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REVIEW

POTENTIAL OF WIDE BORE OPEN TUBULAR COLUMNS IN GAS CHROMATOGRAPHIC ANALYSIS OF DRUGS

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1. INTRODUCTION

The majority of gas chromatographic (GC) drug analyses performed today use packed columns. In spite of certain disadvantages of these columns, such as adsorption effects and the lack of sensitivity, they have been favoured by chromatographers for a long time and it is only relatively recently that wide bore (or 'megabore') capillary columns, especially those with 0.53 mm I.D., have become popular as an alternative to packed columns in routine as well as research applications [1–4]. These columns can be installed in existing packed column instruments with minimum alterations and can be used with direct (on-column) mode of injection. Wide bore columns are made from fused silica column tubing and contain chemically bonded stationary phases with film

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thicknesses up to 8 μm. These columns were first introduced in 1983 [5] as the capillary replacement for packed columns, since then wide bore columns of different polarities, lengths and thicknesses are available from many GC suppliers (e.g. J & W Scientific, Altech Assoc., Chrompack).

Compared to glass capillary columns, fused silica columns have the advantage of flexibility and strength as long as the outside surface remains intact. An external coating of heat-resistant polymer, usually polyimide, is applied to protect the outside surface from damage. This coating has the temperature limit of $\sim 350\,^{\circ}C$, but more recently an aluminium film has been used to give a more thermally stable coating enabling work up to $425\,^{\circ}C$ [6].

2. CHARACTERISTICS OF WIDE BORE CAPILLARY COLUMNS

The degree of resolution and the time necessary for a separation on a wide bore column depend on its characteristics such as length, film thickness and type of stationary phase, as well as experimental parameters such as carrier gas, its flow-rate and column temperature [7]. Careful consideration of these factors can help to achieve optimum separation.

Because wide bore columns are not packed (there is no support), it offers less resistance to gas flow resulting in minimum pressure drop. Long columns are therefore feasible which, compared to packed columns, generate a large number of theoretical plates per column (1000–2000 plates/m). The higher resolving power of wide bore capillaries increases the range of analytes and greatly reduces the need for a large number of stationary phases which are prevalent in packed column GC. Only few columns of different polarities are required for most analyses.

To optimize speed and resolution, wide bore columns are available in different lengths, however, 15 and 25 m being most common. The selection of the column length usually depends on the nature of the sample. For example, for thermally labile compounds, it is advisable to use short columns. Sample capacity can be increased with increased I.D. and/or film thickness, however, as the diameter increases, the column loses its flexibility and ease of handling. The 0.53 mm is the smallest I.D. that allows direct on-column injection with a standard syringe. A thin film coating (0.1 μm) provides a lower sample capacity but shorter retention times since it offers less resistance to mass transfer. Thin film columns are useful for high boiling point or heat-sensitive compounds and are usually selected with a relatively short length (10–15 m). However, such columns are more easily overloaded and are less inert. Thicker films (>1 μm) have sample loading capacity similar to packed column, i.e. in the microgram per component range, but much greater retention. They lower the risk of unwanted interactions between the sample and the residual active sites on the capillary wall thus reducing adsorption and tailing. They are beneficial for separation of low boiling point compounds but give longer retention times

and can have higher bleed rates. Generally speaking, for wide bore columns, a film thickness of 1 μm is preferred because both sample capacity and column efficiency are reasonable at this thickness and a separation of a wide range of compounds can be achieved via temperature programming.

