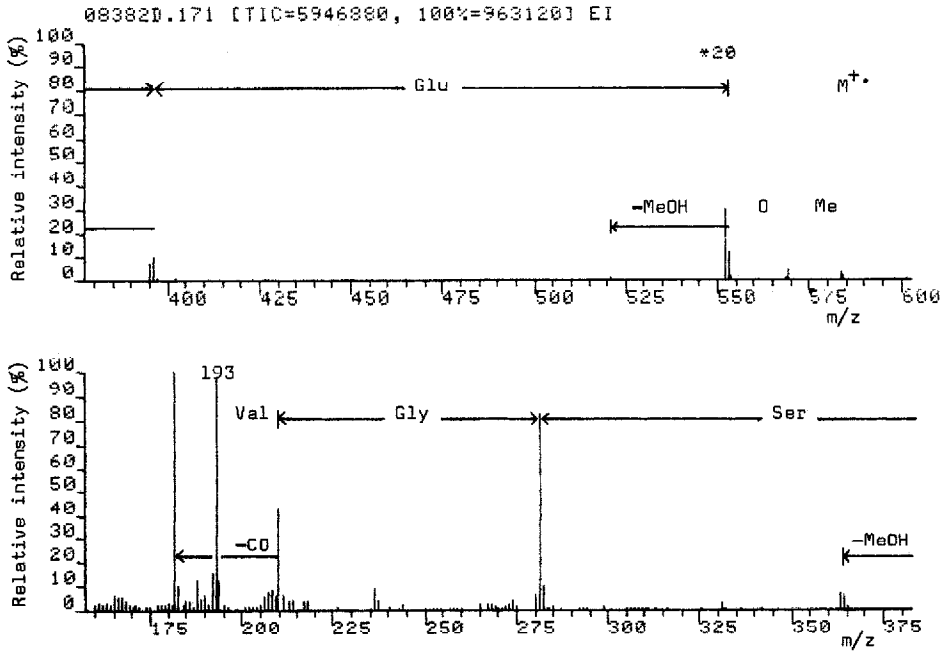

Fig. 2. Mass spectrum of Val-Gly-Ser-Glu obtained using the commercial (jet-separator) interface. An amount of N^{α}-trifluoroacetyl-N,O-permethyl derivative corresponding to 1 nmol of peptide was injected. Although the interface had been treated with Silyl-8, the mass spectrum shows evidence of excessive fragmentation, in particular the ion at m/z 193.

the daily silylation was inconvenient because it led to rapid deterioration of source performance and high background signals. For example, the chemotactic tetrapeptide Val-Gly-Ser-Glu gave a mass spectrum (Fig. 3) which, while the sequence ions are still present, contains an intense ion at *m/z* 193. Similarly, the mass spectrum of the tripeptide Gly-Leu-Tyr showed additional signals at *m/z* 175 and 244. The relative intensities of such non-sequence ions increased with time after silylation of the interface.

By contrast, the new interface permits a clean spectrum of the tetrapeptide to be obtained (Fig. 4). There is a clear improvement over the result obtained with the commercial interface (Fig. 2). Similarly, the spectrum of Gly-Leu-Tyr (not shown) is devoid of signals at *m/z* 175 and 244 and only the sequence ions are prominent.

For the experiments just described, the carrier gas inlet pressure had been maintained at high values (generally 0.04 kg/cm^2 per m of column length) simply in order to keep elution temperatures relatively low. With the peak tailing and sample degradation problems solved by the new direct coupling (Fig. 1), we were able to test the system at flow-rates closer to optimum. For a column of 8 m inserted into the ion source, this requires a sub-ambient inlet pressure. Under such conditions the optimum flow in atmospheric ml/min is below the optimum flow in atmospheric ml/min when the column is operated with the outlet at atmospheric pressure (*e.g.*

