Loading Study on Agilent EnviroPrep Columns

Application Note

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Introduction

This application note describes a loading study performed on EnviroPrep columns to investigate the effect of sample concentration on-column on peak shape.

A mixture of four test probes was used to assess these preparative GPC columns for the separation of small molecules from a high molecular weight matrix. The probes were:

Corn oil

Bis(2-ethylhexyl) phthalate

Methoxychlor

Perylene

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Materials and Methods

Conditions

Column(s):	EnviroPrep, 300 x 25 mm (p/n PL1210-6120EPA) + EnviroPrep, 150 x 25 mm (p/n PL1210-3120EPA)
Eluent:	Dichloromethane
Flow Rate:	5.0 mL/min
Injection Volume:	2 mL
Sample Concentration:	2.5 - 10.0 mg/mL
Temperature:	Ambient
Detector:	Flow split to a differential refractive index (approximate flow in the detector 1.0 mL/min)

The samples were prepared individually in the eluent at 5.0 mg/mL and left overnight to fully dissolve/disperse. A solution containing a mixture of the samples was also prepared with each component present at 5.0 mg/mL.

The samples were injected individually so as to produce on-column loadings of 2.5, 5.0 and 10.0 mg.

Results and Discussion

Figures 1 to 4 show overlays of the chromatograms obtained. Although some of the samples were overloaded on the detector at the concentrations and split-ratios employed, the chromatography gave good peak shapes up to 10.0 mg on-column loading. The only sample that showed a consistent change in chromatography with concentration was the methoxychlor, which displayed a negative dip after the main peak as the concentration was increased.

Figure 5 shows overlaid chromatograms of the mixture of four components. Although some of the components of the sample are off-scale as before, the resolution between the peaks is maintained up to 10.0 mg loading. The peak disturbance noted with the methoxychlor sample is still seen with the mixture, but the overall separation between the four peaks is unaffected. As an indication of the consistency of performance of the columns at different sample loadings, the resolution factor between the bis(2-ethylhexyl) phthalate and methoxychlor peaks in the mixture was assessed at each column loading using the equation:

$$Rs = 2 (V2-V1) / (W1+W2)$$

where V1 and V2 are the elution volume (times) of the two peaks and W1 and W2 are the peak widths subtended to the baseline. Table 1 shows the results for the column set.

Table 1. Rs between bis(2-ethylhexyl) phthalate and methoxychlor at different column loadings for the EnviroPrep column set

Sample Loading mg (each Component)	Rs
2.5	1.91
5.0	1.93
10.0	1.72

Conclusion

The results show that four samples can be run together on an EnviroPrep column set successfully, up to loadings of 10 mg for the individual components. Some disturbance in the baseline is observed at the highest concentration by the methoxychlor, but the separation is still acceptable.

EnviroPrep columns permit a simple, one stage clean-up procedure for the determination of pesticides in a variety of organic matrices, eg soil, animal tissue, etc. The matrix is extracted and the higher molecular weight fractions such as lipids, polymers, natural resins and dispersed high molecular weight components are easily eliminated in the GPC analysis.

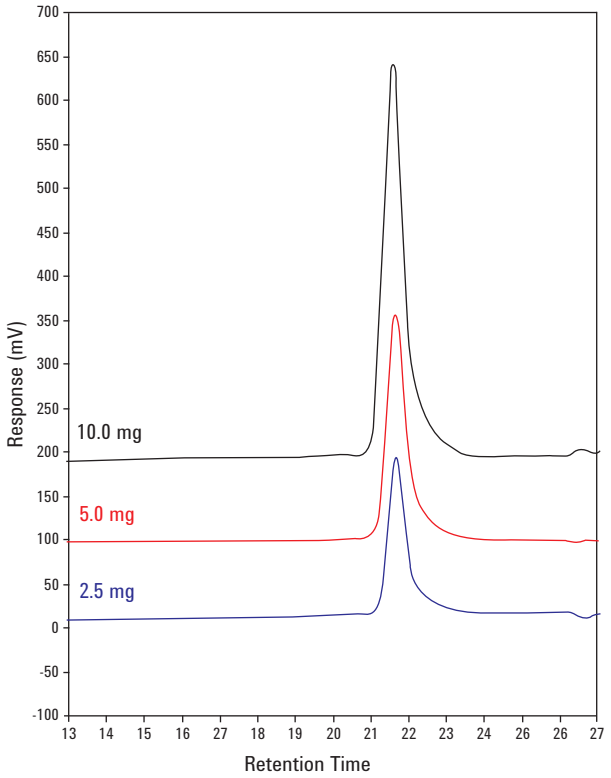

Figure 1. Separation of corn oil at 2.5, 5.0 and 10.0 mg on-column loading on the EnviroPrep column set

