Journal of Chromatography, 142 (1977) 559–574

CHROM. 10,385

DIRECT GAS CHROMATOGRAPH–MASS SPECTROMETER CONNECTION OF GLASS CAPILLARY COLUMNS FOR THE ANALYSIS OF SEROTONIN AND METABOLITES BY SELECTIVE ION MONITORING

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SUMMARY

A direct connection system that requires no modifications to either the gas chromatograph or the mass spectrometer is described for the coupling of glass capillary columns to a commercial mass spectrometer. Vacuum-tight connections can be readily achieved with standard $\frac{1}{16}$-in. fittings and septum discs. The system can be operated with column outlet helium flow-rates of 1.5–2 ml/min at ion source pressures of $2–5 \cdot 10^{-5}$ mmHg. Although an injector splitter of our own design has been used, the initial test results obtained with a splitless injection method based on the use of a solids injection syringe together with a packed column injector assembly indicate the feasibility of developing a totally splitless and connection system that is simple to use.

The system has been applied to the detection of the pentafluoropropionyl derivatives of serotonin, methoxytryptamine, methoxytryptophol and 5-hydroxytryptophol by selective ion monitoring techniques Column performances under both sets of conditions (gas–liquid chromatography alone and combined with mass spectrometry) are compared and the evaluation of direct *versus* indirect coupling showed yields of the order of 50% through the molecular separator.

INTRODUCTION

The advantages of glass capillary columns with respect to their remarkable separating power and chemical inertness are well known. However, although the technology related to the preparation and application of these columns has progressed considerably[1–5], some of the practical problems associated with their connection to commercial instruments and especially to mass spectrometers have not yet been solved satisfactorily. A review of the literature shows various possibilities for achieving a working connection of glass capillaries to mass spectrometers[6–14], which can be classified into three main groups[13]: (a) direct splitless connection, (b) open split connection and (c) separator split. Although each of them has advantages and limitations depending on the user's experience and the intended applications, in principle splitless connection, which gives a 100% yield, would offer better detection limits in the low nanogram and picogram ranges. This factor can be critical when working with samples in which the concentration of the products of interest is of the order of

25 ng/g, as the case with tryptamine in brain tissue[15]. Against this consideration it could be argued that the direct coupling of a glass capillary column to the ion source of a mass spectrometer necessitates the operation of a given length of the column at sub-atmospheric pressure, which may lead to a loss of chromatographic performance, and also that carrier gas flow-rates are difficult to determine under these conditions. In any event, as capillary columns have a high separating power, direct splitless connection has been the subject of several studies[6–11]. Apart from those systems that involve a mechanical modification of the pumping capacity of the mass spectrometer[12,14], in the simpler systems the coupling is effected through a short length of a platinum capillary tubing, allowing all of the effluent to pass into the ion source[8,9]. However, the actual connection of the glass capillaries to the platinum tube has always been a critical step with regards to mechanical and thermal stability. It has been stated that the only practical material for rapid interchange and mounting of columns without breakage is PTFE shrinkable tubing[8]. This type of fitting has been used by various groups [8,12,13,16], although as it becomes permeable to air above 170° it may lead to a loss of vacuum in the ion source and excessive air background if precautions are not taken[8].

On the other hand, even with a splitless direct connection of the column outlet to the mass spectrometer, a significant proportion of the sample is still lost at the inlet by venting it through the injector splitter. The method of splitless injection cannot really be considered as a universal method for both isothermal and programmed-temperature separations. In fact, as the sample is first evaporated in the injector, transferred to the cold column and then vaporized again by starting a programmed run, it may even be considered to diverge from its basic concepts[1].

Taking all of these facts into consideration, a true "ideal" splitless gas chromatographic–mass spectrometric (GC–MS) system would be that which provided for (a) no sample losses at either the column inlet or outlet (100% yield at both ends), (b) no dead volume or excessive loss of chromatographic performance, (c) rapid and easy column mounting and interchange and (d) minimal modifications and technical sophistication together with maximal simplicity of operation. The work presented here includes the results we have obtained with respect to each of these characteristics.

The development of an integrated system as close as possible to this "ideal" has been approached with a practical application in mind. In the past 3 years, the analytical parameters that need to be implemented in order to obtain truly representative GC profiles of biogenic amines have been studied and defined[17–20]. In this context, a method has been developed for the simultaneous determination of the most significant components of the indolealkylamine metabolic pathway in biological samples[18]. This gave us the opportunity to establish the point at which the strict sensitivity and specificity requirements imposed by the complexity of biological samples become too rigorous when one attempts to shorten, simplify and/or avoid the necessary sample handling and clean-up procedures. The results soon compelled us to change from standard electron-capture detection methods to multiple-ion detection (MID) techniques[18]. For this purpose we designed and built a four-channel monitoring system[21] which has allowed the simultaneous quantitative analysis of tryptophan and its metabolites, 5-hydroxytryptophan, serotonin, tryptamine, indoleacetic acid and 5-hydroxyindoleacetic acid in packed columns. Recently we have extended this

work to a study of the chromatographic behaviour of 5-hydroxytryptophol, 5-methoxytryptophol and 5-methoxytryptamine, which should provide the capability of screening the complete metabolic profile of indolealkylamines in biological samples. On the other hand, as capillary columns, in comparison with packed columns, offer a marked increase in the overall resolution of the system, thus being especially suitable for the detection of these metabolites in a complex multi-component pattern, and as this high resolving power can be adequately complemented by the high specificity and sensitivity of mass fragmentographic techniques, we have undertaken a study of the direct coupling of a glass capillary column to a standard mass spectrometer operated in the specific detector mode.

