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SIMPLE DEVICE FOR SOLVENT-FREE INJECTION OF HIGH-BOILING COMPOUNDS IN CAPILLARY GAS CHROMATOGRAPHY

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SUMMARY

The construction and performance of a simple and easily made all glass injector for solvent-free transfer of high-boiling compounds onto capillary columns is described. Samples are applied with a hypodermic syringe onto a deactivated porous sintered glass layer fused to the wall of a glass capillary tube from which the solvent is evaporated prior to installation of the tube in the gas chromatographic system. Good reproducibility of normalised peak areas and retention times were obtained which were unaffected by the boiling points of the solvents used and by sample volumes in the range 1–20 μl.

INTRODUCTION

Satisfactory techniques and commercial devices based thereon have been available for some time to facilitate the transfer of various types of samples onto high-resolution gas chromatographic (GC) capillary columns. Each technique has advantages and disadvantages. For example, conventional splitting of the sample at the point of injection, such that only a small fraction of the sample is transferred onto the column, is unsuitable for the analysis of components present in solutions at only trace levels. At present, the most popular of the injection devices proposed to deal with such problems are those of the splitless type developed by Grob and co-workers[1–3]. Such devices are now available for most types of chromatograph. More recently Grob and Grob[4] have devised an on-column injector by means of which the entire sample is deposited in the capillary column without vaporisation of the solvent. This device, although relatively expensive, appears to be the closest approach so far to the ideal capillary column injector, and is particularly suitable for use with the newer types of fused-silica columns containing permanently bonded stationary phases which are not damaged by condensed solvents.

With practice, our experience in the use of injectors of the splitless type have by and large been satisfactory for many applications. However, at times we have encountered difficulties when dealing with the analysis of trace concentrations of biologically active compounds of low volatility. In essence, we have found that despite

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our best efforts to optimise operating conditions, the injectors have tended to discriminate against later eluting components, especially those emerging from the column at temperatures in excess of 280°C in temperature programmed runs. Apart from the implications for quantitative analysis, one of the most undesirable consequences of the effect was observed in combined GC–mass spectrometry (MS) runs; decreasing peak sizes at high temperatures frequently coincided with an increase in the mass spectrometer background response due to bleed from the column, thereby degrading the quality of the spectra and raising the detection limits of minor high-boiling components.

Solvent-free or solids injection devices are attractive alternatives for the analysis of high-boiling substances present in very dilute solutions. The moving-needle system[5] is perhaps the best known and allows the application of samples up to 5 μl. A precolumn technique using a chemically bonded stationary phase and a special water cooled injection system permits the quantitative transfer of sample volumes up to 20 μl to be made[6]. Yet another approach is the Curie-point injector[7] which is an expensive and sophisticated device. More recently, Vogt *et al.*[8] have described an injector which permits sample volumes as high as 250 μl to be applied with a high degree of reproducibility. Replacement of the existing injection port and heater assembly on the chromatograph is required for the system.

The expedient of removing the solvent from the sample within the chromatographic system is an important consideration which influences the design of most of the solvent-free injectors described in the literature. In our view, this requirement imposes in the design a level of sophistication, which, in its absence, might not be necessary. We are not convinced that there is any particular merit in introducing several microlitres of a solvent near the inlet of a capillary column, only to immediately thereafter effect its complete expulsion from the system. Indeed, we would suggest that, provided a time-consuming and cumbersome procedure is not involved, the removal of solvent before the introduction of the sample into the closed chromatographic system has much to recommend it. Based on this approach we have designed an all-glass injector which can be readily constructed at very low cost using readily available materials. The fabrication and evaluation of this device is the subject of the present paper.

EXPERIMENTAL

Details of construction

The complete injector mounted vertically on a gas chromatograph with a top-entry inlet system is shown in detail in Fig. 1. It was designed to fit into one of the heated injector blocks of the chromatograph from which the injection head had been removed. The carrier gas supply to this head was used as the auxiliary flow for removal of solvent from the sample. The column flow was provided from the second injection head via a length of 1/16 in. O.D. stainless-steel tubing which was silver soldered into a hole drilled through the side of a 1/4 × 1/16 in. Swagelok reducing connector (1), the latter being used to connect the injector to the column (2) using high-temperature Vespel ferrules.