The open tubular columns may contain non-polar (methylsilicones), intermediate polar (phenylmethylsilicones) or polar (cyanopropylsilicones, polyethylene glycols) stationary phases [8]. These phases are thermally and chemically stable and retain their efficiency over a longer period of time if used within the maximum recommended temperatures. They are virtually unextractable, therefore, a contaminated column can be flushed with repeated injections of suitable solvents such as chloroform, diethyl ether or hexane. Low pressure devices which force the solvent into the capillary are available (e.g. from Jones Chromatography, Chrompack) for thorough rinsing of the contaminated column. Like packed columns, wide bore columns do not require extensive conditioning periods and can be operated at very high temperatures (non-polar columns up to 330 °C, polar columns up to 250 °C) with minimum column bleed. Low column bleed allows greater flexibility in temperature programming and use of a lower attenuation setting which is particularly beneficial in trace analysis. On packed columns, often trace components are either unresolved, merged with the background peaks (noise) or adsorbed on chromatographic support.

Since wide bore columns do not contain support, sample loss through support-related adsorption or degradation is eliminated. However, after a continued use, irreversible column contamination may lead to loss of efficiency. In these circumstances, the column may often have its performance restored by removing first coil and creating a new inlet section with no significant loss of efficiency. If used with care fused silica columns will provide reliable service for a long time.

3. INSTALLATION OF WIDE BORE CAPILLARY COLUMNS

The large increase in sample capacity obtained with wide bore capillary columns make them suitable in many instances as an alternative to packed columns. A packed column can be substituted by an equivalent wide bore column in most GC instruments without complicated or expensive modifications. Although narrow bore columns (<0.35 mm I.D.) are more efficient and allow faster analyses than wide bore columns, they impose additional demands on the operator and on the equipment. Owing to their small diameters, gas flow volumes and sample capacities are much more restricted and specialized injection techniques such as split/splitless injection systems are required [8–11]. Further, with narrow bore columns, extra supply of auxiliary (or make-up) gas at the detector end of the column is necessary to reduce band broadening and allow the detector sensitivity to be optimized. Wide bore columns, on the other

hand, can accept relatively large gas flows and no special instrumental modifications are required. The use of inexpensive adaptors (Figs. 1 and 2) connected to the inlet and outlet ends of the capillary by means of appropriate reducing unions allow them to be fitted quickly in the existing packed column instruments [9,12]. In many applications the auxiliary gas supply is not required. As with packed columns, injection is carried out on-column with the syringe needle entering the column itself or the sample is vaporized in the inlet adaptor before entering into the column. Adaptor kits for installation of wide bore columns are commercially available from chromatography suppliers (e.g. J & W Scientific, Altech Assoc.).

A popular inlet adaptor which is suitable for the majority of wide bore column applications involve direct flash vaporization of samples (Fig. 1a). This adaptor has a wide diameter to facilitate vaporization of the sample and a narrow tapered restriction at both ends, the column being snugly positioned into the lower restriction. The sample, which is first flash vaporized, is swept onto the column by carrier gas flow. This mode of injection usually gives a sharp solvent peak with less tailing facilitating baseline separation of components of interest [13]. Another variation of an injector port liner for direct injection is