EXPERIMENTAL

Reagents

Methanol, acetonitrile and benzene "per chromatografia" were supplied by Carlo Erba (Milan, Italy).

Pentafluoropropionic anhydride (PFPA) was supplied by Xpectrix S.A. (Barcelona, Spain). Serotonin (S), 5-methoxytryptophol (5-MTOL), 5-hydroxytryptophol (5-HTOL) and 5-methoxytryptamine (5-MT) were supplied by Regis (Morton Grove, Ill., U.S.A.).

Preparation of derivatives

An aliquot of the stock solution of the four indolealkyl compounds in methanol was evaporated to dryness under a stream of purified helium and the residue was allowed to react for 2 h at 60° with 50 μl of acetonitrile, saturated with sodium hydrogen sulphite, and 50 μl of PFPA[18]. The resulting solution was evaporated to dryness and the residue dissolved in benzene.

Column characteristics and preparation

Pyrex glass tubes (9 mm O.D., 3 mm I.D.) were washed successively with chromic acid, water, acetone, chloroform and methylene chloride, before drawing them with a Hupe-Busch 1045 A glass-drawing machine (Hewlett-Packard, Avondale, Pa., U.S.A.).

The capillary column thus obtained (26 m $\times$ 0.3 mm I.D.) was silanized with hexamethyldisilazane and filled by the static method with a 0.15% solution of GE SE-30 (Supelco, Bellefonte, Pa., U.S.A.) in methylene chloride.

Gas chromatography–mass spectrometry

The mass spectrometer used was a Hitachi RMU-6H equipped with a four-channel MID system of our own design[21], and coupled either conventionally through a Perkin-Elmer single-stage gold-jet-type separator, or directly as described below to a Perkin-Elmer Model 3920 gas chromatograph. The normal operating pressure of the mass spectrometer in the direct capillary connection mode was in the range of $1–5 \cdot 10^{-5}$ mmHg with a column flow-rate of up to 3 ml/min (the normal operating flow-rate was fixed at 1.5–2 ml/min). The mass spectrometric parameters were as follows: chamber voltage, 70 eV; emission current, 80 μA; trap current, 40–50 μA; accelerating voltage, 2400, 1800 and 1200 V, depending on the selected ions focused by

the MID; ion source temperature, 200°; separator and connection system temperature, 250°.

Capillary column connections

Fig. 1 is a schematic diagram of the direct connection system. Vacuum-tight connections are obtained with standard stainless-steel Swagelok fittings as shown. The connection of the column inlet to the injection block splitter assembly (Fig. 1a) as well as the connection of the column outlet (Fig. 1b) to the stainless-steel capillary tubing (No. 8 in Fig. 1) is made with a regular GC septum cut so as to fit the inner dimensions of the $\frac{1}{16}$-in. Swagelok nut. Both ends of the column pass through the septa discs which are used in place of the corresponding back and front ferrules. An adequate leak-tight seal is achieved by tightening the nut.