The outer body of the injector was made by joining together in series a standard Quickfit (Type SQ13) screwthread connector (3), a piece of 6 mm O.D. × 2 mm

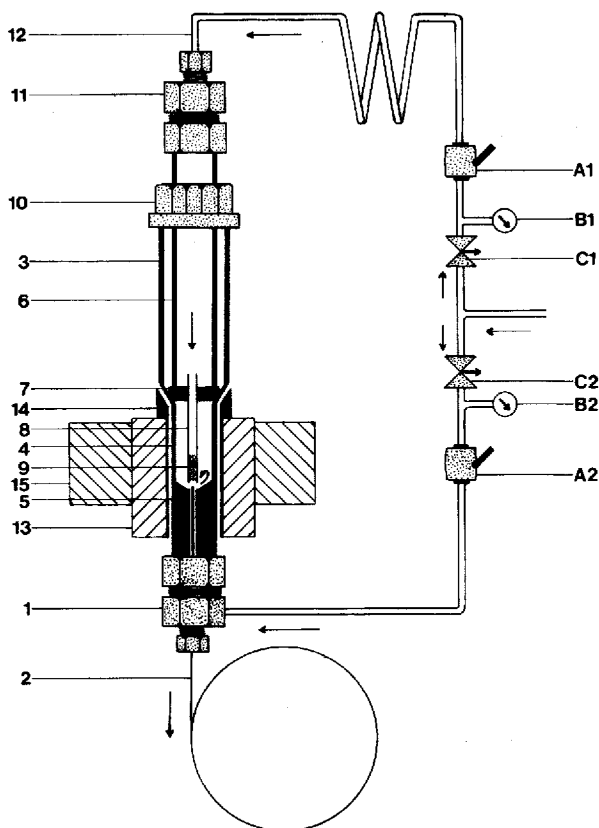

Fig. 1. Schematic representation of injection device. 1 = 1/4 × 1/16 in. Swagelok connector with 1/16-in. stainless-steel sidearm; 2 = capillary column (fused-silica end); 3 = Quickfit (SQ13) screwcap connector, 110 mm, 8-mm bore; 4 = 50-mm glass tube, 6 mm O.D. × 2 mm I.D.; 5 = 50-mm glass tube, 6 mm O.D. × 0.8 mm I.D.; 6 = 6 mm O.D. × 2 mm I.D. glass tube; 7 = silicone rubber septum; 8 = capillary tube; 9 = porous glass sinter; 10 = screwcap + silicone washer; 11 = 1/4 × 1/8 in. Drallim connector; 12 = 1/8-in. nylon tubing; 13 = heated injection block; 14 = 10 mm I.D. glass collar; 15 = oven wall; A1 and A2 = on/off toggle valves; B1 and B2 = pressure gauges; C1 and C2 = pressure regulators. Arrows indicate the direction of auxiliary flow (from C1) and column flow (from C2).

I.D. glass tube (4) and a similar length of 6 mm O.D. × 0.8 mm I.D. capillary tubing (5). The removable inner assembly consisted of a length of 6 mm O.D. × 2 mm I.D. glass tubing (6) onto one end of which was bonded a 6-mm diameter silicone rubber septum (7) using an epoxy resin. A capillary tube (8), containing a porous glass sinter (9) at one end (see below) onto which the sample was desposited, was held in a tight but sliding fit in a small hole drilled through the centre of the septum. The entire inner assembly was connected within the body of the injector by means of a plastic screw-cap nut and silicone rubber washer (10), and gas flow through the sample tube was provided via a 1/4 × 1/8 in. Drallim connector (11) and a length of flexible 1/8 in. Nylon tubing (12). With this arrangement the sample tube could be transferred between cold and heated zones in the injection body by moving tube 6 within the screwcap nut and washer. Since it was necessary for the latter to be reasonably tight in

order to avoid leaks —especially when using columns with a high pressure drop— movement of the inner assembly was best performed by holding the body (3) with the left hand, while grasping the connector 11 with the right to execute the sliding action. When fully pushed down in the sample inject position (Fig. 1), the sample tube was positioned directly over the capillary column inlet and the septum (7) formed a seal at the point where the injector body narrowed. Transfer of heat at this point from the injection block (13) to the septum was avoided by supporting the body at a distance of about 25 mm from the top of the injection block by means of a loose fitting 10 mm I.D. glass collar (14).

