Improving Analysis Sensitivity with Solid Core 4 µm Columns

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Key Words
Solid core, fused core, superficially porous, sensitivity, signal-to-noise, efficiency

Abstract
In this technical note the sensitivity, measured as signal-to-noise ratio, achieved with solid core 4 µm particle packed columns is compared to that of fully porous 5 and 3 µm particle packed columns.

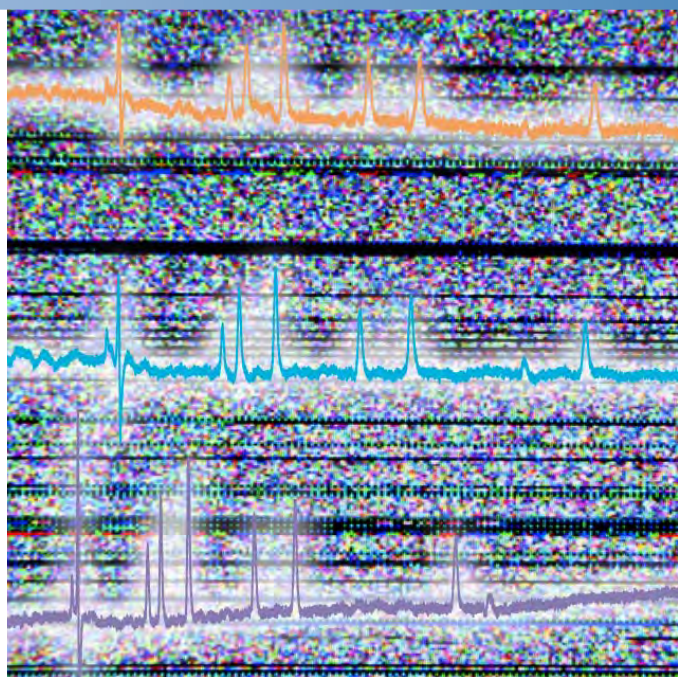
Introduction

Sensitivity is often an important characteristic of a method that needs to be considered in the method optimization strategy. The chromatographic parameters that affect sensitivity are column length and diameter, column performance (peak shape and efficiency), thermodynamic parameters (retention time and temperature), and injection conditions. Sensitivity is related to the concentration at the peak apex C_{max}, which depends on the chromatographic parameters as described by Equation 1 [1].

$$C_{max} = \frac{4}{\varepsilon_t \pi \sqrt{2\pi}} \frac{\sqrt{N}}{L\,(1+k)\,d_c^2} \frac{c_0 V_i}{(T_f - 1)\kappa}$$

Where ε_t – the total column porosity
L – column length
d_c – column diameter
N – peak efficiency
k – peak retention factor
c_0 – sample concentration
V_i – injection volume
T_f – peak tailing factor (measured at a given peak height fraction h)
κ – constant dependent on h

From Equation 1 it is clear that high efficiency and symmetrical peaks produce higher response peaks (higher C_{max}) and therefore higher sensitivity. C_{max} is also inversely proportional to column porosity; thus, lower porosity columns such as those packed with partially porous particles should also produce higher C_{max}, assuming all other conditions remain unchanged.



Based on Core Enhanced Technology™ using 4 µm solid core particles, Thermo Scientific™ Accucore™ XL HPLC columns allow users of conventional HPLC methods to obtain performance far beyond that of columns packed with 5 µm or even 3 µm fully porous particles. Using solid core 4 µm particles packed in conventional column dimensions, significant improvements in the assay performance can be achieved without the need to make changes to the operating parameters or system configuration. Very high peak efficiencies using standard HPLC instrumentation and conditions allow for increased peak resolution and limits of detection.

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Peak efficiency comparison

Figure 1 illustrates the separation of ibuprofen and valerophenone on a Accucore XL C18 4 µm HPLC column and a fully porous C18 5 µm column using the same isocratic method based on the USP monograph [2]. Efficiency for both compounds improved by more than 70% when using the Accucore XL HPLC column compared to the fully porous column (Table 1). This improvement in peak efficiency results in increased signal to noise ratio (112% on average) for exactly the same amount injected on column, representing a dramatic improvement in analysis sensitivity.

The backpressure for the Accucore XL C18 4 µm HPLC column was measured at 312 bar, and the 5 µm fully porous column backpressure was measured at 239 bar. Therefore, the improvement in analysis sensitivity is gained with a small increase in backpressure, which is still within the operating limits of a conventional HPLC system.

