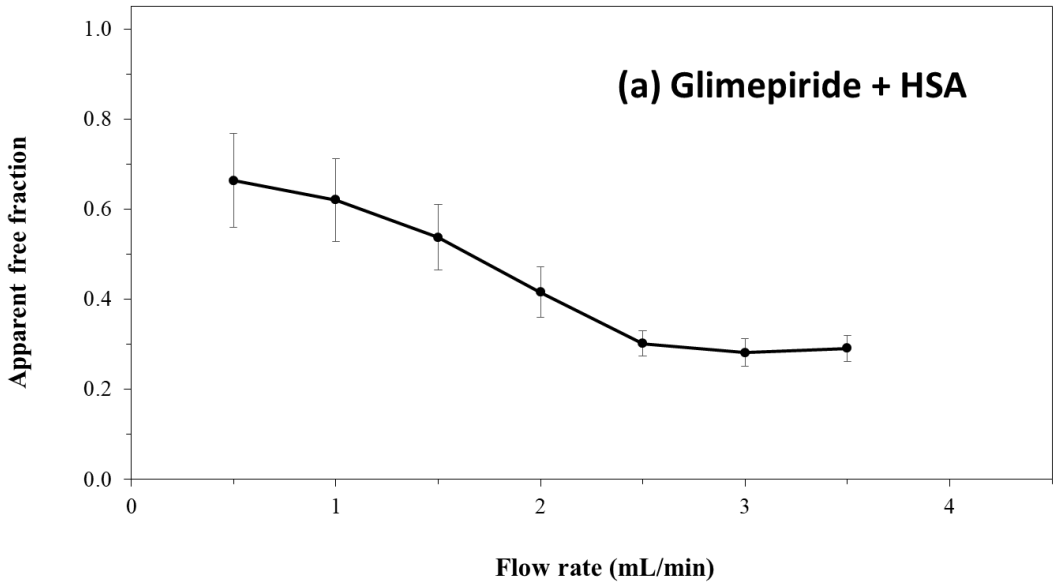
Supplemental Material

Optimization of flow rate in single-column systems for ultrafast affinity extraction

As is described in *Section 3.1*, experiments were conducted with each sulfonylurea drug examined in this study to find the optimum flow rate range that could be used to isolate and retain the drug's free fraction in the presence of soluble HSA. The conditions used are described in the main body of this paper, and the results that were obtained with glibenclamide are shown in Figure 4(a). The results obtained for glimepiride and glipizide are provided in Figure 1S. As was shown for glibenclamide, the apparent free drug fractions in samples containing glimepiride or glipizide plus HSA decreased as the injection flow rate was increased and reached a steady value at higher flow rates. As is illustrated in Figure 1S, glimepiride gave a consistent free fraction at flow rates of 2.5-3.0 mL/min or greater when using a 5.0 mm × 2.1 mm i.d. HSA microcolumn. Glipizide gave a consistent free fraction at flow rates of 3.0-3.5 mL/min or greater when using a 5.0 mm × 2.1 mm i.d. HSA microcolumn.

Figure 1S. Effect of changing the injection flow rate on the measurement of free drug fractions on a single-column affinity systems for samples containing (a) 10 μM glimepiride + 10 μM HSA or (b) 10 μM glipizide + 20 μM HSA. These samples were injected onto 5.0 mm × 2.1 mm i.d. HSA microcolumns. These data were obtained at pH 7.4 and 37°C. The error bars represent a range of ± 1 S.D.