Sintered glass capillary sample tubes

These were prepared from 60-mm lengths of melting point capillary tubes (bore 1–1.5 mm) as follows. A tube was supported in a vertical position against a flat heat-resistant surface, and filled to a height of 10–12 mm with chromatographic grade glass beads (80–100 mesh). A small flame from a microburner was then run up and down the outside of the tube over the packed section until a partial fusion of the glass beads both to one another and to the wall of the tube had taken place. The precise degree of heating required could be assessed fairly accurately after a few trial runs, since the desired effect was that of an even, stable and porous sintered glass section within the tubes. Excessive heating resulted in too much fusion of the beads, leading to a low porosity glassy effect, while too little heat caused much of the packing to be dislodged when the tubes were rinsed with liquids.

Deactivation of the sintered capillary tubes was carried out by first heating them in 20% sulphuric acid at 110°C for 12 h to remove metal ions, followed by washing with distilled water and acetone and purging with nitrogen gas to dry them out. Subsequently, the tubes were coated with a 2% solution of Carbowax 20M in dichloromethane and, after removal of the solvent, were heated for 12 h at 280°C under a flow of nitrogen. An alternative treatment involved coating with SE-30 and heating at 400°C. The heat treatment was most conveniently carried out in the injection device after first disconnecting the column. Finally the tubes were rinsed with distilled dichloromethane and dried. High temperature deactivation of glass and other surfaces using stationary phases such as Carbowax 20M[9] and polymethylsiloxanes[10] is an effective and well established procedure in the preparation of high-performance capillary columns.

Sampling technique

For application of the sample, the sintered glass capillary tube was removed from the injector by unscrewing the plastic nut (10) and lifting out the entire inner assembly, including the nut. With the sample tube pointing upwards at an angle of about 30° from the horizontal, the assembly was placed in a clamp, which may be either attached to the chromatograph or held in a support stand beside the instrument. Since carrier gas flow through the column was diverted to the atmosphere on removal of the inner assembly, the outer body was immediately sealed with a second plastic nut fitted with a silicone rubber blanking plug, thereby restoring column flow.

With a small flow (2–3 ml/min) of carrier gas through the sample tube from the auxiliary gas line, sample was applied to the sintered glass layer using a hypodermic syringe fitted with a flat-tip needle. The porosity of the glass was such that, depending

on the solvent, up to 2 μl of sample could be applied directly without any tendency of the liquid to pool. Larger volumes should be applied more slowly, step by step, over some minutes to allow time for the solvent to evaporate. The inclusion of an on/off toggle valve (A1) in the gas line was useful when applying larger volumes, since momentary interruption of the gas flow facilitated the dispersion of the solvent over a greater surface area of the sintered sample tube, thereby enhancing its subsequent rate of evaporation. When the latter step was complete, the sample tube was reinstalled in the injector body so that its tip was approximately in line with the top of the glass supporting collar (14). With the capillary column at ambient temperature, the sample was then volatilised and cold trapped on the column by simply pushing down the sample tube into the heated injection position as shown in Fig. 1. After allowing a minute or two for stabilisation of the flow, the column temperature was raised to the starting value and the run commenced. Initial cold trapping of the sample was not essential when using wide-bore capillary columns (see Results and discussion).

Chromatographic analysis

A temperature-programmed Pye Unicam (Model 204) gas chromatograph with a flame ionisation detector was used in conjunction with a Spectra-Physics computing integrator for quantitation of the data obtained. Analyses were performed with a 20 m × 0.3 mm bore glass capillary coated with SE-30 and also with a 10 m × 0.6 mm wide-bore glass support-coated open tubular (SCOT) column containing diethylene glycol succinate (DEGS) plus phosphoric acid on a layer of deactivated silica. To permit easy connection to the injector and detector, the columns were terminated at both ends with 0.5-m lengths of coated flexible fused-silica tubing. The latter were sealed into the columns using a high temperature polyimide adhesive.

RESULTS AND DISCUSSION

The performance of the injector was evaluated using a mixture of *n*-alkanes (C_{18}–C_{32}) possessing a relatively wide boiling range, as proposed by Schomburg *et al.*[11] and also with a sample of long chain fatty acid methyl esters.