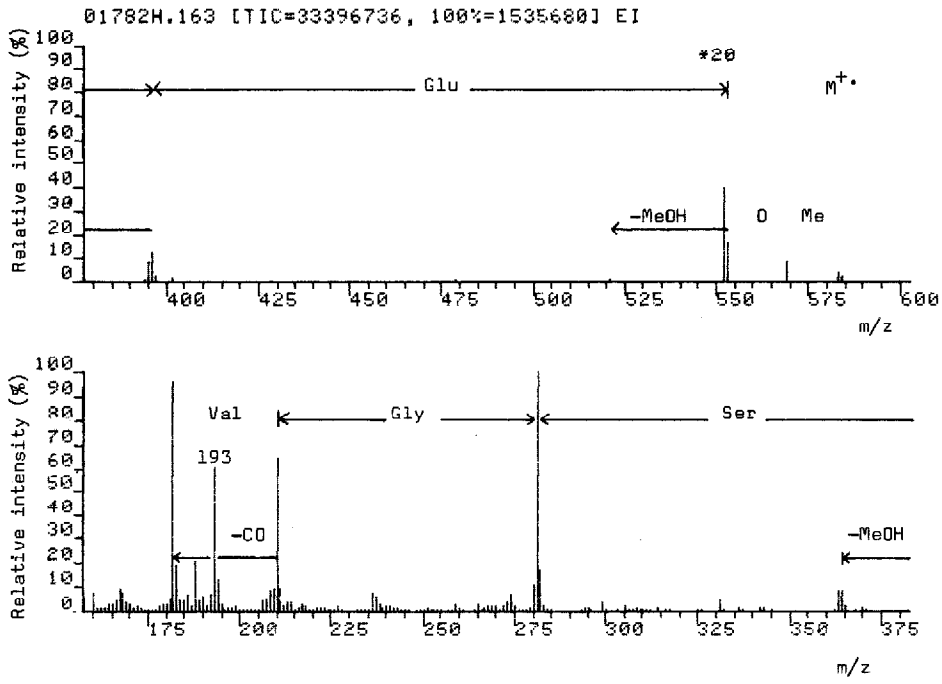

Fig. 3. Mass spectrum of Val-Gly-Ser-Glu obtained using a direct coupling and a glass transfer line. This spectrum was obtained just after silylation of the transfer line using Silyl-8 and HMDS. There is less fragmentation than in Fig. 2. The loss of CO from *m/z* 210 is less prominent, as is the non-sequence ion at *m/z* 193, and an ion of higher mass becomes base peak (*m/z* 281 rather than *m/z* 182). An amount of derivative corresponding to 500 pmol of peptide was injected. Me = Methyl.