(a) Glimepiride + HSA
Apparent free fraction
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
3
4
Flow rate (mL/min)

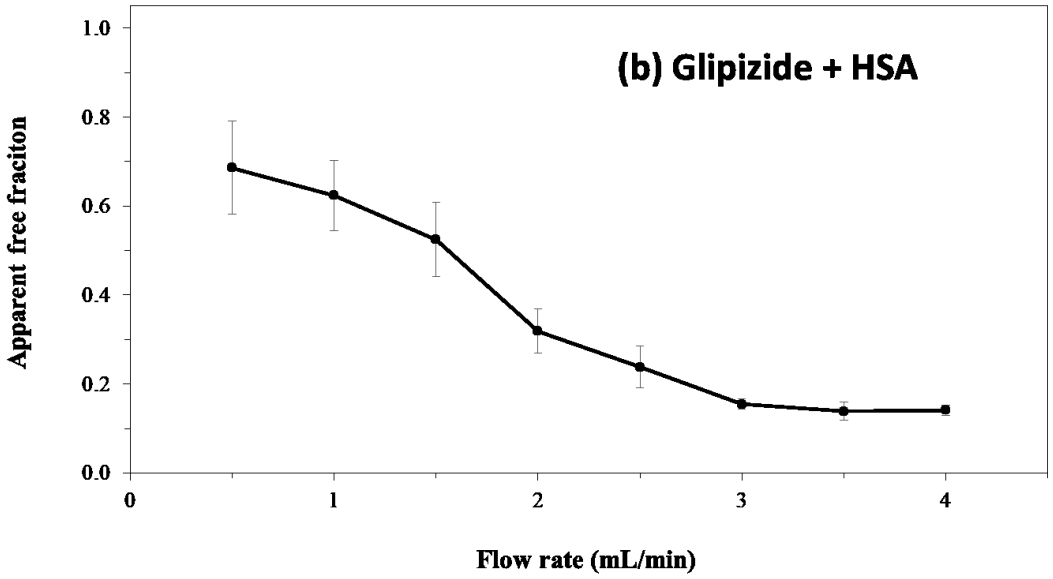
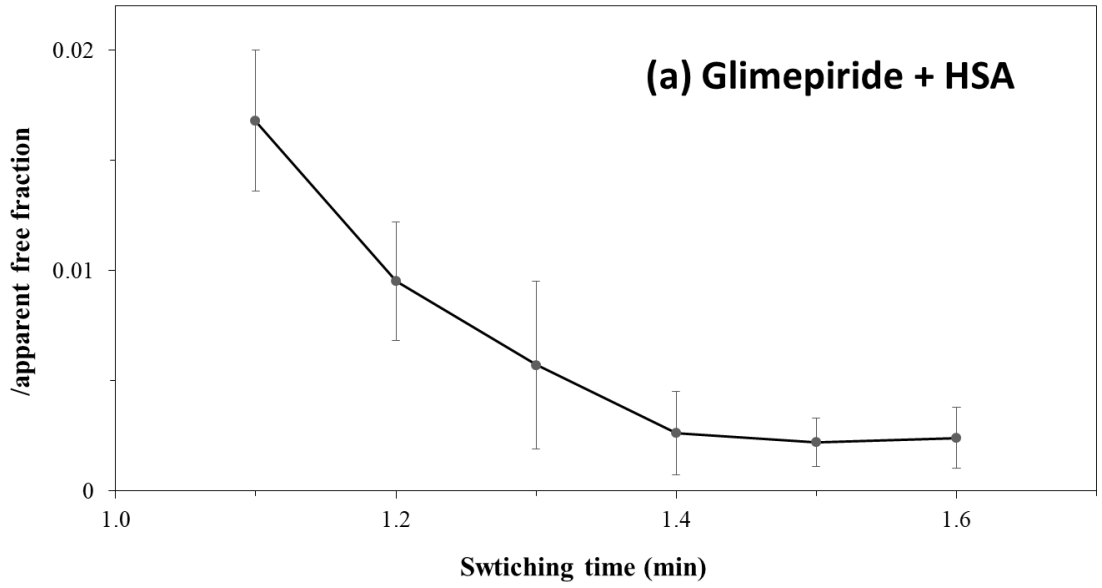

(b) Glipizide + HSA
Apparent free fraciton
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
3
4
Flow rate (mL/min)

Figure 1S

Optimization of switching time in two-column systems for ultrafast affinity extraction