The effects of sample size and solvent boiling point on the reproducibility of relative and total peak areas and of retention times were examined with the alkane mixture on the SE-30 column under temperature programmed conditions; the results are presented in Table I. Alkane mixtures were prepared in different solvents having concentrations in the range 5–100 ng per component. The data in Table I show clearly that neither the volatility of the solvent nor the volume applied to the sintered capillary sample tube had any significant effect either on the relative or total peak areas or on the absolute retention times (measured for C_{32}). It has been proposed[12] that high-performance capillary systems should satisfy the following criteria: normalised peak areas should be reproducible to 1% or better, relative standard deviation and retention times to 0.1% or better. The coefficients of variation calculated for the data in Table I show that, despite substantial differences in the volatility of the solvents and the sample volumes applied, the present system largely fulfilled both of these stringent requirements.

In preliminary studies with the injection device, somewhat variable recoveries for the C_{18} and (to a lesser extent) C_{20} alkanes were observed. Having verified that

TABLE I

EFFECT OF SOLVENT AND SAMPLE VOLUME ON RELATIVE AND TOTAL PEAK AREAS AND RETENTION TIME

Solvent	*Sample concentration (ng per peak)*	*Volume applied (μl)*	*Relative peak area (%)*								*Total peak area (integrator counts)*	*Retention time of C_{32} (min)*
			C_{18}	C_{20}	C_{22}	C_{24}	C_{26}	C_{28}	C_{30}	C_{32}		
Diethyl												
ether,	100	1	11.4	12.0	12.2	12.6	12.5	13.5	13.0	12.7	2893	18.48
b.p. 35°C	20	5	11.5	12.2	12.5	12.7	12.7	13.2	12.7	12.5	2856	18.46
n-Hexane	100	1	11.5	12.2	12.7	12.7	12.6	13.3	12.7	12.3	2838	18.49
b.p. 68°C	5	20	12.0	12.0	12.4	13.1	12.9	13.2	12.4	12.1	2872	18.48
Toluene,												
b.p. 110°C	100	1	11.9	12.3	12.6	12.6	12.6	13.0	12.5	12.4	2935	18.44
$\bar{x}$			11.7	12.14	12.48	12.74	12.66	13.24	12.66	12.4	2885	18.47
S.D.			0.24	0.12	0.17	0.18	0.14	0.16	0.2	0.2	34	0.019
C.V. (%)			2.1	0.99	1.4	1.4	1.1	1.2	1.6	1.6	1.2	0.1

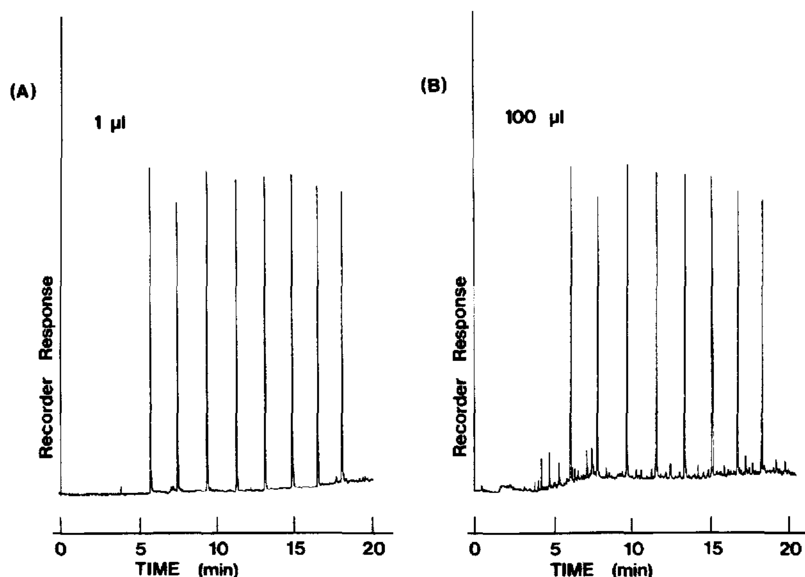

Fig. 2. Gas chromatograms from (A) a 1-µl injection of a C_{18}–C_{32} alkane mixture (100 ng per component) and (B) a 100-µl sample (1 ng per component) dissolved in diethyl ether. Column: 20 m × 0.3 mm I.D. glass capillary coated with SE-30. Carrier gas: hydrogen at 2 ml/min. Column temperature programmed from 150 to 310°C at 8°/min. Injection block temperature: 250°C.