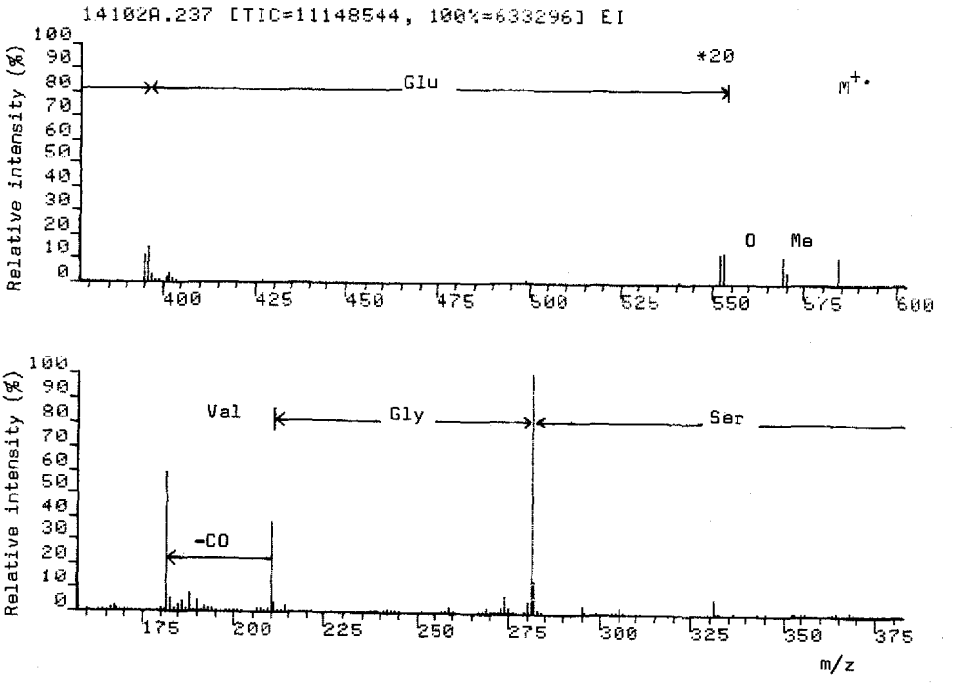

Fig. 4. Mass spectrum of Val-Gly-Ser-Glu obtained using the direct coupling schematized in Fig. 1. No silylation was necessary since the column extends into the ion source. There is less fragmentation than in Figs. 2 and 3, *i.e.* more relative intensity in the fragments of higher mass. The non-sequence ion at *m/z* 193 is no longer prominent. An amount of derivative corresponding to 500 pmol of peptide was injected.

FID)[6]. Fig. 5 shows the total ion current (TIC) trace of part of a run of *n*-alkanes using a sub-ambient inlet pressure. Owing to the high sensitivity of the MS 50 mass spectrometer, mass spectra were obtained up to the molecular ion in all cases. Fig. 6 shows the molecular ion regions of the later alkanes, up to *n*-$C_{54}H_{110}$. (By comparison, difficulty was experienced in obtaining full spectra from alkanes above *n*-$C_{36}H_{74}$ on a quadrupole instrument[1].) The peaks in Fig. 5 do not tail significantly in spite of the fact that the interface temperature is below that of the elution temperature of the later alkanes. This is probably because of the very low pressure in the column in the interface, and also possibly due to there being very little stationary phase left in this region. When a column is first installed, the phase is seen to arrive in the source in bursts, producing a spiky TIC trace and spectra characteristic of the phase. This subsides after a few hours conditioning at 285–300°C.

Our simple and inexpensive modification preserves the convenience, when changing the source, of the vacuum-loaded re-entrant, and column changing is also easy. The metal coupling, always a potential site for leaks, is, in this interface, placed inside the oven and so is easily checked and tightened. Should, during column changing, the interface break by accident where it enters the metal coupling, it is easily repaired in a few minutes at the cost of a few cm of A45 glass. By using A45 glass no graded seals are required. The reason why the glass is drawn out to pass through a 1/16 in. coupling rather than attached via a 1/4–1/16 in. reducing union is to create