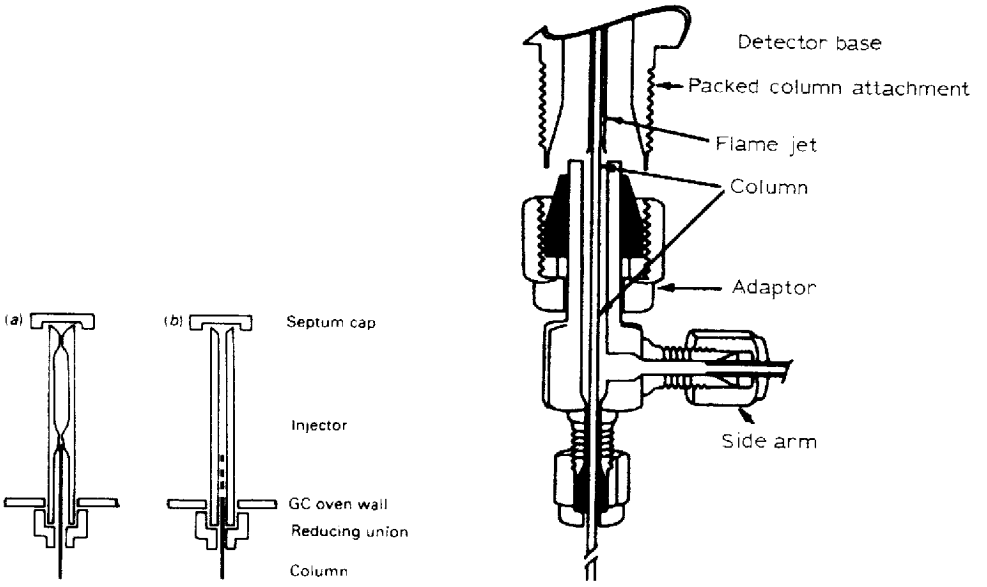

Fig. 1. Inlet adaptors for installing wide bore columns into a packed column injector port: (a) direct flash vaporization injector (J & W Scientific); (b) hot on-column injector. Reproduced from ref. 13 with permission.

Fig. 2. Typical detector adaptor for conversion of the packed column instrument to wide bore open tubular columns. Reproduced from ref. 1 with permission from Academic Press and J & W Scientific.

hot on-column injector (Fig. 1b). This adaptor is a narrow bore tube of constant I.D. with the column mounted at the base of the liner or moved up so that the syringe needle can enter the column itself. Since there is no vaporization chamber in this liner, it has very little expansion volume, therefore very small volume (0.5–1 μl) of sample is injected particularly at lower flow-rates. The hot on-column liner is particularly advantageous for thermally labile or high boiling point compounds.

If the column is operated at high flow mode (>10 ml/min) then neither auxiliary gas nor a special capillary flame jet is necessary. However, auxiliary gas, capillary flame jet and a low range (0–10 ml/min) flow controller is recommended for low flow mode (<10 ml/min) of operation [10,12]. A side arm for auxiliary gas is usually provided with detector adaptor (Fig. 2) for those instances where make-up gas is required. The side arm is capped off when the column is operated in high flow mode.

4. PRACTICAL CONSIDERATIONS

Wide bore columns are open tubes and therefore are more permeable to gases than the packed columns. Although efficiency is the same for both column types, wide bore columns are longer and generate a large number of plates per column. Compared to packed columns the Van Deemter plots for wide bore columns are relatively smooth which means that HETP (height equivalent to a theoretical plate) values rise slowly as the carrier gas flow increases. Thus, columns can be operated at two to three times their optimum flow-rates (i.e. up to 30 ml/min) without losing much efficiency [4]. Consequently one can get results with a shorter analysis time than for packed columns but with equivalent or improved separation (Fig. 3). Packed column mode of operation (i.e. higher flow-rates) is useful for many drug analysis applications which do not require very high resolution. Low flow-rates (capillary mode, 0.5–2.5 ml/min) on the other hand can be used to maximize resolution, albeit at the expense of speed. Both modes offer benefits, the choice depends upon application. In general, the flow-rates between 10 and 20 ml/min seem to be a reasonable compromise between resolution and separation time.

Because of their higher diffusion rates hydrogen and helium are suitable carrier gases for wide bore capillary columns. Slow carrier gas such as nitrogen is not generally recommended for capillary work. For hydrogen and helium the HETP versus linear velocity curves are relatively flat which means that higher gas velocities can be achieved with these gases which give shorter retention times without significant loss in resolution or efficiency [6,14]. Hydrogen produces faster separation than helium but helium is a lot safer. Proper safety precautions must be taken if hydrogen is employed as the carrier gas. As with