losses of these compounds were not occurring during evaporation of the solvent, it appeared that the difficulty arose at the point of injection. The most likely explanation was that as the sample was being volatilised at the column inlet, the carrier gas may have carried a small amount of the more volatile alkanes into the cooler upper part of the injection block where condensation occurred. The problem was overcome by the simple expedient of closing the toggle valve (A2) on the carrier gas line during the injection step and then restoring the flow a minute or so later. Interruption of the latter was not necessary for quantitative transfer of compounds with boiling points higher than C_{20} (343°C). It may also be noted that an injection block temperature of 250°C was sufficiently high to ensure complete transfer of the C_{32} alkane (b.p. 467°C). For most applications with this injector, sample volumes in the range 1–10 µl should be adequate; however in certain instances it may be desirable to handle much larger volumes and the device is suitable for this purpose. As an example, a comparison of the analysis of 1 µl of the standard alkane mixture with a 100-µl aliquot of the same sample, diluted 100-fold in diethyl ether, is shown in Fig. 2. The solution of the latter was applied in 10-µl aliquots over a period of about 15 min. Apart from some background peaks due to the solvent in the latter sample, the two chromatograms were identical.

Application with wide-bore capillary columns

The use of cold on-column trapping of the injected samples is necessary with the higher-performance capillary columns (bore 0.2–0.3 mm), if some loss in the separating power of the system —either through peak broadening or tailing— is to be avoided. Such considerations applied to the SE-30 column used in the present work.

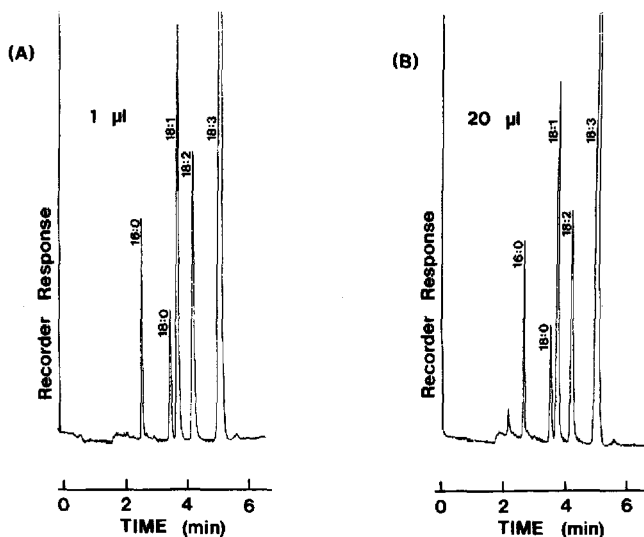

Fig. 3. Gas chromatograms from (A) a 1-μl injection of linseed oil fatty acid methyl esters (FAME) and (B) 20 μl of the same sample (diluted 20-fold) in hexane, cold trapping not employed. Column: 10 m $\times$ 0.6 mm I.D. glass SCOT column coated with DEGS + phosphoric acid. Carrier gas: hydrogen at 6 ml/min. Oven temperature: 170°C. Injection block temperature: 250°C.

However, for the purpose of achieving rapid analyses, we also make considerable use of relatively short wide-bore (0.5–0.6 mm) SCOT columns which, while displaying only moderate efficiencies, have low pressure drops and may be operated at high gas velocities. Initial on-column trapping is not a prerequisite for these columns, since identical column performances were obtained using solvent-free and direct liquid-injection. In addition, it was observed that even when quite large volumes were applied to the sintered glass sample tube, with considerable dispersion of the sample in the tube after evaporation of the solvent, no obvious impairment of the separation process occurred. This is illustrated in Fig. 3 which shows the analysis of 1- and 20-μl samples of the fatty acid methyl esters (FAME) of linseed oil.

A consequence of the design of the present injector is that a certain amount of compression of the gas within the system takes place when the tube holding the sample capillary is depressed downwards during the injection step. This leads to a temporary increase in the gas flow through the column. In relation to the repeatability of retention times, this effect is not of any consequence in the case of cold on-column trapping, since a suitable time may be allowed thereafter for flow conditions to stabilise before commencing the run. However, since this stabilisation period is not available when samples are injected directly onto heated wide-bore capillary columns, it was of some importance to evaluate what the implications might be for reproducibility of retention times. Accordingly, replicate 2-μl samples of the linseed oil FAME mixture were run on the DEGS column; the retention time data as well as the relative concentrations of the individual components are given in Table II. The average coefficient of variation, C.V. = 0.2, for the retention measurements, while quite satisfactory, was clearly not as good as that obtained in the hydrocarbon experiments, presumably for the reason stated above. However, the quantitative data (average C.V. = 0.62) were excellent.

