

## A Shielded Hydrophobic HPLC Phase for Drugs in Biological Matrices

Direct injection of untreated biological samples onto a conventional reversed phase HPLC column causes clogging and rapid deterioration of the column. A shielded hydrophobic phase (SHP) excludes proteins while interacting with small molecules. SHP consists of a polymeric surface containing hydrophobic pockets enclaved by a hydrophilic network. Small analytes, such as drugs, penetrate the water-solvated interface of the hydrophilic network to interact with the hydrophobic regions. The hydrophilic shielding prevents larger water-solvated molecules, such as proteins, from entering into interactions with the hydrophobic groups.

### Key Words:

- drugs • biological fluids • Hisep HPLC column
- shielded hydrophobic phase

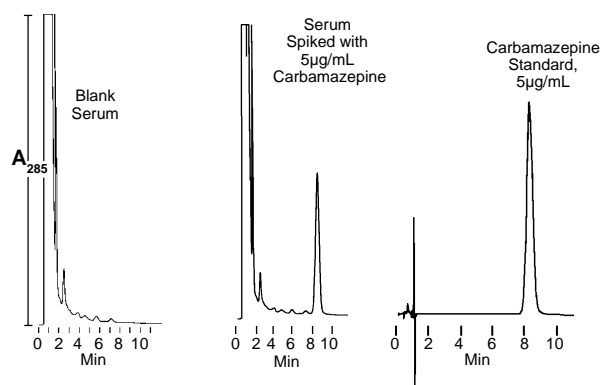
Direct sample injection in HPLC analyses of drugs, drug metabolites, and other substances in biological matrices would eliminate extensive sample cleanup prior to analysis. One review summarizes methods for direct injection (1). In the earliest applications, direct sample injection of the biological matrix was performed; however, the column required thorough washing after every few injections to remove precipitated proteins (2,3). In later applications, samples were injected directly onto standard columns using micellar mobile phases that solubilize the proteins (4). Other methods involve pre-columns or two-column techniques, employing a variety of column switching methods that allow only the drug(s) to elute onto the analytical column (5-9). Other approaches have been used, each with its own drawbacks (10-13).

The retention of the smaller analytes is unaffected under appropriate chromatographic conditions because the bulk of the protein matrix elutes as an unretained, or nearly unretained, band. A hydrophilic network of bonded polyethylene oxide, embedded with hydrophobic phenyl groups, forms the Hisep™ packing.

Human serum and plasma samples, spiked with therapeutic levels of carbamazepine and phenobarbital, demonstrate the application of the Hisep column for drug analyses (14). We used a 15cm x 4.6mm ID Hisep column for direct injection chromatography of these drug-spiked biological fluids. Figure A shows chromatograms of blank serum, carbamazepine-spiked serum (5µg/mL), and a carbamazepine standard (5µg/mL). Figure B includes chromatograms of phenobarbital-spiked plasma (12.5µg/mL) and a phenobarbital standard (12.5µg/mL). In each case, most proteins eluted unretained from the serum or plasma matrix.

Figure A. Human Serum: Direct Injection

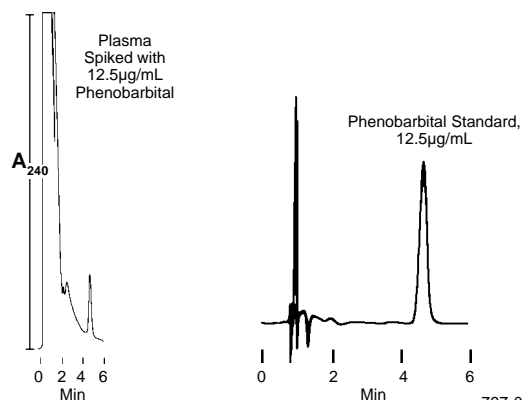
Column: Hisep, 15cm x 4.6mm ID, 5µm particles  
Cat. No.: 58935  
Mobile Phase: acetonitrile:180mM ammonium acetate (12:88)  
Flow Rate: 2mL/min  
Temp.: ambient  
Det.: UV, 285nm  
Inj.: 50µL



797-0536, 0537, 0538

Figure B. Human Plasma: Direct Injection

Column: Hisep, 15cm x 4.6mm ID, 5µm particles  
Cat. No.: 58935  
Mobile Phase: acetonitrile:180mM ammonium acetate (5:95)  
Flow Rate: 2mL/min  
Temp.: ambient  
Det.: UV, 240nm  
Inj.: 50µL



797-0539, 0548

Mobile phase conditions determine the retention of the low-molecular mass components of the blood fluid matrices. Protein precipitation is the limiting factor in changing mobile phase conditions. The pH range of the mobile phase is about 2.5 to 7 because the SHP is based on a silica support. The mobile phase typically consists of no more than 15-20% organics and 0.5M buffered solutions.

