

NOTE NO. 43 - Sept 15 1993

## VALIDATED ASSAY OF AN LTD4 RECEPTOR ANTAGONIST

From a poster presented to the Fourth International Symposium on Pharmaceutical and Biomedical Analysis, Baltimore, MD, April 18-21, 1993, by Senior Research Fellow John Hsieh, Merck Research Laboratories.

**Analytes:** LTD4 receptor antagonist I (0.1-5.0  $\mu\text{g/mL}$ , validated, linear); and its metabolites II and III (0.7  $\mu\text{g/mL}$ ).

**Sample Matrix:** Human plasma

**Sample Size:** 20  $\mu\text{L}$

**GFF II Columns:** Guard cartridge: 5 micron, 1 cm x 3 mm ID

**Regis Product Number:** 731475

**Analytical column:** 5 micron, 5 cm x 4.6 mm ID

**Regis Product Number:** 731470

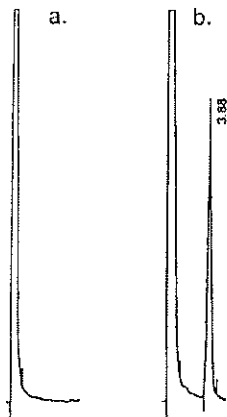
**Mobile Phase:** 64% 0.1M  $\text{KH}_2\text{PO}_4$  (pH 6.5), 36% Acetonitrile

**Flow Rate:** 0.9 mL/min, ambient temperature

**Detection:** Fluorescence, Exc 359 nm, Em 400 nm

**Centrifugation:** 1. Microcentrifuge<sup>TM</sup> tube, 12,000 rpm, 8 min;

2. Aliquot from 1, 8,000 rpm, 7 min. through Microcentrifuge<sup>TM</sup> tube, 0.2  $\mu\text{m}$  Nylon-66.



**Column life:** Fine. After some 350 injections, the GFFII was "still in good shape" (1).

**Resolution:** GFFII resolves Comp. I well from II, III, and interfering human plasma substances (IHPSs); GFF, not so well; a competitor's RAM, not from IHPSs.

**Efficiency:** Superior GFFII-over-GFF efficiency yields about 10% better sensitivity S (see below), much better reproducibility R. GFFII chromatographic efficiency is over 60,000 plates per meter; GFF, 35,000 (2). S: the GFFII/GFF same-sensitivity peak-height ratio is about 1.10. R: at 0.1  $\mu\text{g/mL}$ , the coefficient of variation with GFF is 31.3, intraday; with GFFII, 9.7, interday.

Column: 5 cm Pinkerton GFFII

Sample: (a.) control plasma,

(b.) 1  $\mu\text{g/mL}$  Compound I in human plasma

(c.) 1  $\mu\text{g/mL}$  of I and II, 0.7  $\mu\text{g/mL}$  III in human plasma

Inj. Vol.: 20  $\mu\text{L}$

Flow Rate: 0.9 mL/min, ambient temperature

Mobile Phase: 64% 0.1M  $\text{KH}_2\text{PO}_4$  (pH 6.5), 36% Acetonitrile

Detection: Excitation = 359 nm, Emission = 400 nm

**References:** (1) Hsieh, J. Personal communication, July 19, 1993.

(2) Perry, J. A.; Invergo, B.; Wagner, H.; Szczerba, T. J.; Rateike, J. D. *J. Liq. Chromatogr.* **1992**, *15*, 3343-3352.

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