Smaller is better - How to use 0.15mm Capillary Columns

by J. Kuipers, N. Reuter - Varian B.V. The Netherlands*
** corresponding author, johan.kuipers@varianinc.com*

There are still obstacles to switch from the very common internal diameters of 0.32 mm or 0.25 mm to the "faster" columns with 0.15 mm ID. This article will show how easy it is to adapt your methods and to what you have to pay attention to when doing the conversion.

Introduction

The most popular capillary column dimensions in modern day chromatography are beyond doubt the 25 m/30 m x 0.25 mm versions. These columns are usually coated with either polydimethylsiloxane (VF-1ms) or poly(phenylmethyl)siloxanes types (VF-5ms) of liquid phases with layers of 0.25 µm.

The wide spread use of this particular column dimension is mainly based on their applicability in a wide variety of samples types, injection modes and detector types. In addition, they offer a good compromise between speed of analysis, resolution, and sample loadability. As such they are normally the first choice for lab techni-cians.

However, the traditional 250 µm ID columns are often outperformed in terms of speed of analysis, sensitivity and bleed performance by 150 µm ID columns. These 150 µm ID columns were carefully developed with a fo-cus on improving this speed of analysis while at the same time maintaining much of the sample capacity, retain-ing a wide applicability and instrument compatibility.

This article offers some practical guidelines for updating your existing application into a high speed application based on 0.15 mm columns using the same equipment:

Column ID and Efficiency

The number of theoretical plates, Nth, generated by any capillary column is mainly dependent on its length and internal diameter. Columns with a smaller column ID will provide more theoretical plates due to reduced diffu-sion rates in the gas phase during the separation process. In a simplification of the Golay equation the number of theoretical plates in inversely proportional to the square of the internal diameter (eq. 1)

$$N_{th} \approx \frac{1}{ID^2}$$

Equation 1

Table 1 below shows some typical column parameters indicating the relationship between column internal di-ameter, length and number of theoretical plates.

Internal Diameter [mm]	Film Thickness [µm]	Column length [m]	Total Number of Theoretical Plates	Nth/m
0.10	0.10	13	115000	8500
0.15	0.15	18	115000	6400
0.25	0.25	30	115000	3900
0.32	0.32	38	115000	3000
0.53	0.53	64	115000	1800

Table 1: Influence of column diameter on theoretical plates and column length

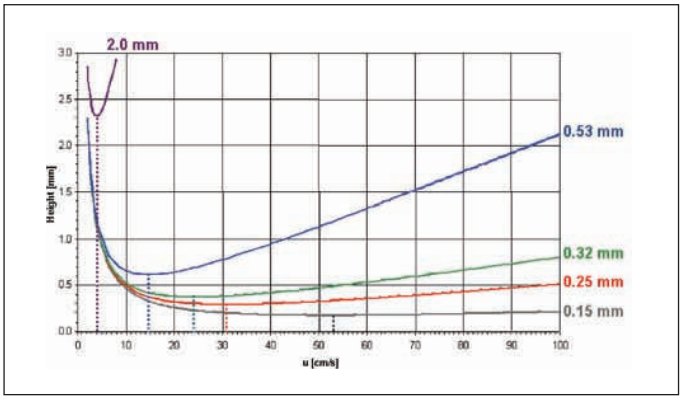

Figure 1: Golay plot or H/u curves for various internal diameter columns

This means that in order to obtain an identical separation in terms of column efficiency and thus component resolution a 30 m x 0.25 mm column is comparable to a 18 m x 0.15 mm column. The 40 % reduction in column length will therefore automatically result in an almost 40 % drop in analysis time.

At the same time column efficiency is also depends on carrier gas flow. The capillary column will generate its maximum efficiency and will provide its maximum resolution near to its optimum average linear gas velocity.
This relationship between theoretical plates (efficiency), column internal diameter and linear gas velocity can be illustrated by the Golay plot (table 2). Column efficiency can also be expressed independently from its length by the Height Equivalent to one Theoretical Plate (HETP)

The steepness of the slope decreases with decreasing internal diameter, i.e. small ID columns can be operated at linear gas

Internal Diameter [mm]	Optimum linear gas velocity [cm/s]
0.53	15
0.32	24
0.25	31
0.15	53
0.10	> 60

Table 3: Optimum linear gas velocities for various internal diameter columns

velocities or flows far above their optimum without much loss of efficiency. This feature of 150 µm columns can be fully exploited by applying high column flows of 1.5 – 3.0ml/min (helium) resulting in significant additional gains in speed of analysis compared to 0.25mm columns (Figure 2).