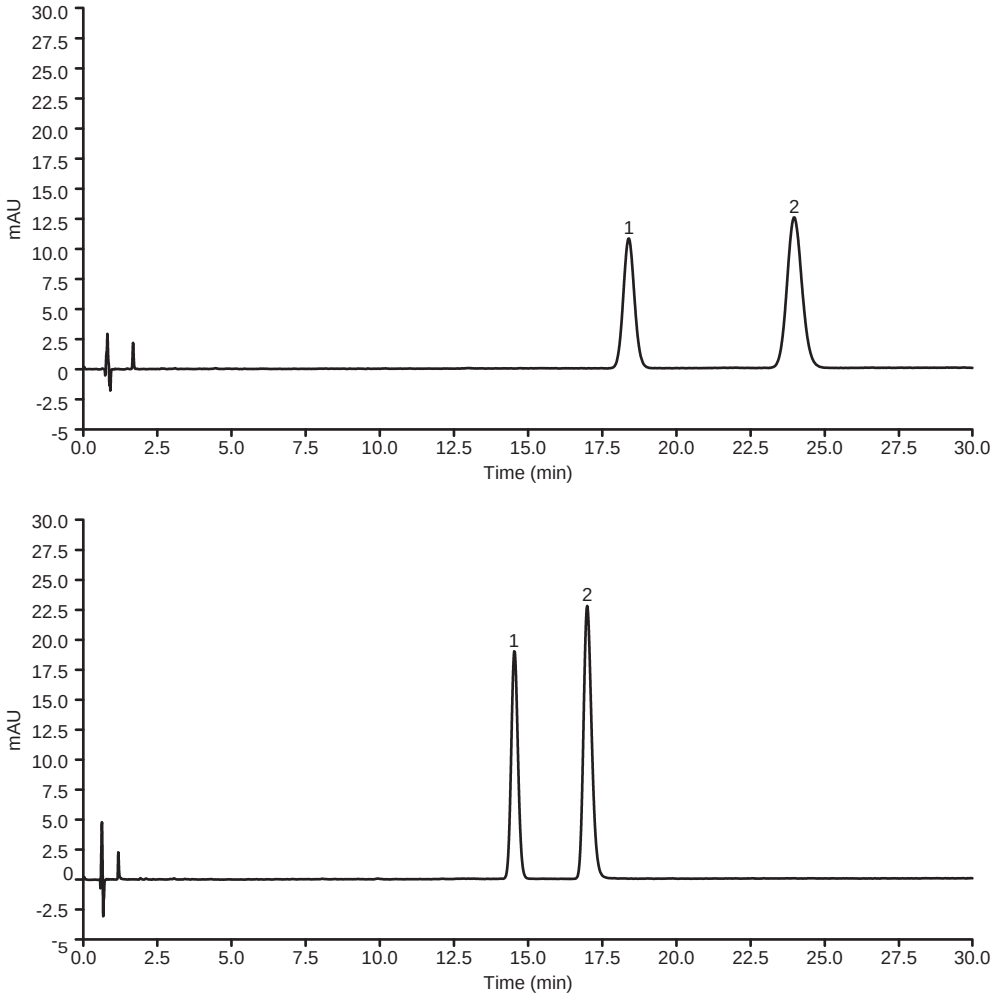

Figure 1: Chromatogram of valerophenone (1) and ibuprofen (2) analyzed using an Accucore XL C18 4 µm HPLC column (bottom trace) compared to a fully porous C18 5 µm column (top trace)

Experimental conditions: columns – 150 × 4.6 mm; mobile phase – water with phosphoric acid, pH 2.5 / acetonitrile (66.3:33.7 v/v); flow rate – 2 mL/min; column temperature – 30 °C; UV detection – 214 nm; injection volume – 5 µL

Compound	Plates (USP)			Signal-to-Noise Ratio		
	Accucore XL	Fully Porous	% improvement	Accucore XL	Fully Porous	% improvement
Valerophenone	19532	11218	74	908	462	96
Ibuprofen	18274	10538	73	1202	534	125

Table 1: Efficiency and signal-to-noise ratio data for valerophenone and ibuprofen

Sensitivity comparison with gradient mobile phase conditions

In Figure 2 the analysis performance of an Accucore XL C8 4 µm HPLC column is compared to that of a fully porous C8 5 µm using gradient mobile phase conditions, and maintaining all other experimental conditions for the two columns. The peak widths for the seven triazines narrowed significantly (on average by 29%) when using the Accucore XL HPLC column compared to the fully porous column (Table 2). As a result, the signal-to-noise ratio increased by 140% on average for exactly the same amount injected on column, significantly improving analysis sensitivity (Figure 3). Additionally, the resolution between the critical pair (peaks 5 and 6) on the fully porous column was 1.92, which improved by 54% to 2.95 with the Accucore XL C8 HPLC column.

The backpressure for the Accucore XL C8 4 µm HPLC column was measured at 215 bar, and the 5 µm fully porous column backpressure was measured at 165 bar. The improvement in performance was gained with a small increase in backpressure, which was still within the operating limits of a conventional HPLC system.

