

High Throughput Medicinal Library Screening against Human Serum Albumin

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INTRODUCTION:

Protein-drug binding greatly influences absorption, distribution metabolism and excretion (ADME) properties of typical drugs. Human Serum Albumin (HSA) is the most abundant protein in human blood (60% in plasma). Drug binding to HSA is an important determinant in identifying potential drug-free fraction within systemic circulation. Relative binding screening of combinatorial libraries against HSA during the preclinical stage is essential in eliminating compounds with potentially poor ADME properties.

Here we describe a model online technique to determine relative ligand binding of compounds against HSA. For proof of concept, a structurally diverse set of compounds: Indomethacin, Warfarin, Imipramine, Acetylprocainamide and Quidine with a wide range of respective association constants were selected. These were used to validate the method. The validated method was then run with a five component beta-lactam library. PE-Sciex API-III + triple quadrupole tandem mass spectrometer with APCI was used for the determination of MRM transition intensities.

Online determination was performed using 2 valco six-port switching valves (see Figure 1): (a) Incubations of HSA-ligand complexes (10 µL) were isolated from excess HSA @ 0.4 mL/min on a custom packed Amersham Biosciences 1ml HiTrap Desalting column in mobile phase 1. (b) In turn the complexes were retained and washed for 2 minutes @ 1.0 mL/min with mobile phase 2 on a protein macrotrap 3mm x 8mm (Michrom BioResources). (c) Subsequently, the macrotrap was placed inline and mobile phase 3 carried the unbound ligands @ 0.4 mL/min to a Luna C18 50 x 2.1 mm, 5µ (Phenomenex) for separation. LC conditions are described in Table 1. Total cycle was 7.5 minutes. Compounds were purchased from Sigma-Aldrich (St. Louis, MO) or donated by Procter and Gamble (Cincinnati, OH).

Beta-lactams were studied using a modified method (see Table 1). Small changes were made in the trap column phase to permit multiple applicability. We used a Waters Oasis HLB 30 x 2mm, 5µ as the trap column. The ionization mode was positive ESI and mobile phase 3 was 1/1 solvent A and B, isocratic. Cycle time was 6 minutes. These results are described extensively.

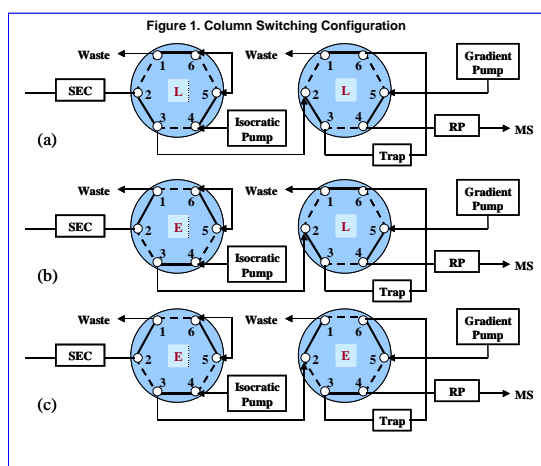


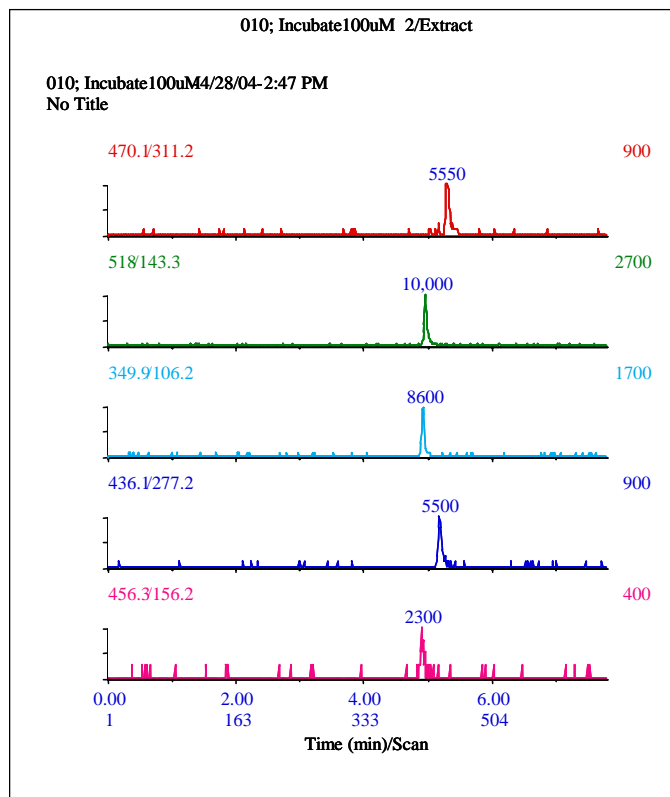
Table 1. LC/MS/MS Conditions

Conditions	Generic Method		Beta-lactam Method	
Solvent A	10 mM ammonium acetate in water			
Solvent B	10 mM ammonium acetate in acetonitrile			
Buffer B	10mM Tris-HCl, pH=7.4, 10mM MgCl ₂ , 10mM KCl			
Mobile phase 1	95/5 Buffer B/methanol		95/5 Buffer B/methanol	
Mobile phase 2	0.1% TFA in water		0.1% TFA in water	
Mobile phase 3	5-80% B over 3.5 min		50% B/A	
SEC column	HiTrap 1 ml		HiTrap 1 ml	
Trap column	Protein Trap		Oasis HLB	
Reverse-Phase (RP) column	Luna C18		Luna C18	
Ionization mode	APCI +		ESI +	
Increasing binding affinity ↓	Compounds	SRM	Compounds	SRM
	Quindine	325.2>160.2	Ampicillin	349.9>106.2
	Acetylprocainamide	278.1>205.2	Piperacillin	518.0>143.3
	Imipramine	281.2>208.3	Cefazolin	456.3>156.2
	Warfarin	309.1>250.9	Cloxacillin	436.1>277.2
	Indomethacin	358.1>139.0	Dicloxacillin	470.2>311.2

DISCUSSION-

Table 2 summarizes the binding data for the beta-lactam experiment. Figure 2 is a typical SEC-UV-LC-MS/MS of an incubated sample. Peak area ratios (Incubation/REF) illustrate the relative binding of each ligand to the receptor HSA. As mentioned earlier, the incubation peaks correspond to the ligands bound

Figure 2. SEC-UV-LC-MS/MS of 100µM Incubation



to HSA. Therefore, a larger Incubation/REF ratio for a particular ligand indicates a higher binding affinity to HSA. Among the five beta-lactam compounds in our study, dicloxacillin exhibited the highest relative binding affinity to HSA while ampicillin had the lowest binding affinity. The results obtained through this experiment and summarized in Table 2 were consistent with the binding constants and percent binding characteristics of each ligand against HSA. Monitoring of relative binding affinity of a combinatorial libraries can be achieved using our on-line SEC column switching configuration.

Table 2. Relative binding ratios

Ligand	Ref. (10 µM) Peak Area	Incubation (100 µM) Peak Area	Peak Area Ratio (Incubation/Ref)
Ampicillin	103350	8600	.08
Piperacillin	89300	10000	.11
Cefotaxime	17950	2300	.13
Cloxacillin	20950	5000	.24
Dicloxacillin	16400	5550	.34

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