As is discussed in *Section 3.3*, another factor that was optimized prior to use of a two-column system for ultrafast affinity extraction was the time at which the second microcolumn was switched on-line with the first. The effect of changing this parameter on the apparent free drug fraction for glibenclamide was shown in Figure 4(b), in which the apparent free drug fraction reached a minimum and a steady value when using a valve switching time of 4.9 min or longer. The results that were obtained in the same types of experiments for glimepiride and glipizide are shown in Figure 2S. For glipizide, a consistent free fraction was acquired when using a switching time of 1.3-1.4 min or more to place the first microcolumn on-line with a second HSA microcolumn that had a size of 10.0 mm × 2.1 mm i.d. and was used at a flow rate of 1.5 mL/min. Glimepiride gave a consistent free fraction when using a switching time of 1.4 min or greater and a second HSA column that had a size of 10.0 mm × 2.1 mm i.d. and was operated at 1.0 mL/min.

Figure 2S. Effect of changing the switching time for placing a second column on-line during the measurement of free drug fractions by two-column affinity systems for samples that contained (a) 0.40 μM glimepiride + 500 μM HSA or (b) 2.24 μM glipizide + 600 μM HSA. These measurements were made at pH 7.4 and 37 ℃ using 5.0 mm × 2.1 mm i.d. HSA microcolumns at 3.0 mL/min for glimepiride or 3.5 mL/min for glipizide. This was followed by the addition of a second 10.0 mm × 2.1 mm I.D. HSA microcolumn at 1.0 mL/min or 1.5 mL/min, respectively, at the given switching time. The error bars represent a range of ± 1 S.D.


(a) Glimepiride + HSA
/apparent free fraction
0.02
0.01
0
1.0
1.2
1.4
1.6
Swtiching time (min)

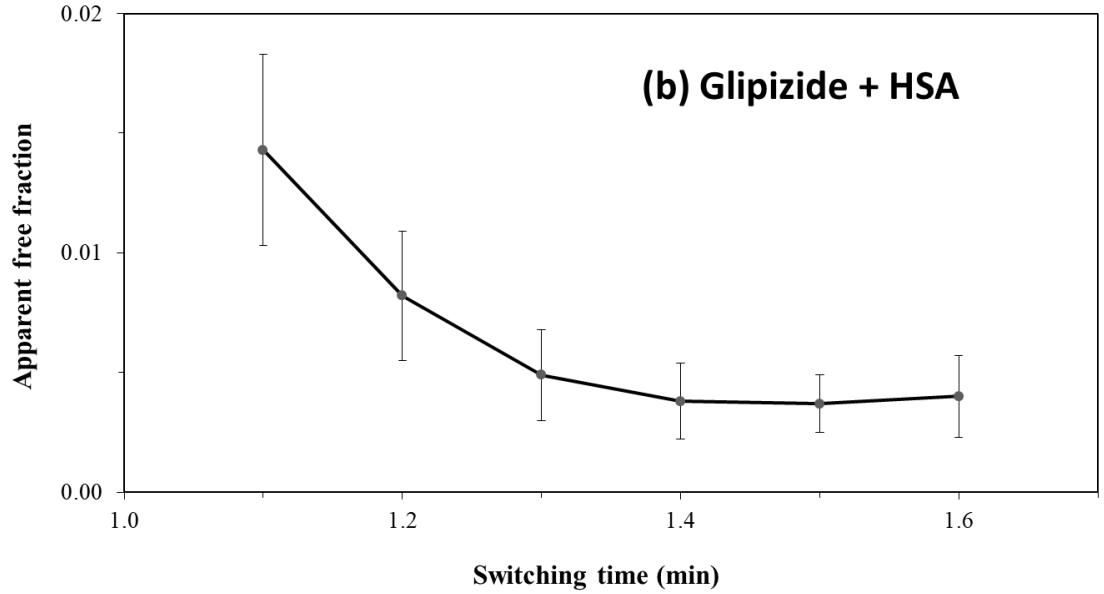
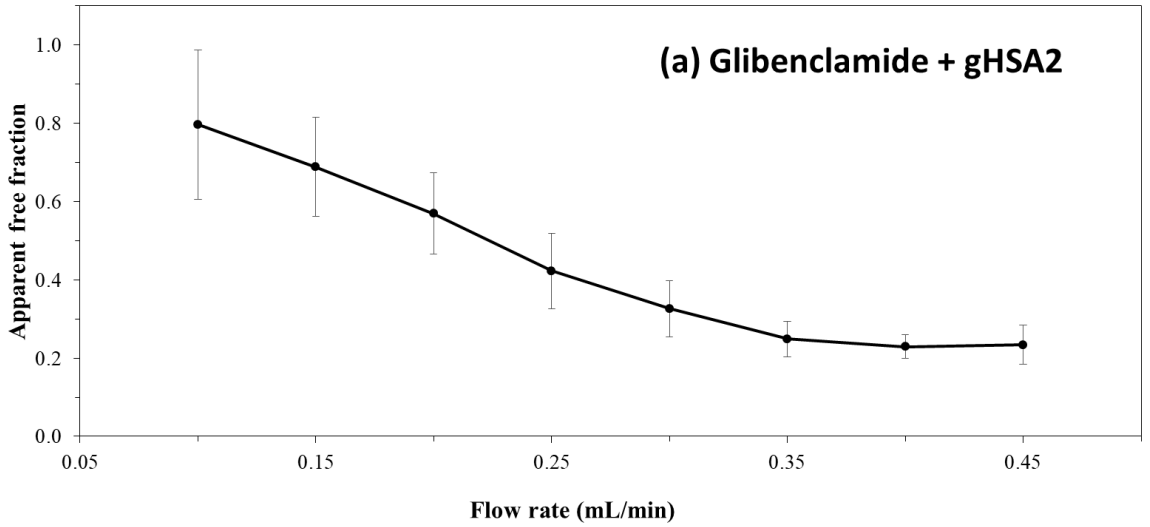