TABLE II

REPRODUCIBILITY OF RETENTION TIMES (t_R) AND NORMALISED PEAK AREAS (%) ON A WIDE-BORE CAPILLARY COLUMN

Run No.	*FAME mixture (linseed oil)*									
	16:0		*18:0*		*18:1*		*18:2*		*18:3*	
	t_R *(min)*	*(%)*	t_R *(min)*	*(%)*	t_R *(min)*	*(%)*	t_R *(min)*	*(%)*	t_R *(min)*	*(%)*
1	3.09	6.41	4.20	4.80	4.49	18.97	5.13	14.39	6.21	55.41
2	3.12	6.40	4.22	4.78	4.52	19.03	5.15	14.36	6.24	55.41
3	3.11	6.26	4.20	4.85	4.50	19.15	5.13	14.36	6.22	55.38
4	3.11	6.16	4.21	4.76	4.50	19.05	5.13	14.46	6.21	55.56
5	3.11	6.43	4.20	4.81	4.50	19.04	5.12	14.30	6.21	55.41
$\bar{x}$	3.11	6.33	4.20	4.80	4.50	19.05	5.13	14.37	6.22	55.43
S.D.	0.01	0.10	0.011	0.03	0.01	0.06	0.01	0.05	0.012	0.06
C.V. (%)	0.32	1.65	0.26	0.63	0.22	0.31	0.19	0.36	0.19	0.12

GC–MS applications

An injection device of the type described has been used extensively during the past year or so in a capillary column based high-performance GC–MS system. Major areas of analysis have included trace levels of pesticides in environmental samples and metabolic steroids in biological materials. The injector has proved particularly successful when dealing with dilute solutions containing sub-nanogram levels of components eluting from the column at high temperatures. The use of relatively large sample volumes allied to an absence of discrimination against high-boiling components, has allowed excellent signal to noise ratios and high quality spectra to be obtained for eluates emerging at temperatures in excess of 300°C. We have had much less success in applications of this type when using a conventional splitless injector.

It was mentioned earlier that on removal of the inner assembly for the application of the sample, the carrier gas was momentarily diverted to the atmosphere, before the outer body of the injector was capped. By monitoring the masses at m/e 28 and 32 while the cap was removed, it was confirmed that as long as the carrier gas was allowed to sweep through the open injector, no air entered the column and subsequently the mass spectrometer. Thus, under normal operation, no air enters the system at any stage during the removal and reinstallation of the sample tube in the injector.

Contamination of sample tube

A problem common to heated injection systems —especially when large sample volumes are involved— is contamination of the injection block, glass liner, etc., with non-volatile material, which may slowly decompose and bleed into the column giving rise in time to increased background responses and impaired performance. Harmful effects of this type can be minimised with the present injector, since if it is suspected that a sample may contain unstable non-volatile material, it is only necessary to withdraw the sintered tube into the cold region of the apparatus as soon as the sample has been injected onto the column. At the end of the run the sample tube may be

replaced with a fresh one, while the contaminated tube is regenerated by flushing with a suitable pure solvent and then drying under a stream of nitrogen.

CONCLUSIONS

The principal features and attributes of the injection device described may be summarised as follows:

(a) The device is simple and inexpensive to construct and requires only minor modifications on the gas chromatograph for its installation.

(b) The technique of sample application and solvent removal is straightforward and easy to perform and is suitable for either small or large sample volumes.

(c) Solvent free samples are transferred to capillary columns to yield good quantitative data and reproducible retention times.

(d) Excellent results may be obtained in the analysis of trace high-boiling substance in GC–MS.

(e) Contamination of the column by decomposition of heat labile non-volatiles may be minimised.

A disadvantage of the device, common to all solvent-free injection systems, is that a sufficient difference between the boiling point of the solvent and solutes must exist, so the technique is limited to higher-boiling components. Another possible disadvantage is that since samples have to be vaporised in the heated injection zone before being deposited on the column, it is possible that in certain cases labile, heat-sensitive substances may be adversely affected. However, our experiences to date have indicated that the latter effect is frequently more serious when using hot metal hypodermic needles with conventional injection devices. Cold on-column liquid injection is probably the only way that this difficulty can be completely avoided.

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