System operation

Direct coupling of the capillary column to the mass spectrometer. The samples

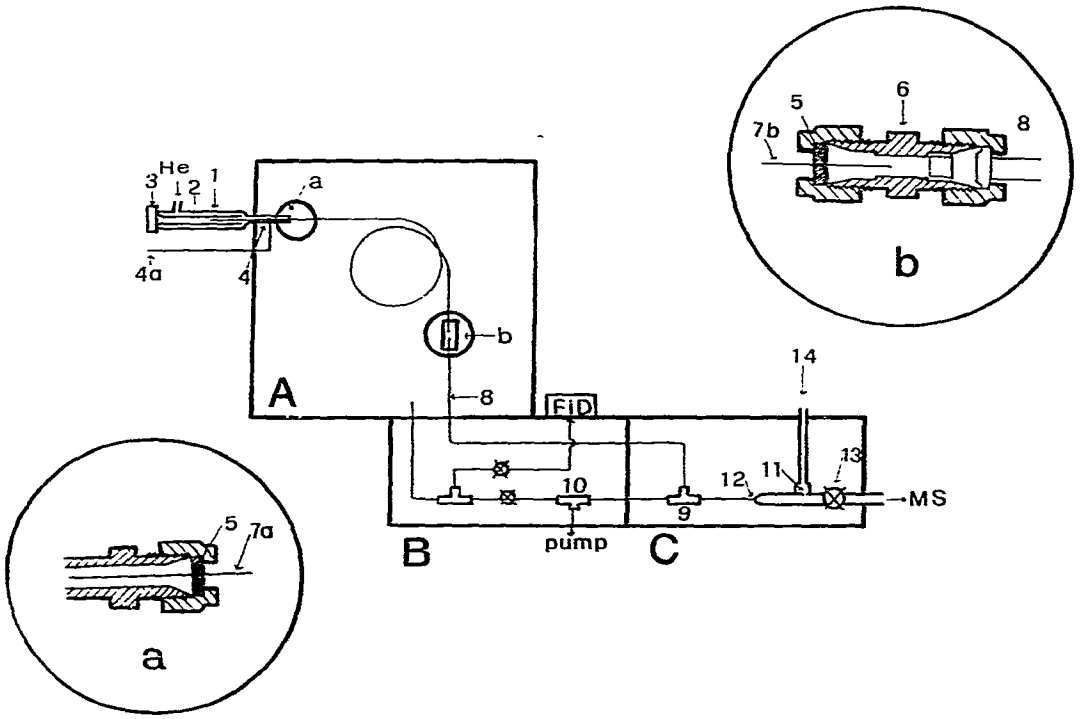

Fig. 1. Arrangement for direct or indirect connection (through molecular separator) of glass capillary columns to the mass spectrometer. The system is divided into three distinct zones: (A) GLC oven, (B) standard manifold of the Perkin-Elmer Model 3920 gas chromatograph and (C) extra manifold chamber. 1 = Standard packed column injector; 2 = $\frac{1}{8}$-in. I.D. glass liner; 3 = septum; 4 = home-built splitter assembly; 4a = vent restrictor (Perkin-Elmer No. 009, 0110); 5 = septum disc; 6 = $\frac{1}{16}$-in. Swagelok union; 7a = capillary column inlet; 7b = capillary column outlet; 8 = stainless-steel capillary (50 cm × $\frac{1}{16}$-in. O.D., 0.15–0.20 mm I.D.) coated with OV-101; 9 = Swagelok "tee"; 10 = single-stage jet separator; 11 = molecular leak; 12 = glass-to-metal seal; 13 = on–off bellows valve (Nupro 4 BW-SW); 14 = batch inlet system.

are injected through the regular packed column injector, which was fitted with a specially designed splitter unit made out of a 2-cm long piece of stainless-steel tubing ($\frac{1}{8}$-in. O.D.). One end of it can be connected to the injector via a $\frac{1}{8}$-in. nut and ferrule assembly while the other end is welded to a $\frac{1}{16}$-in. union. The $\frac{1}{8}$-in. tubing has a $\frac{1}{16}$-in. O.D. side-arm that ends in a vent restrictor (Fig. 1,4a) (Perkin-Elmer part No. 009.0110). The sample is injected with a restrictor (Perkin-Elmer No. 009.0110) in place capping the vent restrictor after 30 sec. A splitting ratio of 1:20 was used throughout this work.

The inlet end of the capillary column is inserted through the splitter unit and into the vaporization chamber of the injector. A tight seal is obtained with the small GC septum disc (5) placed inside the $\frac{1}{16}$-in. nut as shown in Fig. 1. This arrangement is also used at the outlet of the capillary column which is interfaced directly with the mass spectrometer through a 50-cm long stainless-steel capillary. Column changes are very simple to perform and when the direct connection line is not in use the $\frac{1}{16}$-in. union (No. 6 in Fig. 1b) is conveniently plugged with a regular $\frac{1}{16}$-in. cap. The connection capillary No. 8 ends in a Swagelok "tee" (9). From this "tee" there is a "line of sight" connection to the ion source of the mass spectrometer. The molecular leak of the batch inlet line is built into the side of the wide-bore glass tube in order to avoid any dead volume or unswept spaces[22]. The mass spectrometer can be isolated from the entire system by an on–off bellows valve which can be closed, for instance, while changing columns or to keep the solvent and/or unwanted peaks from reaching the ion source.

Indirect coupling of the capillary column to the mass spectrometer. Manifold B (Fig. 1) contains a single-stage jet separator. The effluent from the capillary or packed column can either be split for simultaneous flame-ionization detector (FID)/GC–MS operation or be passed to the separator unit. The exit to the FID can be shutt off with a micro-capillary valve with zero dead volume. Another of these valves is installed upstream of the separator. When capillary columns are run through the jet, pre-heated helium make-up gas is added at the column outlet at the rate of 12 ml/min.

Splitless injection technique

The samples are injected with a solids injection syringe equipped with a plunger fitted with a 1-cm long spiral section (Scientific Glass Engineering, London, Great Britain; Order Code SI-1RDS). A small measured volume of the liquid sample (*ca.* 1 μl) is deposited on the spiral section of the injector, where it is evaporated to dryness, and then the plunger containing the solid residue is retracted into the needle. After the needle has pierced the septum the plunger is depressed, exposing the sample to the heated chamber of the injection block.

RESULTS AND DISCUSSION

Connection techniques and operating parameters

Although the connection of capillary columns to a mass spectrometer in many instances involves sophisticated technology and/or operator expertise that are sometimes difficult to reproduce by other workers, the simple system described here makes use of commercially available parts of low dead volume (no mechanical modification or adjustment is needed other than the cutting of a septum disc in such a way that it fits inside a $\frac{1}{16}$-in. stainless-steel nut). A chromatographer experienced in using $\frac{1}{8}$- and

$\frac{1}{4}$-in. fittings for packed GC columns would have no problems in handling the $\frac{1}{16}$-in. nut and septum arrangement.

These fittings have no limitations within the useful temperature range of capillary columns and provide a leak-tight connection that, contrary to PTFE tubing[8], improves with temperature, as shown in Fig. 2. This property is probably due to an effect resulting from expansion of the septum by heat. On capping union No. 6 in Fig. 1, the pressure in the ion source is of the order of $2–3 \cdot 10^{-7}$ mmHg. With the glass capillary column connected, the operating pressure in the ion source is of the order of $2–5 \cdot 10^{-5}$ mmHg in the temperature range 150–250° and at a helium flow-rate of 2 ml/min into the column. The maximum flow-rate of helium through the capillary column that the ion source can tolerate is 3 ml/min measured at room temperature at the column outlet.