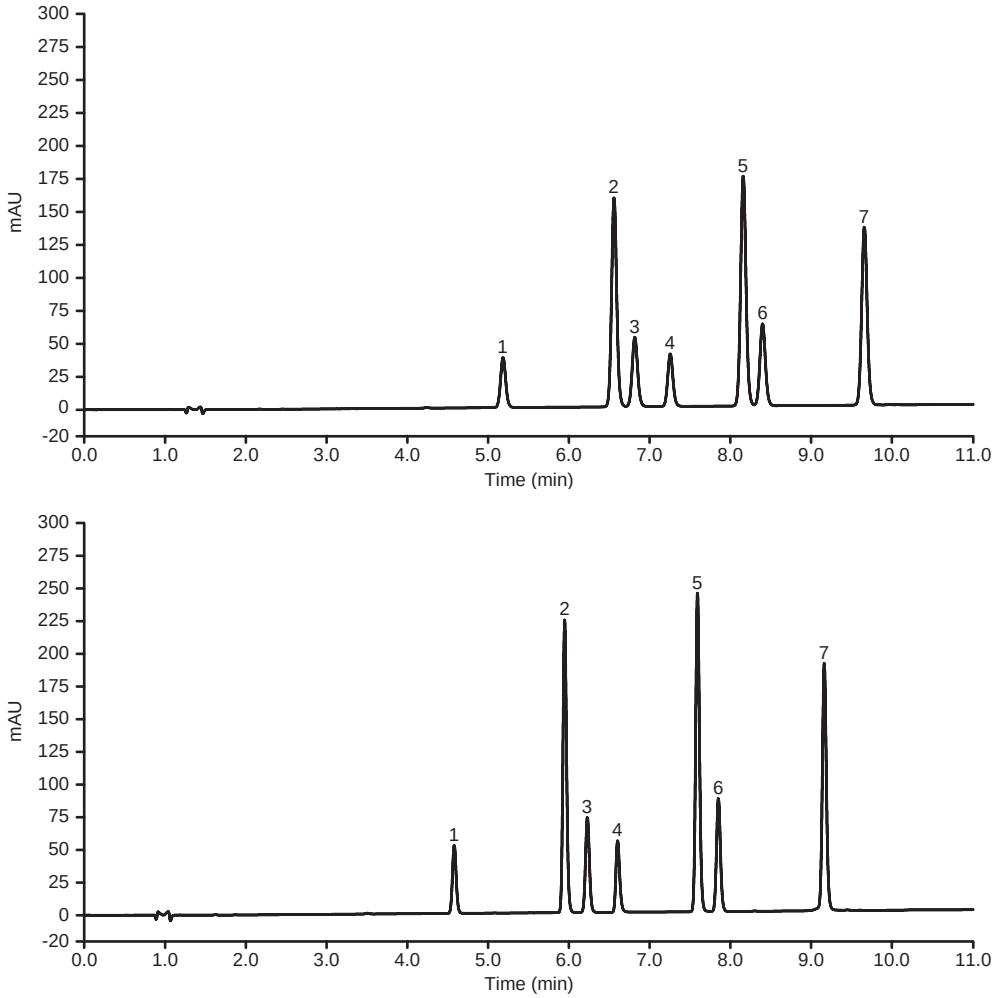

Figure 2: Chromatogram of simazine (1), simetryn (2), atrazine (3), prometon (4), ametryn (5), propazine (6), and prometryn (7) analyzed using an Accucore XL C18 4 µm HPLC column (bottom trace) compared to a fully porous C18 5 µm column (top trace)

Experimental conditions: columns – C18, 150 × 4.6 mm; mobile phase – water and acetonitrile; gradient – 20% to 60% acetonitrile in 10 min; flow rate – 1.5 mL/min; column temperature – 25 °C; UV detection – 220 nm; injection volume – 5 µL

Compound	Peak Width		Resolution		Signal-to-Noise Ratio	
	Accucore XL	Fully Porous	Accucore XL	Fully Porous	Accucore XL	Fully Porous
Simazine	0.108	0.151	N/A	N/A	3453	1468
Simetryn	0.106	0.151	16.42	11.75	14790	6109
Atrazine	0.112	0.160	3.31	2.13	4828	2027
Prometon	0.112	0.153	4.30	3.61	3603	1534
Ametryn	0.111	0.158	11.56	7.49	16170	6718
Propazine	0.114	0.163	2.95	1.92	5745	2390
Prometryn	0.115	0.162	14.64	9.98	12363	5200

Table 2: Peak width, resolution, and signal-to-noise ratio data for seven triazines