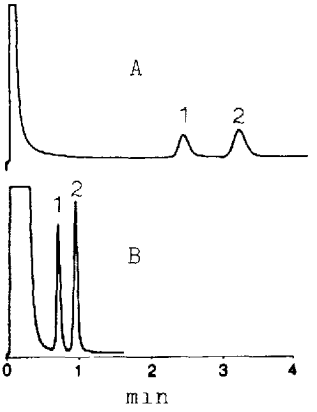

Fig 3 Chromatograms of amphetamine (1) and methamphetamine (2) generated by packed (A) and wide bore (B) capillary column Both systems used non-polar columns, helium carrier gas at 30 ml/min and column temperature of 120 °C Note the differences in sensitivities and retention times Reproduced from ref 9 with permission of Preston Publications

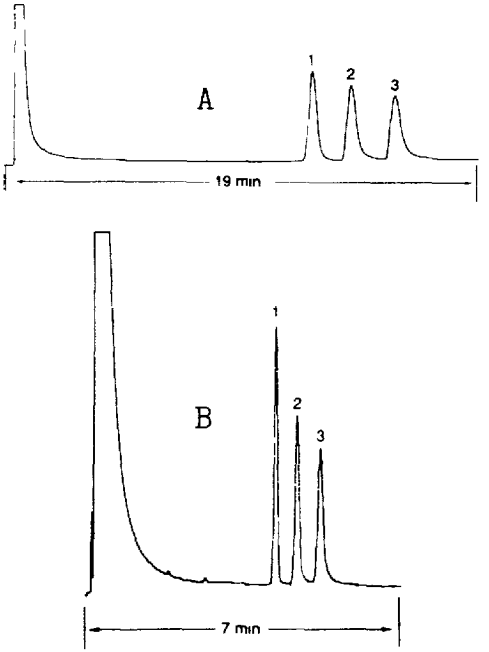

Fig 4 Chromatograms of underivatized amitriptyline (1), nortriptyline (2) and protriptyline (3) generated by packed (A) and wide bore (B) capillary column A 1-μl volume containing 300 ng of each drug in methanol was directly injected Both systems used similar stationary phases and experimental conditions Helium (20 ml/min) was used as the carrier gas Reproduced with permission from Jones Chromatography

packed column work, the purity of carrier gas (hydrogen or helium) should be monitored, and oxygen- and moisture-removing systems should be installed in the carrier gas supply.

Wide bore columns can tolerate direct injection of large sample volumes (up to 10 μl) and standard syringes with 0.47 mm O.D. needle can be used for on-column injection. The injection process should be slow particularly for large volumes, e.g. >1 μl, and the syringe needle should be left in position for some time (approximately 1 s/μl injected) to allow the pressure pulse to be dissipated into the column. This will prevent possible backflow of rapidly vaporizing solvent into the injector resulting in a tailing solvent peak. To achieve and maintain good sample recovery, reproducibility and chromatographic efficiency, the inlet liner should periodically be cleaned. Inlet liner can be cleaned by immersion in concentrated nitric acid followed by thorough rinsing with deionized water.

Wide bore columns are fully compatible with all common modes of detection, however, since most drugs contain nitrogen, a nitrogen–phosphorus detector (NPD), which gives selective response to nitrogen–phosphorus-containing compounds, is particularly useful in drug analysis. Though not as sensitive as an electron-capture detector (ECD), NPD has a wider linear range and its use simplifies sample clean-up procedure and gives fewer interfering peaks compared to ECD.

5. APPLICATIONS FOR DRUG ANALYSIS

Wide bore capillary columns with 0.53 mm I.D. are ideal not only for analysing simultaneously a number of drugs, but also for simpler separations where high efficiency and sensitivity are desired, for example, in the measurement of trace concentrations of drugs in biological fluids. They have a particular advantage in the area of trace analysis since direct on-column injection enables transfer of entire sample into the column. This, coupled with high efficiency and low column bleed, yields narrow peaks of greater height leading to lower limit of detection. Since analyses with wide bore columns are quicker and direct on-column injection can easily be automated, the great potential of these columns can be exploited in the analysis of drugs of abuse and in toxicological screening [15–17] where rapid results are often required. The isothermal retention indices of 75 drugs and other compounds of toxicological interest measured on packed columns (SE-30) and equivalent narrow and wide bore columns are shown to be highly correlated. Retention indices on wide bore columns obtained from two different manufacturers (SGE and J & W Scientific) are also shown to be highly correlated [16].