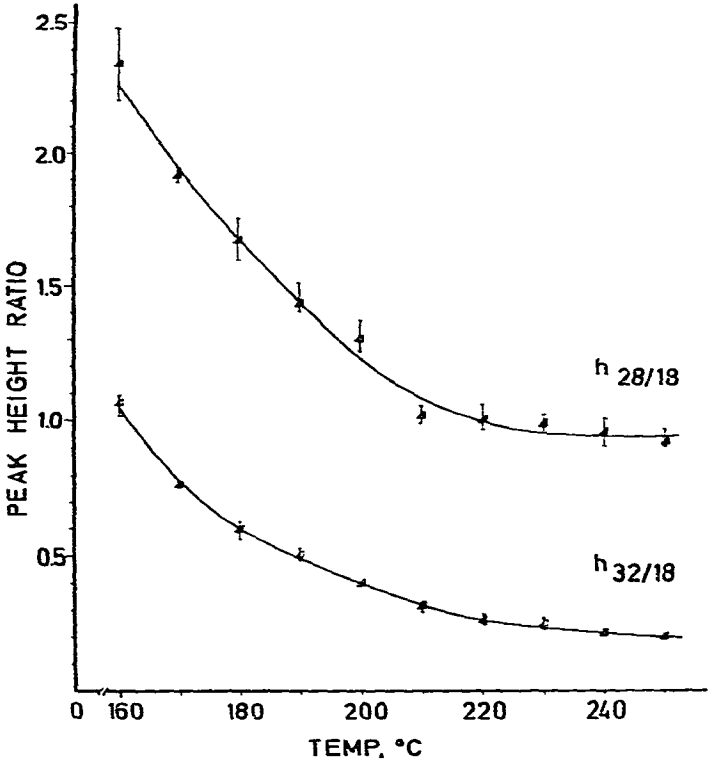

Fig. 2. Peak-height ratios of air to water (*m/e* 28, 32 and 18) background ions in the mass spectrometer as a function of the temperature of the capillary column fittings (same temperature as that of the GC oven). Ion source and coupling line temperatures constant at 200° and 250°, respectively.

Owing to the low splitting ratio used (1:20), the access of the solvent front to the ion source may shut off the vacuum protector of the mass spectrometer if the pressure increases above $5 \cdot 10^{-5}$ mmHg. For this reason, before injecting a sample either the bellows valve (No. 13 in Fig. 1) is closed or the protector circuit is by-passed by setting the vacuum gauge range at 10^{-4}. In the first instance the flow is diverted backwards towards the separator (No. 10) and most of the solvent front is evacuated