Additional applications can be conducted by injecting a wide variety of drug compounds onto the Hisep column under simple chromatographic conditions. Each drug in Table 1 was eluted in a single binary mobile phase. Compounds not eluted under these conditions can be expected to elute upon adjustment of the mobile phase.

**Table 1. Retention Data for Drugs on a Hisep Column**

Compound	Capacity Factor	Compound	Capacity Factor
Acetaminophen	1.35	Naproxen	8.13
Acetylsalicylic acid	0.22	Nifedipine	30
Amitriptyline	12.6	Phenobarbital	2.60
Atenolol	0	Phenylbutazone	18.6
Butorphanol	2.05	Phenytoin	NE*
Caffeine	0.81	Prazosin	17.6
Carbamazepine	8.73	Primidone	1.35
Chlorpheniramine	3.62	Procainamide	0.29
Chlorpropamide	2.05	Propoxyphene	1.93
Chlorthalidone	10.2	Propranolol	2.85
Cimetidine	1.04	Quinidine	4.16
Codeine	0.72	Salicylic acid	2.00
Desipramine	7.80	Sulfamethoxazole	2.02
Diflunisal	46	Sulfapyridine	2.81
Dipyridamole	40	Sulfasalazine	NE*
Fenopropfen	8.45	Sulfipyrazone	22.7
Furosemide	19.6	Terbutaline	0.21
Hydrochlorothiazide	8.91	Theophylline	0.67
Hydroxyzine	7.99	Timolol	0
Ibuprofen	4.46	Tolbutamide	2.35
Imipramine	9.62	Tolmetin	5.37
Indomethacin	44.4	Trimethoprim	2.29
Lidocaine	0.30	Trimipramine	9.74
Nadolol	0	Valproic acid	NE*
		Verapamil	5.53

Column: **Hisep, 15cm x 4.6mm ID, 5µm particles**  
(with 0.5µm in-line frit filter)  
Cat. No.: **58935**  
Mobile Phase: acetonitrile:180mM ammonium acetate (10:90)  
Flow Rate: 2mL/min  
Temp.: ambient  
Det.: UV, 254nm  
Inj.: 10µL

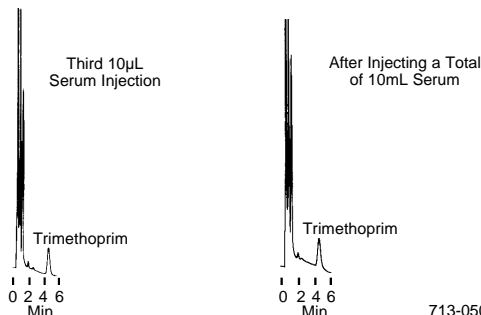
\*NE – not eluted under these conditions.

Figure C illustrates the chromatographic stability and reproducibility of the SHP. After more than 1000 injections of trimethoprim-spiked bovine serum, retention of the drug did not change significantly, nor was there a significant change in column backpressure.

The high degree of physical stability and chromatographic reproducibility demonstrated by the Hisep column indicates the column's usefulness for routine or experimental work in separating proteins from smaller analytes.

### Figure C. Hisep Columns Perform Consistently for Many Injections

Column: **Hisep, 15cm x 4.6mm ID, 5µm particles**  
Cat. No.: **58935**  
Mobile Phase: acetonitrile:180mM ammonium acetate (15:85), pH 7  
Flow Rate: 2mL/min  
Temp.: ambient  
Det.: UV, 254nm  
Inj.: 10µL spiked serum (25µg/mL trimethoprim)



713-0504,0505

### Ordering Information:

Description	Cat. No.
<b>Hisep SHP Columns</b>	
25cm x 2.1 mm ID, 5µm particles	<b>57932</b>
5cm x 4.6mm ID, 5µm particles	<b>59143</b>
15cm x 4.6mm ID, 5µm particles	<b>58935</b>
25cm x 4.6mm ID, 5µm particles	<b>58919</b>

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Note 145

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