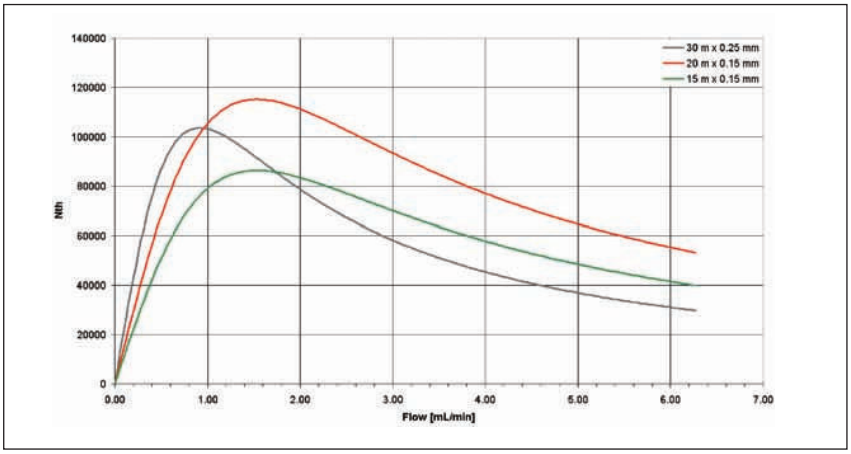

Figure 2: Efficiency / flow (helium) relationship for 0.15mm and 0.25mm columns

Also note that for temperature-programmed runs, using constant pressure conditions, the linear gas velocity decreases due to increased viscosity of the carrier gas at higher temperatures. The column may no longer operate at the optimum flow conditions within the H-u curve with the possible loss of efficiency as a consequence.

Relative Loadability

The sample capacity of a capillary column is proportional to the volume or amount of the liquid phase available and as such is related to the columns internal diameter, length and film thickness (Figure 3 and Equation 2 – 5)

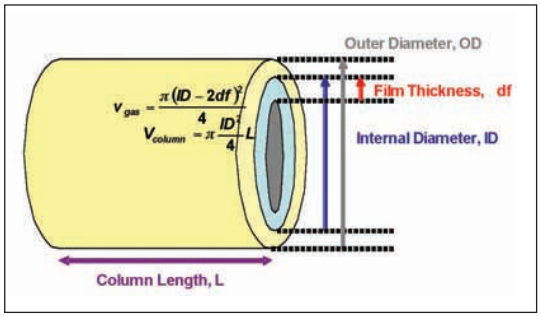

Figure 3: Column dimensions and phase ratio

Column Volume, Eq. 2

Gas Volume Eq. 3

Phase Volume, Eq. 4

Phase Ratio, Eq. 5

The relative loadability in Table 4 stands for the relative amount of sample which can be loaded onto the capil-lary column without affecting resolution, peakshape or performance. The loadability factor can be used to adapt injection volume or split ratios. As an example, changing the internal diameter from 0.25 mm column to the 0.15 mm equivalent an initial injection volume of 1ul should be reduced by almost a factor 5 to 0.22 µL. Alterna-tively, the split ratio can be increased with the same factor to obtain the same effect.

Internal Diameter [mm]	Film Thickness [µm]	Column Length [m]	Relative Loadability
0.10	0.10	12	6
0.15	0.15	18	22
0.25	0.25	30	100
0.32	0.32	38	210
0.53	0.53	64	960

Table 4: Internal diameter and relative loadability

Relative column loadability plays a role in impurity analyses where limited sample capacity can result in lower dynamic concentration ranges that can be handled by the column. This can be addressed by applying a thicker film column with the same internal diameter. The maximum sample capacity of a column is usually not ex-ceeded for trace level analyses and column loadability is therefore less important for these type of analyses. Please note that on smaller ID columns peaks elute earlier and therefore do not undergo as much diffusion as on larger ID columns which leads to smaller the peak widths. For rate sensitive detectors like the FID this means that the peak height increases because the peak area remains constant. The loss of sensitivity caused by the reduction of sample introduced on the column itself is partially compensated by this effect.

How to do it?

How fast is the analysis?

For isothermal analyses at the same temperature Table 5 shows the speed gain or loss using helium as carrier gas:

Internal Diameter [mm]	Film Thickness [µm]	Column Length [m]	Speed Factor
0.10	0.10	12	3.0
0.15	0.15	18	2.0
0.25	0.25	30	1.0
0.32	0.32	38	0.7
0.53	0.53	64	0.3

Table 5: Speed of analysis and internal diameter

How to adapt the Temperature Program?

That depends on what is needed. The basic requirement is to keep the retention factors, k, constant. The sim-plest temperature programming consists of 3 steps:

1. isothermal starting period, e.g. 5 minutes at 100 °C.
2. ramping (heating rate), e.g. heat with 10 °C/min.
3. final isothermal period, e.g. 10 minutes at 250 °C.