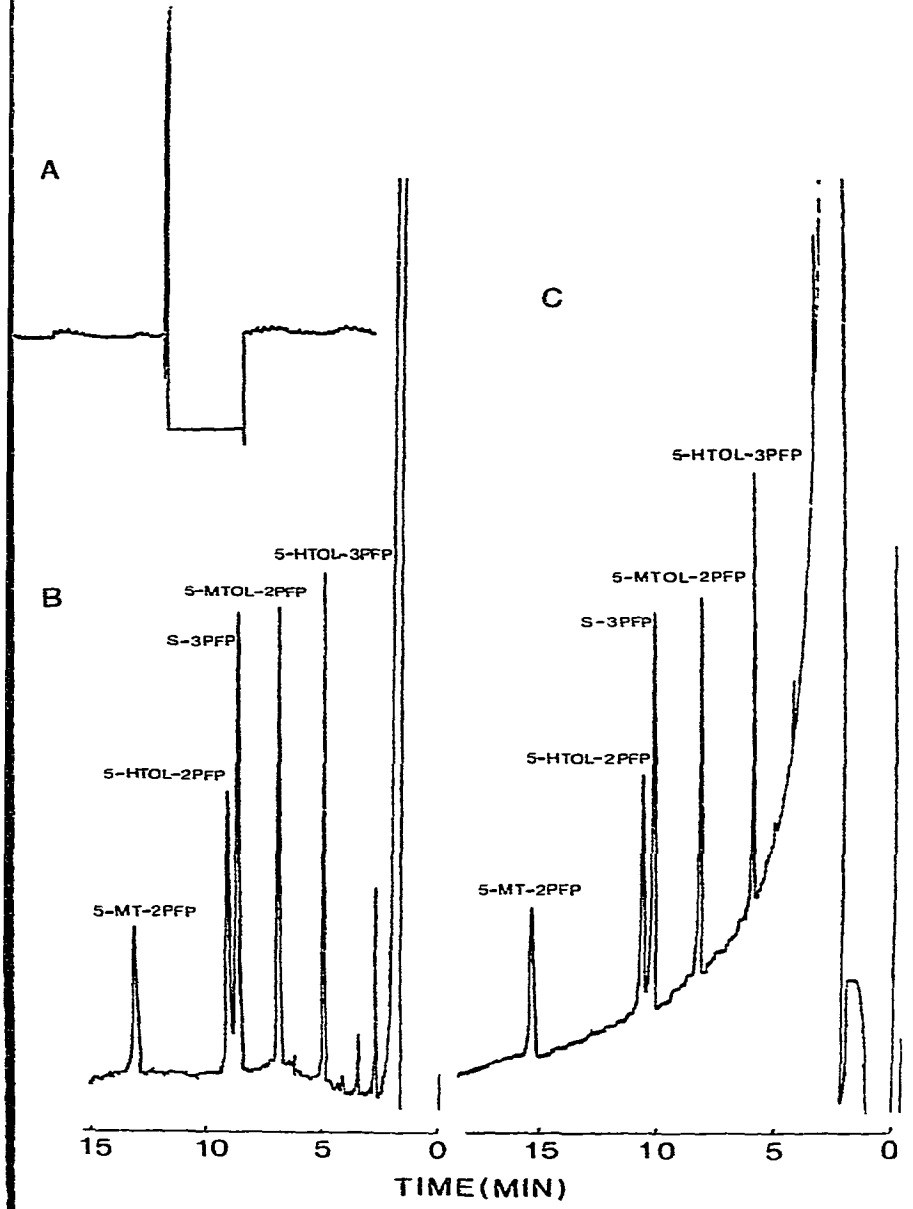

Fig. 3. (A) Baseline stability to changes in ion source operating pressure conditions on direct GC–MS coupling. Main isolation valve closed for 3 min (No. 13 in Fig. 1). (B) Total ion monitor profile of a sample of the four compounds (5-HTOL partially and fully perfluoroacylated) separated on a 26 m × 0.3 mm I.D. glass capillary column at 170°. Injector at 250°, splitter at 250°. Helium carrier gas flow-rate measured at the column outlet, 2.6 ml/min. Coupling manifold at 250°. The column is directly coupled to the mass spectrometer, as described in the text. Mass spectrometer operating conditions: chamber voltage, 70 eV; emission current, 70 μA; trap current, 45–50 μA; accelerating voltage, 2400 V. Splitting ratio, 1:20. Amounts in the range 400–900 ng of each were injected. (C) FID profile of the same sample under identical GC conditions.

TABLE I

MOST CHARACTERISTIC IONS OBSERVED IN THE ELECTRON-IMPACT MASS SPECTRA OF EACH OF THE DERIVATIVES UNDER STUDY

Relative abundances are given in parentheses.

*Ion**	*5-MTOL-2PFP*	*5-MT-2PFP*	*S-3PFP*	*5-HTOL-3PFP*	*5-HTOL-2PFP*
M^+	483 (87)	482 (20)	614 (5)	615 (4)	469 (16)
$M - HX\text{-}COC_2F_5$	319 (96)	319 (90)	451 (100)	451 (26)	305 (74)
$M - CH_2\text{-}X\text{-}COC_2F_5$	306 (100)	306 (85)	438 (48)	438 (14)	292 (100)
$M - CH_2\text{-}X\text{-}COC_2F_5 - COC_2F_5$	159 (85)	159 (100)	291 (16)	291 (7)	145 (51)

* X represents the NH group in the amines and the –O– group in the alcohols; M is the molecular ion.

by the separator forepump. This valve can also be closed in the middle of a run in order to eliminate any unwanted zone of the chromatogram. This does not cause any loss of stability in the system, as shown in Fig. 3A. The chromatogram in Fig. 3B shows the type of response obtained by allowing the solvent ($\leqslant 1\ \mu l$) into the source without closing the Nupro valve. Although the pressure may increase up to *ca.* $3 \cdot 10^{-4}$ mmHg in the source, the baseline is very stable and the solvent does not produce a significant tailing effect compared with the corresponding FID trace shown in Fig. 3C.

With regard to the home-built splitter assembly, no detailed evaluation of its overall performance was attempted, mainly in view of our ultimate aim of eliminating it as part of our project to develop a true, if not "ideal", splitless GC–MS system. Nevertheless, the quantitative results obtained in tests carried out over more than 1 years' use were consistent with the degree of molecular size discrimination and repeatability that could be expected from such a design under the experimental conditions used (Fig. 3), that is, a negligible level of peak area discrimination by molecular weight, at a splitting ratio of 1:20, between the PFP derivatives whose molecular weights differ by one mass unit [*e.g.*, between 482 (5-MT) and 483 (5-MTOL) and between 614 (S) and 615 (5-HTOL)] (see Table I) with larger differences between the peak area responses at both extremes (*e.g.*, 482 and 614). In any event, such quantitative errors would be eliminated by MID quatitation using deuterated analogues as internal standards.

The derivatization conditions described in the experimental section give rise in all instances to the fully perfluoroacylated derivatives. However, when the reaction time is reduced below the optimum of 2 h the partially diacylated form of 5-HTOL appears in the chromatograms, as shown in Fig. 3C (5-HTOL-2PFP). This is in agreement with the results reported for a 20-min reaction time[23].

It is also interesting that the values of the retention time differences (Δt_r) calculated by measuring the t_r of each substance in both direct capillary GC–MS and GC are similar for each one substance at a given flow-rate, with values decreasing at higher carrier gas flow-rates as shown in Fig. 4. The retention time of a substance in combined GC–MS could be defined by a expression such as

$$t_{ri} = \frac{L}{V_i} + \frac{L'}{V'_i}$$

where

L = column length not affected by vacuum;
V_i = average flow velocity in length L;
L' = length that the substance travels under sub-atmospheric conditions until it reaches the ion source ($L' \ll L$);
V_i = average flow-rate in length L'.

The ratio L'/V_i is constant for all compounds and varies with the column flow-rate[6], which would explain the fact that the Δt_r values of all substances are very similar at a given flow-rate. When the carrier gas flow-rate is increased, L' would be expected to decrease, thus reducing the influence of the second term in the above equation at the higher flow-rates tolerated by the mass spectrometer.

TABLE II

COMPARISON OF CAPILLARY COLUMN PERFORMANCES IN GC AND DIRECTLY COUPLED GC–MS OPERATION

Helium flow-rate through the capillary column measured at the column outlet at room temperature. Column performance expressed as total number of plates, N. Values given in parentheses correspond to the data obtained by direct coupling of the glass capillary column (26.5 m × 0.3 mm I.D.) to the mass spectrometer. The numbers shown between pairs of values of N for each flow-rate indicate the percentage decrease in performance found on direct GC–MS operation.