(b) Glipizide + HSA
Apparent free fraction
0.02
0.01
0.00
1.0
1.2
1.4
1.6
Switching time (min)

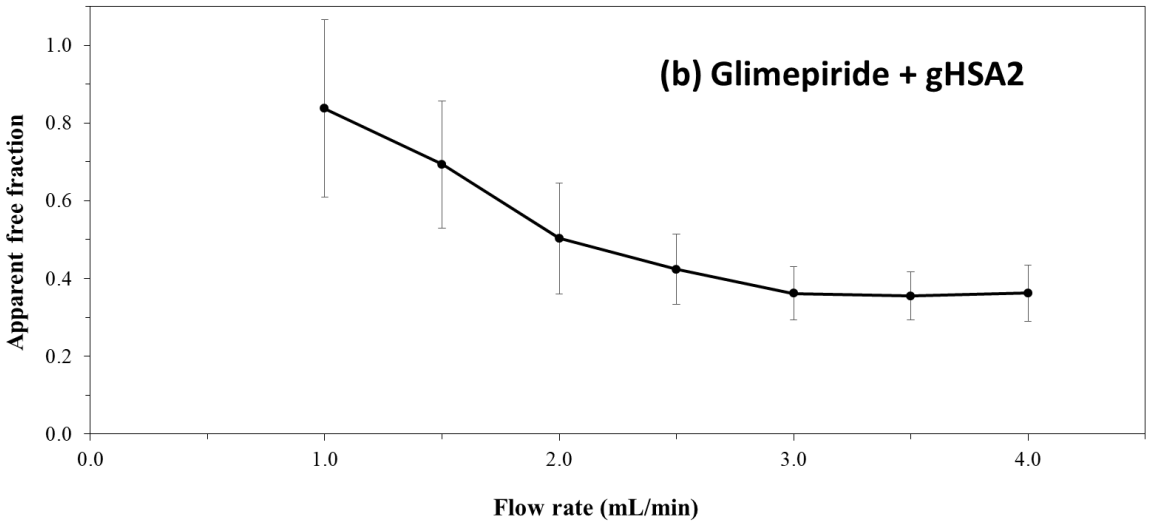
Figure 2S

Analysis of sulfonylurea binding to glycated HSA by ultrafast affinity extraction