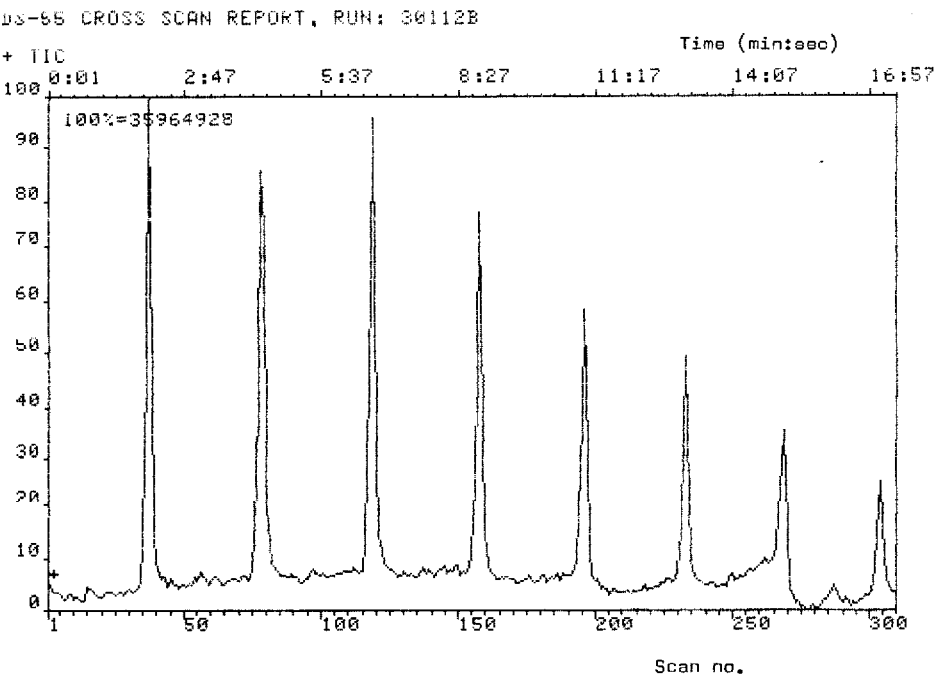

Fig. 5. Part of the total ion current trace of a GC–MS analysis of mixture of *n*-alkanes up to *n*-$C_{54}H_{110}$. These are the raw data. No background subtraction has been performed.

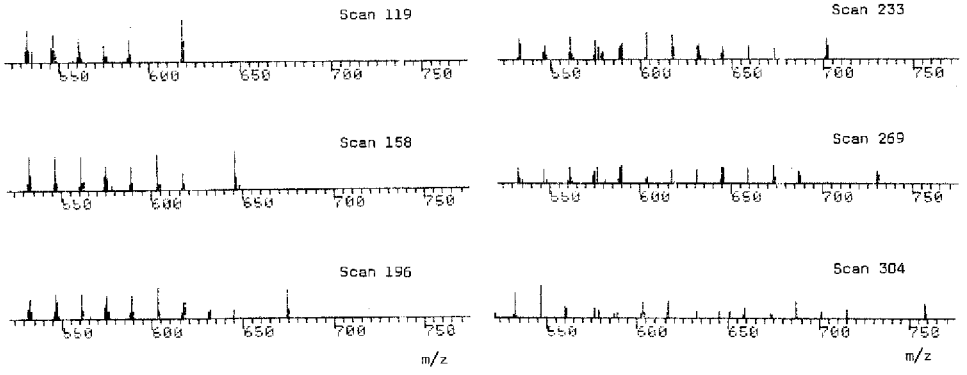

Fig. 6. Molecular ion regions of the later alkanes in the GC–MS run shown in Fig. 5. These are the raw data. No subtraction or averaging has been performed. The spectra continue down to below *m/z* 32. Masses have been rounded down for plotting purposes but are stored by the data system to four decimal places. For example, the masses found for the molecular ions are (with calculated values in parentheses) 758.8476 (*n*-$C_{54}H_{110}$ = 758.8607), 730.8109 (*n*-$C_{52}H_{106}$ = 730.8294), 702.7962 (*n*-$C_{50}H_{102}$ = 702.7981), 674.7121 (*n*-$C_{48}H_{98}$ = 674.7668), 646.7309 (*n*-$C_{46}H_{94}$ = 646.7355) and 618.6734 (*n*-$C_{44}H_{90}$ = 618.7042).

a weaker point and so avoid possible breakage inside the re-entrant. We have tried using a 1/16–1/16 in. coupling in place of the composite coupling but found that the graphitized Vespel ferrules could become jammed in the coupling, necessitating cutting of the glass interface to remove the column. Such events do not occur with the composite coupling, since the mating surface is flat and not conical and pure graphite is used: the column can always easily be removed or repositioned. If it is necessary to use glass capillary columns, these may be connected to a short piece of fused silica for passage through the interface, by means of an appropriate butt connector (*e.g.* the one supplied by Supelco) but we have not yet tried this. We warn that it is in principle more difficult to interface to an 8 kV instrument (such as the MS 50) than to the quadrupole instruments which have been used until now[1–3,5,6]. The absence in the new interface of an annular leak in the re-entrant may lead to breakdown at full accelerating voltage at very high source pressures [*e.g.* during GC–chemical ionization (CI) MS]. Although CI reagent gases may be used to adantage as carrier gas[8], it may nevertheless be necessary to isolate the metal coupling, permitting it to float. Under GC–electron impact (EI) MS conditions, there is no problem.

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