Flow-rate (ml/min)	*5-HTOL-3PFP*		*5-MTOL-2PFP*		*S-3PFP*		*5-MT-2PFP*	
	N (170°)	*t_r (min)*	*N (170°)*	*t_r (min)*	*N (180°)*	*t_r (min)*	*N (180°)*	*t_r (min)*
1.4	66,000	7.1	72,800	9.8	37,000	8.7	47,750	13.4
	1.5		36		32		67	
	(65,000)	5.4	(46,500)	7.6	(25,000)	6.6	(16,000)	9.4
1.9	84,000	5.6	89,000	7.6	41,500	7.1	50,000	9.7
	20		47		25		57	
	(67,000)	4.9	(46,000)	6.7	(31,000)	6.1	(21,500)	8.7
2.6	99,000	4.6	74,000	6.2	48,000	5.7	47,500	8.0
	22		31		29		37	
	(77,000)	4.5	(51,000)	6.1	(34,000)	5.4	(29,700)	7.7
≥3	81,000	3.5	66,600	4.7	40,750	4.7	47,666	6.9

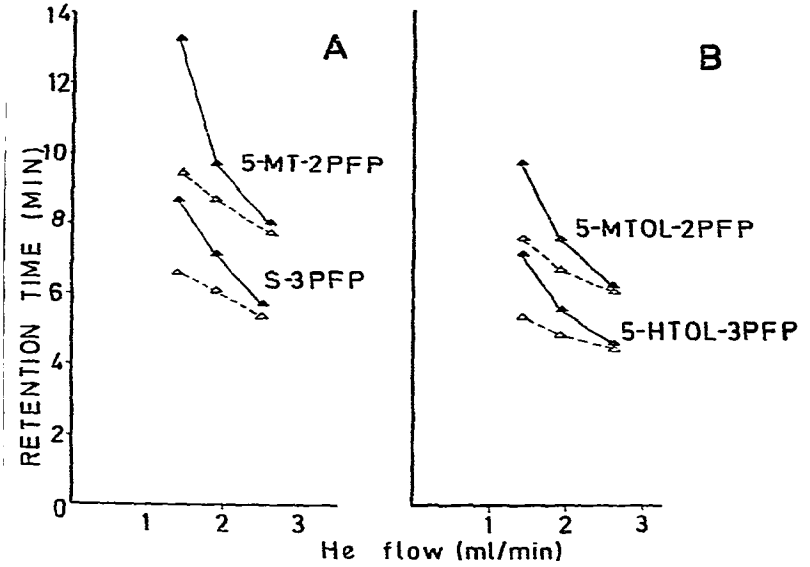

Fig. 4. Plots of the retention times of each of the four derivatives measured under the conditions in Fig. 3. Solid line obtained by GC; broken line obtained by direct GC–MS coupling. (A) Data at 180°; (B) data at 170°.

Table II summarizes the efficiency parameters of the chromatographic column in both modes of operation (GC and directly coupled GC–MS). The highest operating efficiencies lie in the flow-rate range of 1.9–2.6 ml/min, depending on the nature of the substances. The data in Table II indicate (a) that the alcohol (5-HTOL and 5-MTOL) derivatives are chromatographed much more satisfactorily than their corresponding amines in both systems (GC and direct GC–MS) and (b) that the 5-methoxy compounds show higher efficiencies at 1.9 ml/min while the performance of the 5-hydroxy compounds is better at 2.6 ml/min. In GC the column efficiencies at a flow-rate of 2.6 ml/min range from a maximum of 3736 to a minimum of 1972 plates/m, while in direct GC–MS the corresponding values are 2906 and 1120 plates/m. If the flow-rate is increased to about 3 ml/min the number of plates decreases in all instances indicating that it is possible to achieve optimal GC–MS coupling conditions.

The calculated percentage differences in the column efficiencies range from 22 to 37% at optimal flow-rates. Nevertheless, from a practical point of view this decrease in performance is not reflected in terms of peak shape and resolving power as shown by the virtually identical chromatograms in Figs. 3B and 3C. It would be interesting to compare these values with those obtained with other directly coupled GC–MS systems in practical applications. However, to our knowledge there are no detailed reports on the experimental comparison of capillary column performances in GC and in combined direct GC–MS. Although the theoretical treatment predicts that the differences should not be very large[6], and this prediction has been supported by a comparison of the HETP values of *n*-heptane in a 110 m × 0.25 mm I.D. column coated with squalane at atmospheric outlet pressure and vacuum outlet pressure, the data in Table II seem to indicate that this is not the case, at least in this work.