More complicated oven programs repeat the steps 2-3 multiple times.

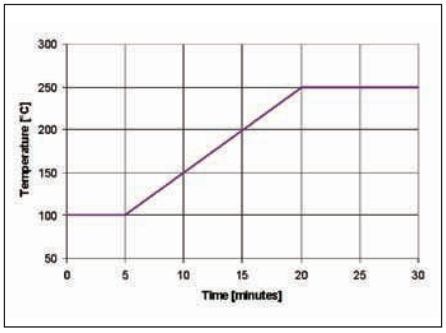

Figure 4: Example temperature program

A tool is needed to adapt head-pressures, isothermal times and temperature ramps from the original method on a 0.25 mm column to the new settings for the 0.15 mm column. The following nomogram provides some guide-lines for this.

The x-axis depicts the ratio between the column lengths of the 0.25 and 0.15mm columns. For example switch-ing from 30 m x 0.25 mm to 15 m x 0.15 mm, the ratio is 30/15 m = 2.0. In the nomogram you will find 3 multipli-cation factors for the ratio = 2:

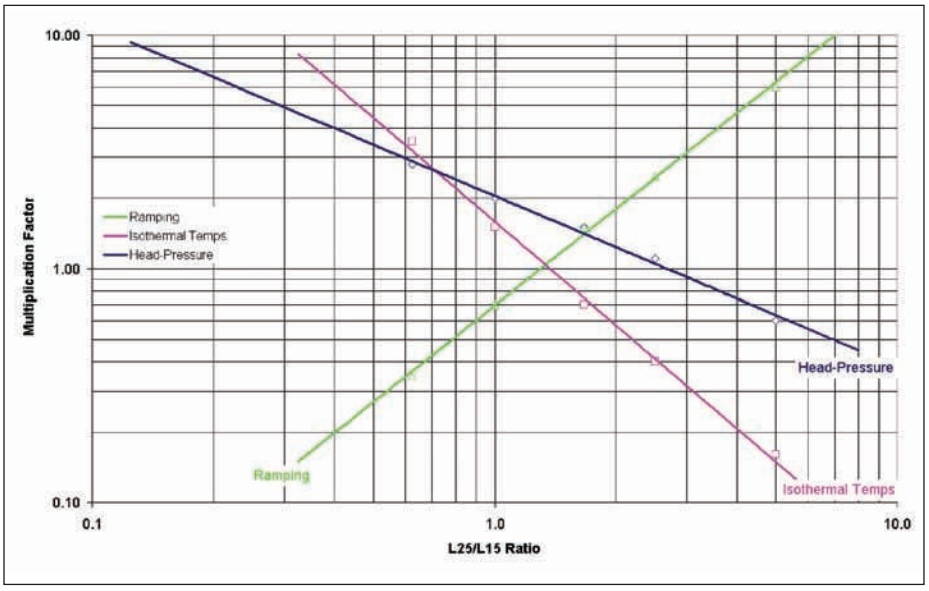

Figure 5: Nomogram relating column pressures and temperature programming between 0.25mm and 0.15mm columns

- Head-Pressure: x 1.20
- Time of Isothermal Temps: x 0.58
- Ramping: x 1.80

In the example above the 2 isothermal times are multiplied by 0.58, the ramp is multiplied by 1.8 and the head-pressure is multiplied by 1.2 resulting in the new settings:
2.9 Minutes at 100 °C, heating by 18 °C/min and 5.8 minutes at 250 °C.

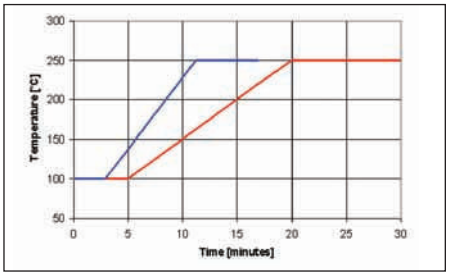

Figure 6; Adapted temperature program 0.15mm vs. 0.25mm

The red line is the original temperature program and the blue line the adapted program for the 0.15 mm column. The speed gain in temperature-programmed runs is slightly less than for isothermal analyses, in this case not a factor of 2, but a factor of 1.8. Using these simple tools and rules it becomes quite easy to implement the fast 0.15 mm columns and to im-prove your speed of analysis.

Conclusions

0.15 mm columns can outperform all larger diameter columns. They can be used with almost all injection and detection techniques including splitless injection and MS detection. Adapting the temperature program is easy with the tools above. These 0.15mm columns provide significant gains in speed of analysis and improved pro-ductivity.

For more information on the use of 150 µm columns contact the author johan.kuipers@varianinc.com

The 0.15mm columns are available in a wide range of liquid phases and different dimensions.

More information can found at www.varianinc.com