The conditions to be used for ultrafast affinity extraction and single-column systems in examining the binding by second- and third-generation sulfonylurea drugs with glycated HSA were optimized in the same manner as described in *Section 3.1*. It was found that the same types of microcolumns and flow rates as were used for samples containing normal HSA could be used for samples with glycated HSA. This is illustrated by comparing the results that were given in Figure 4(a) and Figure 1S for samples containing normal HSA with the data that were obtained with glycated HSA in Figure 3S. In each case, the same flow rate ranges gave consistent free fraction measurements. The similarity in the conditions obtained with the different types of protein samples was due to the fact that the same types of HSA microcolumns were used to capture the free drug fractions, along with the similar (although not identical) binding parameters and rate constants that were measured in *Sections 3.2-3.5* for interactions of the given drugs with normal versus glycated HSA.

Figure 3S. Effect of changing the injection flow rate on the measurement of free drug fractions on a single-column affinity systems for samples containing (a) 10 μM glibenclamide + 10 μM gHSA2, (b) 10 μM glimepiride + 10 μM gHSA2, or (c) 10 μM glipizide + 20 μM HSA2. The samples were injected onto (a) 1.0 mm × 2.1 mm i.d. or (b-c) 5.0 mm × 2.1 mm I.D. HSA microcolumns. These data were obtained at pH 7.4 and 37°C. The error bars represent a range of ± 1 S.D.


(a) Glibenclamide + gHSA2
Apparent free fraction
1.0
0.8
0.6
0.4
0.2
0.0
0.05
0.15
0.25
0.35
0.45
Flow rate (mL/min)


(b) Glimepiride + gHSA2
Apparent free fraction
1.0
0.8
0.6
0.4
0.2
0.0
0.0
1.0
2.0
3.0
4.0
Flow rate (mL/min)

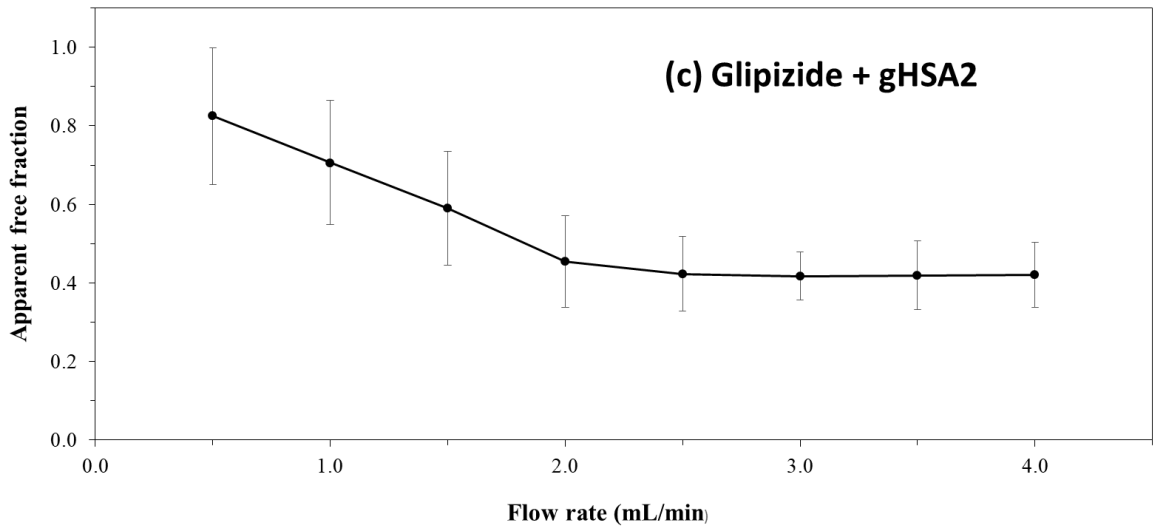

(c) Glipizide + gHSA2
Apparent free fraction
1.0
0.8
0.6
0.4
0.2
0.0
0.0
1.0
2.0
3.0
4.0
Flow rate (mL/min)

Figure 3S