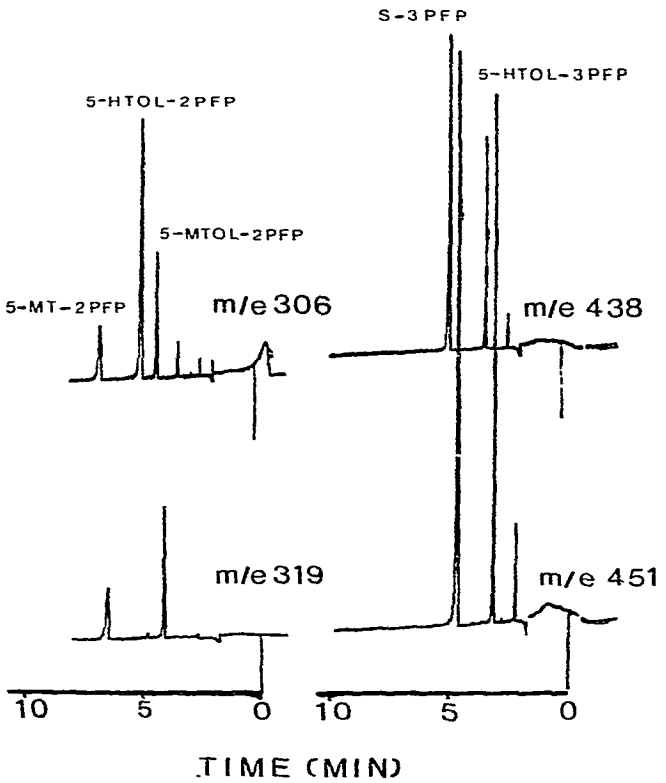

Fig. 5. MID profiles of the perfluorocylated derivatives of 5-HTOL, S, 5-MT and 5 MTOL obtained with an SE-30 glass capillary column (26.5 m × 0.33 mm I.D.) directly connected to the mass spectrometer. Column at 190° with a helium flow-rate of 2 ml/min. Injector at 250°. Ionizing voltage, 70 eV; nominal accelerating voltage, 1800 V. Multiplier voltage, 4 V. The four profiles were registered on two dual-pen recorders; in both the top trace is slightly offset. The true zero injection times are indicated by the downward spikes.

Direct GLC–MID of serotonin and metabolites

Fig. 5 shows the mass fragmentographic profiles obtained by focusing the MID system upon ions at *m/e* 306, 319, 438 and 451, which are among the most prominent and characteristic ions in the mass spectra, as summarized in Table I. The ions at *m/e* 306 and 319 are used to detect the 5-methoxyindole PFP derivatives while those at *m/e* 438 and 451 characterize the 5-hydroxyindole PFP derivatives. When the derivatization reaction has not gone to completion, the ion profile at *m/e* 306 also detects the presence of 5-HTOL-2PFP, as shown in Fig. 5. Thus in one injection the presence of each compound is verified by its response to at least two different masses, providing a high degree of reliability.

Quantitative analysis

The response curves of both alcohol PFP derivatives in the low nanogram range are given in Fig. 6. These curves were obtained by selected monitoring of the ions indicated. However, one must take into account that, at a splitting ratio of 1:20

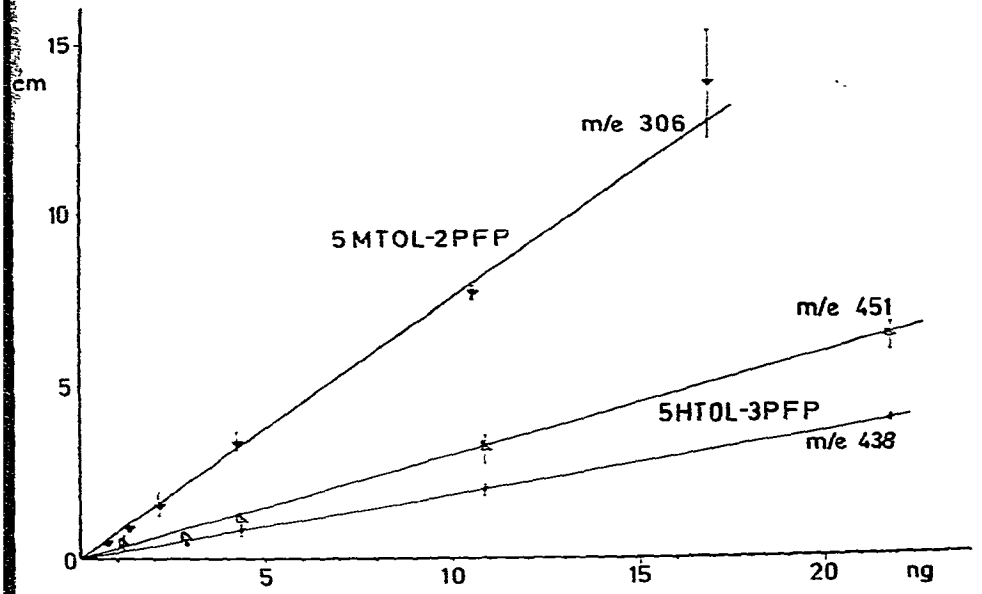

Fig. 6. MID response curves for 5-MTOL-2PFP at *m/e* 306 and 5-HTOL-3PFP at *m/e* 438 and 451. Ordinate, peak height (cm); abscissa, amount injected (ng). Each point where a dispersion range is shown represents the mean value of at least three determinations.

an injection of 0.6 ng (see *m/e* 306) represents a detection level of 30 pg in the ion source, which underlines the importance of combining the operation of a directly coupled GC–MS system with a truly splitless injection technique. Detection levels of the order of 5–30 pg have been obtained with packed columns (no injection splitting) coupled through the molecular separator.

However, as is evident on applying any of the splitless injection methods described in the literature[24,25], in comparison with the injection systems designed for packed columns they have much to lose in terms of sample size, simplicity, reproducibility, discrimination according to molecular weight and concentration and ease of operation. An attempt was therefore made to try to acommodate a capillary column with a regular metal injection assembly equipped with a reduced-bore glass liner. This approach necessitates that the solvent be removed before introducing the sample into the glass liner, because after this step there is no way out other than through the column itself. For this purpose a solids injection syringe was operated in the manner described in the experimental section. As shown in Fig. 7, the initial results obtained in tests of the stability of these derivatives with this mode of injection in a packed column are promising. Likewise, preliminary tests run on capillary columns gave good results, although the column performance decreased. Work is under way to correct this effect by reducing the inner bore of the glass liner to match the capillary dimensions of the columns used.