A number of drugs require derivatization for their analysis on packed columns, whereas the increased inertness of fused silica open tubular columns may allow in some cases direct analysis of the underivatized drug further reducing the time of analysis (Fig. 4). In spite of these advantages it appears

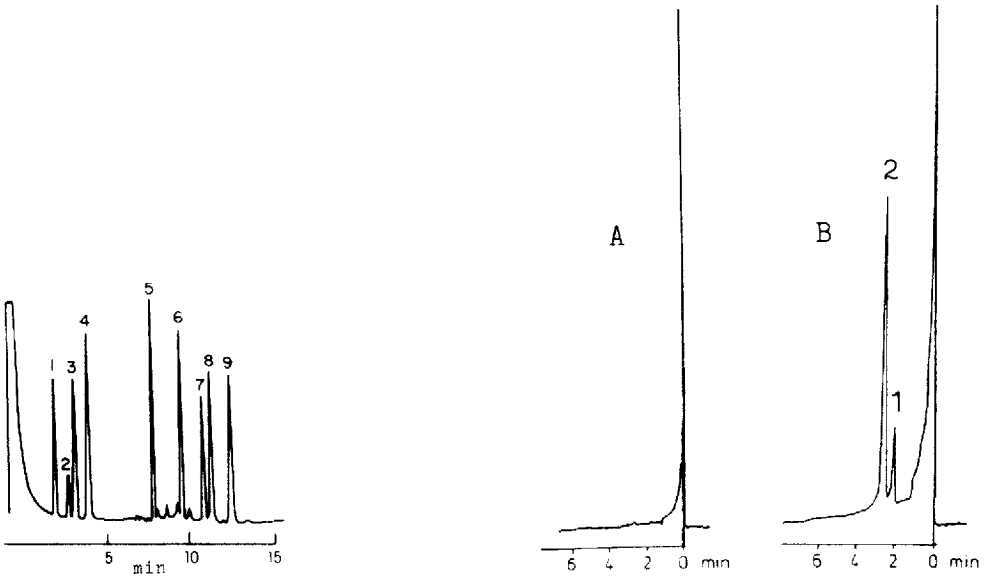

Fig. 5. Separation of a mixture of cardiac drugs on a wide bore column. Peaks: 1 = tocainide; 2 = monoethylglycinexylidide; 3 = lidocaine; 4 = orphenadrine (internal standard); 5 = procainamide; 6 = disopyramide; 7 = N-acetylprocainamide; 8 = *p*-chlorodisopyramide; 9 = N-butyrylprocainamide. Reproduced from ref. 20 with permission.

Fig. 6. Chromatograms of (A) a drug-free control from a human plasma sample and (B) an extract of a human plasma sample containing 123.0 ng/ml thromboxane A_2 receptor blocker (1). Propionic acid analogue of the drug (1 μg/ml) was used as an internal standard (2). Reproduced from ref. 21 with permission.

that the pharmaceutical analysts have not fully exploited the potential of wide bore capillary columns. Perhaps they are quite content with their packed columns which may have served them well over a long time. A packed column method for a given drug can be employed as a starting point for developing a wide bore capillary method using an equivalent column, however, once the preliminary results are obtained, the experimental conditions for all steps in the latter must be optimized. Suppliers of wide bore columns do in house development work to expand the applications and the range of their products. It is sometimes beneficial to consult them (or their catalogues) for advise on specific problems.

