

# Analysis of NAPROXEN in Serum: Zorbax SPE C18 Cartridge

Application Brief Pharmaceutical

Naproxen is a pharmaceutical compound used for the treatment of pain caused by arthritis, gout, menstrual cramps, and other medical problems. It may be of interest to determine levels of naproxen or naproxen-like compounds in the bloodstream with a reduced background matrix (e.g., blood proteins). As shown below, it is possible to significantly reduce levels of interfering substances through the use of a Zorbax SPE C18 cartridge. The analyte of interest may then be quantitated by reversed-phase HPLC. Recovery was high, though naproxen undergoes significant binding to serum proteins.



*Figure 1.* Comparison of naproxen-spiked serum before (panel A) and after extraction (panel B) using the Zorbax SPE C18 cartridge. Notice effectiveness of the extraction. In panel A, the large amount of protein in the sample interferes with detection of naproxen. Once the sample has passed through the cartridge, naproxen is clearly detected and quantifiable (panel B). Nitrobenzene is included in samples as an internal standard.

### Solid Phase Extraction Method

- Cartridge: 100 mg, 1 ml Zorbax SPE 18 (P/N 5184-3590, Box of 100)
- PRETREAT:
  -2.0 mL 1% acetic acid in Methanol
  -1.0 mL Deionized Water
- LOAD: -Sample (see sample preparation)
- WASH:
  -1.0 mL 4% Isopropyl alcohol in
  100 mM formic acid
  -0.5 mL Deionized Water
- ELUTE: -1.0 mL 50% Acetonitrile in 40 mM ammonium acetate
- INTERNAL STANDARD:
  -10 μL (2 mg/mL) nitrobenzene in methanol as internal standard for High Load.

-10 μL (0.4 mg/mL) nitrobenzene in methanol as internal standard for Low Load.

## Sample Preparation

-500 µL Serum

-500 µL 4% Isopropyl alcohol in 100mM formic acid

-10µL Naproxen (as below)

High load,

-1 mg/mL in 50% Acetonitrile 25 mM formic acid

Low load,

*-0.2 mg/mL in 50% Acetonitrile 25 mM formic acid* 



### HPLC Method

LC Instrument: Agilent 1100 Column: Zorbax SB-C8, 4.6 mm x 150 mm, 3.5 µm (P/N 863953-906) Eluent: 50% Acetonitrile in 25 mM Ammonium acetate UV: 240 nm Flow: 1.0 mL/min. Temperature: Ambient Inj. Vol.: 10 µL

## Recovery

• HIGH LOAD (10 µg/mL): X = 99.25%, n = 3

• LOW LOAD (2 μg/mL): X = 102.2%, n = 3

Recovery of analyte in these experiments is an indication of 'goodness' of technique, as well as quality of the SPE packing. For best results, solutions should not be drawn completely through the cartridge until the final elute step.

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Publication Number 5988-2525EN