The possibility of quantitative losses through the separator was checked by means of a capillary valve installed between points 9 and 10 in Fig. 1. By closing this valve, the line to the separator was isolated so that there was no possibility of back-diffusion towards the separator unit. Under these conditions replicate samples of

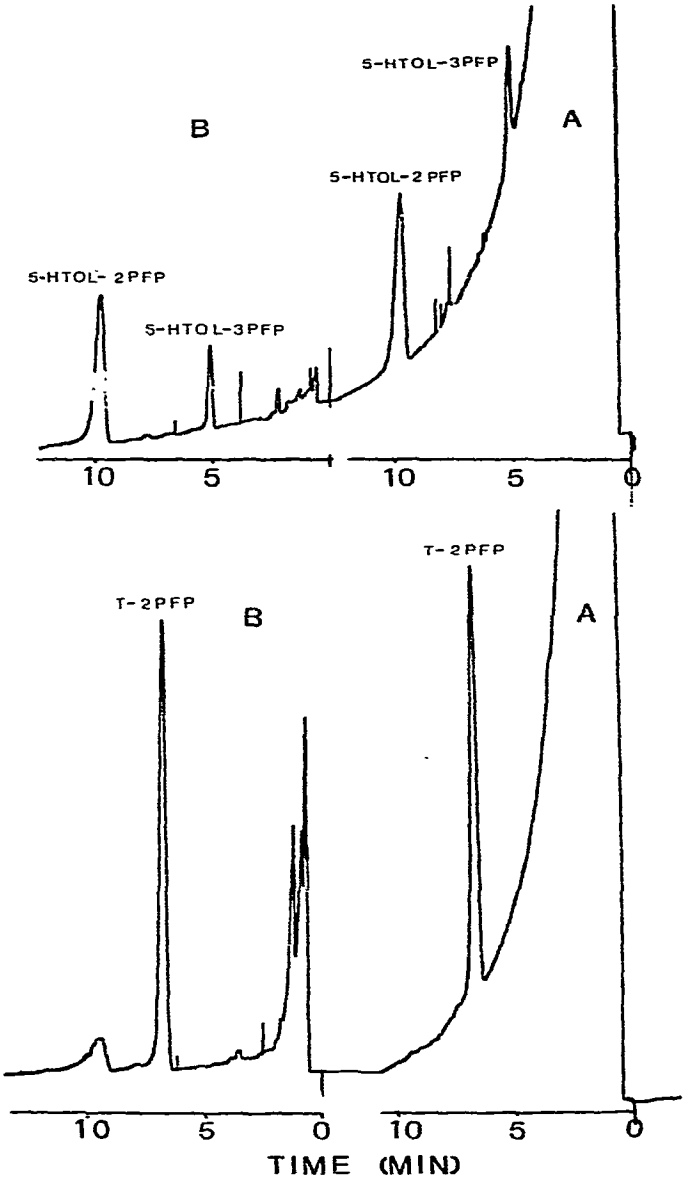

Fig. 7. Upper trace: (A) Chromatographic separation of the two acylated forms of 5-HTOL in a packed column filled with 5% SE-30 on Chromosorb W (100–120 mesh). Column temperature, 180°; injector, 250°; detector (FID), 250°. A 1-μl volume of the sample (340 ng) was injected with a 1-μl Hamilton Model 7001 syringe into the regular metal injector, equipped with a glass liner, of the Perkin-Elmer Model 3920 gas chromatograph. (B) Same sample injected with an SGE SI-1RDS solids injector after pre-evaporation of the solvent. Note the virtual disappearance of the solvent peak. Lower trace: (A) and (B) Same conditions as above except that perfluoroacylated tryptamine (T) was used.

10.5 ng of 5-MTOL-2PFP were injected into the column with the capillary valve closed (all effluent to the ion source) and with the valve open (possibility of flow towards the jet). Peak heights were calculated as the sum of the heights of each of the steps produced by each MID scan[21]. For the ion at *m/e* 319 these values were 24 ± 0.9 cm with the valve open and 25.1 ± 1.6 cm with the valve closed, which represents a difference of only 4%. This was confirmed by focusing also on the ion at *m/e* 306. Therefore, in order to avoid any extra fittings in the line, the valve was removed.

Indirect GLC–MID (with jet interface)

A comparison was made between the performances of glass capillary columns coupled directly and through the single-stage jet to the ion source of the mass spectrometer. The operation of these columns through the jet required the addition of a make-up flow of 12 ml/min of helium to the column exit by means of an arrangement similar as that used for the inlet splitter, although in this instance the make-up gas was added through the side-arm. The necessary flow was determined from the response given by the ion at *m/e* 319 for 5-MTOL-2PFP at various flow-rates while maintaining a constant column flow-rate of 2 ml/min. The decrease in response thus obtained for 5-MTOL-2PFP and 5-HTOL-3PFP gave values of the order of 52% and 63.5%, respectively, in relation to the responses obtained through the direct connection. These values are in agreement with the predicted efficiency of this type of separator[26].

ACKNOWLEDGEMENTS

We thank the Instituto Químico de Sarriá, Barcelona, for the use of a capillary drawing machine. C.S. is also grateful to Prof. G. Guiochon for her short stay at his laboratory and for fruitful discussions. This work was supported by a Research Grant from the Comisión Asesora de Investigación Científica y Técnica.

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