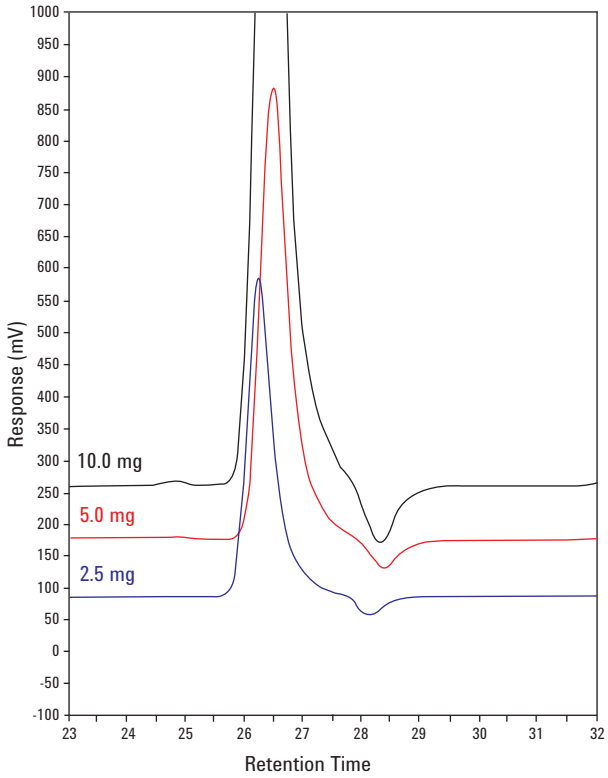

Figure 3. Separation of methoxychlor at 2.5, 5.0 and 10.0 mg on-column loading on the EnviroPrep column set

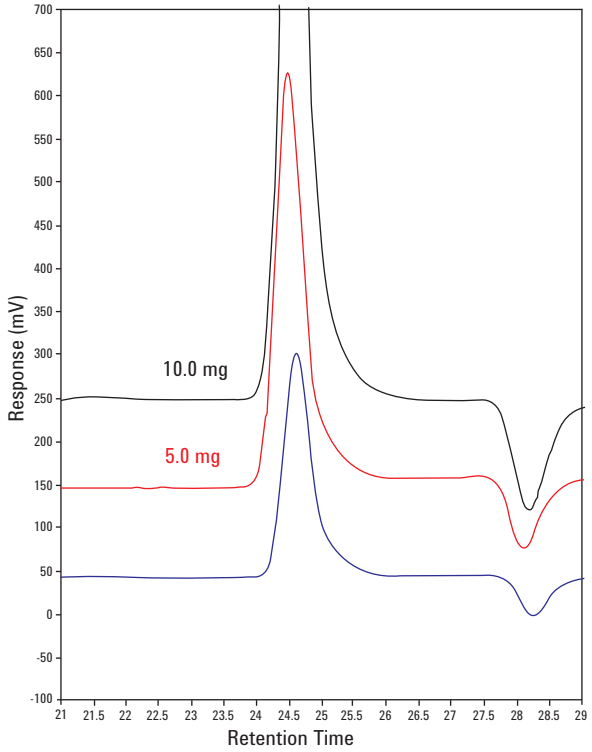

Figure 2. Separation of bis(2-ethylhexyl) phthalate at 2.5, 5.0 and 10.0 mg on-column loading on the EnviroPrep column set

