Analysis of Clozapine: Zorbax SPE C18 EC Cartridge

Application Brief

Pharmaceutical

Dr. David Freeman*

Clozapine is a pharmaceutical compound used for the treatment of severely ill schizophrenic clients who do not respond adequately to other drug therapies. It may be of interest to determine levels of clozapine or clozapine-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 EC cartridge. The analyte of interest may then be quantitated with little interference by reversed-phase HPLC. Recovery was high for all analytes tested.

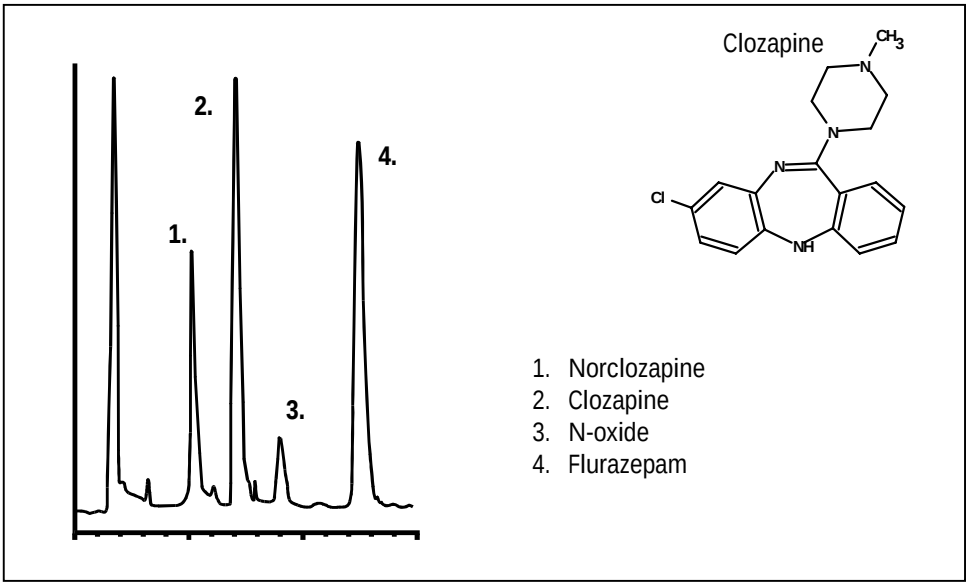

Figure 1. *Analysis of clozapine and its metabolites using the Zorbax SPE C18 EC cartridge. