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## COMPARISON OF CLASSICAL GFF AND NEW GFFII ISRP PACKINGS

**Analytes:** #I: Phenobarbital and carbamazepine in human serum

#II: Barbiturate standards in human serum

<b>Flow Rate:</b>	#I: 0.6 ml/min	<b>Detection:</b>	#I: 254 nm	<b>Sample Size:</b>	#I: 10 microliters
	#II: 1.0 ml/min		#II: 240 nm		#II: 10 microliters

**Column A:** 5 micron GFF ISRP, 15 cm x 4.6 mm ID

**Regis Product Number:** 731451

**Column B:** 5 micron GFF II ISRP, 15 cm x 4.6 mm ID

**Regis Product Number:** 731471

### Mobile Phase:

#I: 80% 0.1M  $\text{KH}_2\text{PO}_4$  (pH 6.8), 20% Acetonitrile

#II: 95% 0.1M  $\text{KH}_2\text{PO}_4$  (pH 7.5), 5% Methanol

**Discussion:** The ISRP packing which Regis is commercially marketing has an outer surface of glycine and an inner surface of the tripeptide glycine-phenylalanine-phenylalanine (GFF). The tripeptide is bonded on the amino side leaving a free carboxylic acid group. GFF is therefore, a hydrophobic weak cation exchanger.

GFF is now widely used in many applications involving the separation of small molecules in the presence of proteins and other large molecules. However, in order to improve the usefulness of this first generation ISRP phase, we undertook a detailed study of the synthesis and performance of the GFF packing. As a result of this effort, we developed a new GFF packing, GFFII, that has these improved qualities:

1. Greater hydrophobicity to improve the retention of the more hydrophilic drugs.
2. Efficiencies greater than the 35,000 plates/meter that the GFF column generally delivers.
3. Lot-to-lot reproducibility which is more easily controlled.

Figure 1 shows chromatograms of human serum spiked with phenobarbital and carbamazepine run on the original GFF column and the new GFFII. The retentions of both drugs have substantially increased. The efficiency of the GFFII column is also much higher.

The improved performance of the GFFII packing is dramatically shown in Figure 2. This is a serum sample spiked with a standard mix of six barbiturates: 1: Barbitol; 2: Phenobarbital; 3: Butobarbital; 4: Amobarbital; 5: Pentobarbital; and 6: Secobarbital. The unsatisfactory analysis on the GFF column is now workable on the GFFII.

In comparing the two packings, efficiency can be seen to have increased from 35,000 plates/meter to about 63,000 plates/meter and the retention of carbamazepine has more than doubled.

Since the original GFF columns are being used in many applications, Regis will continue to offer the original GFF

columns along with the new GFFII columns.

## Figures:

Figure 1

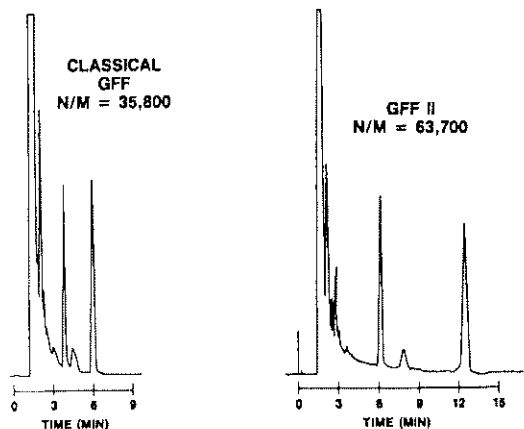
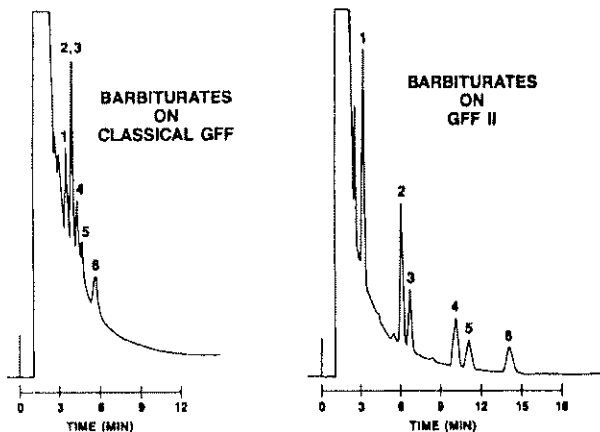


Fig. 1. The 10  $\mu$ ls serum-phenobarbital carbamazepine test mix, our standard for testing GFF columns, on the classical GFF column (left) and the new GFFII column (right).

Fig 2. 10  $\mu$ ls of a 6-component mix of barbiturates, each at 10  $\mu$  gms/ml. 1: Barbital; 2: Phenobarbital; 3: Butabarbital; 4: Amobarbital; 5: Pentobarbital 6: Secobarbital. GFF (left) and GFFII (right) columns.



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