Wide bore columns have not yet been very widely used in the pharmaceutical field though sufficient use has been made of them to confirm a trend. They have been used for the separation of mixtures of drugs as well as individual drugs and their metabolites. For example, antiepileptic drug mixture has been separated on a non-polar wide bore column and detected by a flame ionization detector (FID). Helium (15 ml/min) was used as the carrier gas [18]. As for individual drug and a metabolite, verapamil and norverapamil are determined

TABLE I

APPLICATIONS OF WIDE BORE (0.53 mm) CAPILLARY COLUMNS IN DRUG ANALYSIS IN BIOLOGICAL SAMPLES

Drug	Column polarity (length, m)	Carrier gas (flow-rate, ml/min)	Detector	Reference
Morphine and diazepam	Intermediate (12)	He (8)	FID/NPD	17
Antiepileptics	Non-polar (10)	He (15)	FID	18
Verapamil and norverapamil	Non-polar (30)	N_2 (24)	NPD	19
Antiarrhythmic drugs	Intermediate (10)	He (10)	FID	20
Thromboxane A_2 receptor antagonist	Intermediate (10)	H_2 (15)	ECD	21
Epomedial	Non-polar (25)	–	FID	22
Nifedipine and its metabolites	Non-polar (30)	He (1)	ECD	23
Valproic acid	Non-polar (25)	He (10)	FID	24
Orphenadrine	Intermediate (30)	N_2 (15)	NPD	25
Buflomedil	Non-polar (15)	N_2 (–)	NPD	26
Ethanol	Polar (15) Non-Polar (15,30)	He (25)	FID	27

in post-mortem specimens and body fluids using a 30 m, 5% phenylmethylsilicone column, NPD and nitrogen as the carrier gas [19].

Smith [20] has determined tocainide in serum using a 10 m long OV17 bonded fused silica column, FID and helium (10 ml/min) as the carrier gas. The method has sufficient sensitivity for monitoring of this drug in cardiac patients; using temperature programming it can also be applied for monitoring other antiarrhythmic drugs such as lidocaine or procainamide (Fig. 5).

A very sensitive (limit of detection, 8 ng/ml) method for the determination of thromboxane A_2 receptor antagonist, 4-[2-(4-chlorophenylsulphonylamino)ethyl]phenylacetic acid, has been developed using a wide bore capillary column [21] (Fig. 6). After extraction and derivatization, the drug is separated on a 10 m, 50% phenylmethylsilicone column at 260°C and detected by an ECD. Hydrogen is used as the carrier gas. The method permits the routine analysis of a large number of samples required for pharmacokinetic study. Further examples of the applications of wide bore capillary columns in drug analysis are presented in Table I.

6 CONCLUSIONS

Wide bore open tubular columns in GC analysis provide the chromatographer with a simple route to medium to high resolution GC with a significant improvement on packed column GC Overall, they are more versatile than packed columns and are very convenient to use Although not as efficient as the conventional (narrow bore) capillary columns, they are ideal when relatively uncomplicated samples are being analysed, such as in routine drug monitoring work or in pharmacological studies where the presence of extremely low concentrations of drugs require a selective and sensitive technique Several benefits such as better separation, higher sensitivity, increased sample loading capacity and faster analysis can be achieved by using these columns The capital cost can be justified on the grounds of the increased productivity (sample throughput) Dedicated capillary instrument is not required for wide bore capillary columns, since they can be easily accommodated in existing packed column instruments Thus, they are a good compromise for those on the restricted budget or working in a small laboratory with limited resources but want to use capillary columns Wide bore columns should be regarded as complementary to conventional capillary columns and it seems likely that with time they will find a place in every drug analysis laboratory using GC

7 SUMMARY

The recent introduction of wide bore flexible fused silica columns and the development of chemically bonded stationary phases for gas chromatography (GC) has opened up new possibilities for carrying out medium resolution capillary GC on packed column instruments. The favourable chromatographic properties of wide bore capillary columns make them ideal for applications in many areas of GC This review discusses the characteristics of more popular 0 53 mm I D wide bore columns, experimental conditions necessary for their use and their applications in the pharmaceutical field with an emphasis on the analysis of drugs in biological fluids

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