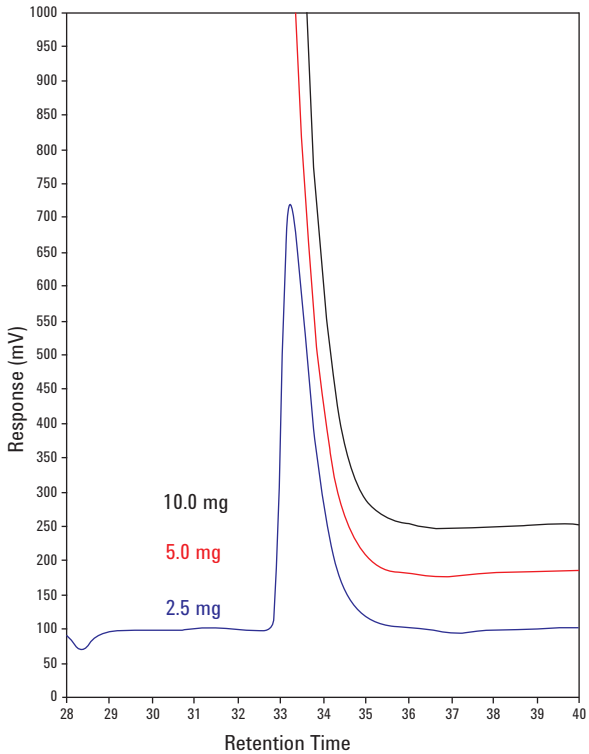

Figure 4. Separation of perylene at 2.5, 5.0 and 10.0 mg on-column loading on the EnviroPrep column set

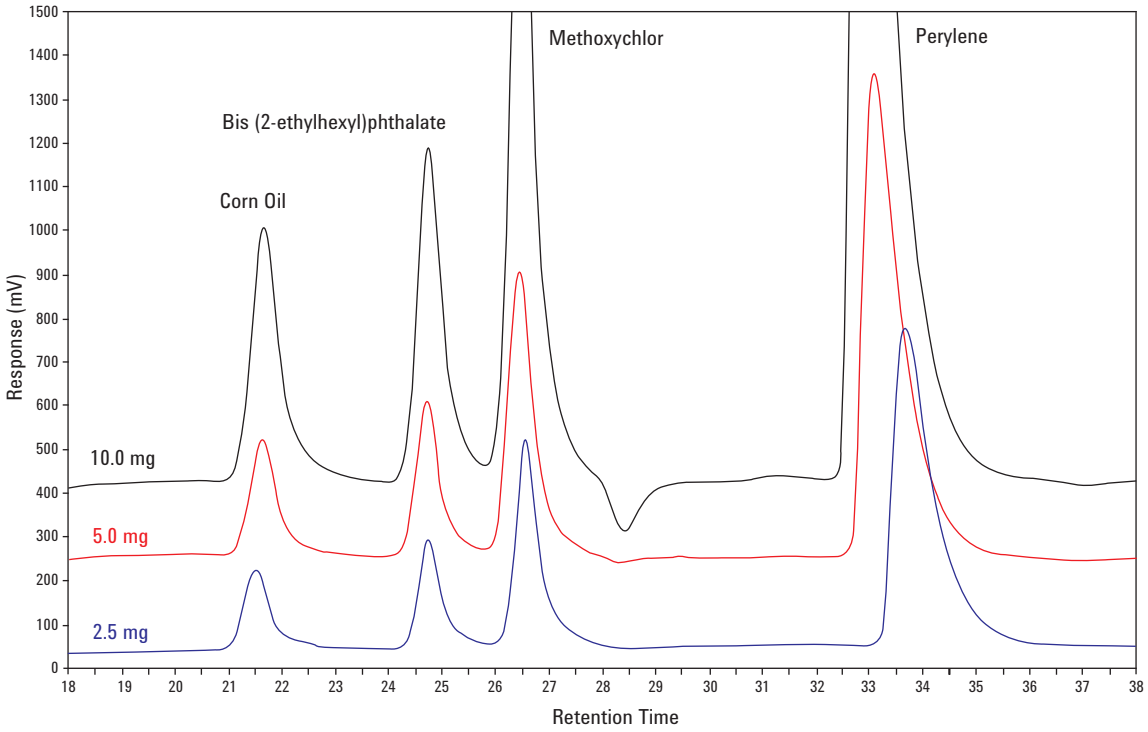

Figure 5. Separation of the four components at 2.5, 5.0 and 10.0 mg on-column loading on the EnviroPrep column set

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