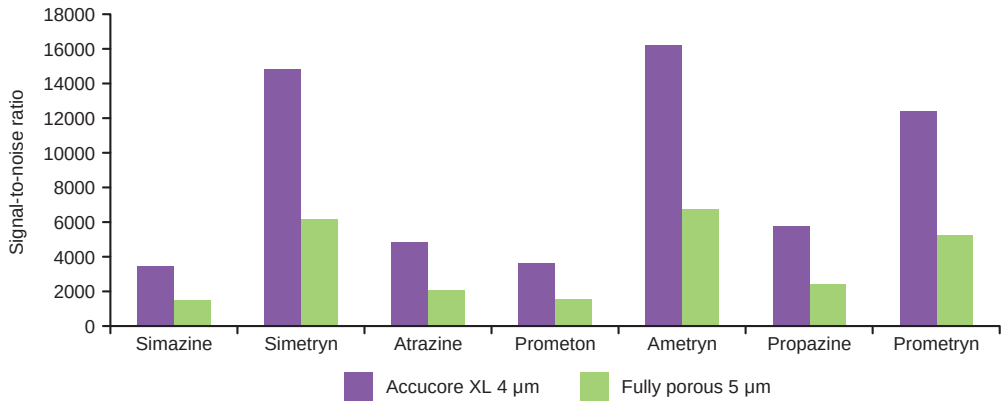

Figure 3: Signal-to-noise ratio comparison for the triazines gradient method in Figure 2, showing improvements between 135% and 142% for the 7 triazines

Sensitivity comparison in trace analysis

In trace analysis, the analyst is challenged to achieve the lowest possible limit of detection (LOD), the lowest concentration that can be detected, and the limit of quantification (LOQ), the lowest concentration that can be reliably quantified at a given signal-to-noise. For example, LOD is typically defined as the concentration on column that gives S/N = 3 and LOQ where S/N = 10. To achieve this goal, it is important to select the chromatographic parameters that will maximize C_{max}, namely high efficiency columns that produce symmetrical peaks, and which are not excessively retained.

In Figure 4, a comparison is made of the signal-to-noise ratios obtained for a series of triazines at trace level (1 ng injected on column) separated on Accucore XL 4 µm and fully porous 3 and 5 µm columns. It can be seen that the higher efficiency of the chromatographic peaks on the Accucore XL HPLC column enables greater signal-to-noise ratios, which are on average 116% and 100% higher than those obtained on fully porous 5 and 3 µm columns, respectively. The derived LODs and LOQs (based on this data) are listed on Table 3.

Column	LOD (ng) S/N = 3	LOQ (ng) S/N = 10
Fully porous 5 µm	0.6	2.0
Fully porous 3 µm	0.6	1.9
Accucore XL 4µm	0.3	0.9

Table 3: Derived LODs and LOQs

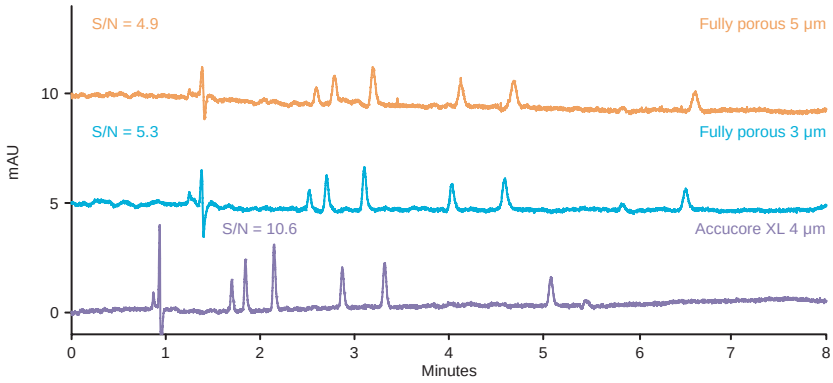

Figure 4: Comparison of average signal-to-noise ratios (S/N) on fully porous 5 and 3 µm and Accucore XL 4 µm columns for 1 ng of each solute loaded on column

Experimental conditions: columns – 150 × 4.6 mm; mobile phase – water and acetonitrile; gradient – 35% to 60% acetonitrile in 7.5 min; flow rate – 1.3 mL/min; column temperature – 30 °C; UV detection – 247 nm; injection volume – 5 µL; solutes – 1. Uracil, 2. Tebuthiuron; 3. Metoxuron; 4. Monuron; 5. Chlorotoluron; 6. Diuron; 7. Linuron

Conclusion

- The solid core 4 µm particles in Accucore XL HPLC columns provide significant improvements over fully porous 5 µm and 3 µm particles in terms of separation efficiency and sensitivity of the analysis.
- The Accucore XL 4 µm columns significantly improve sensitivity over fully porous 5 µm and 3 µm columns, with no changes to methodology or HPLC system configuration.

References

1. U. D. Neue, HPLC Columns: Theory, Technology, and Practice, Wiley-VCH, New York, 1997, page 334

2. USP-32, Ibuprofen, Chromatographic Purity

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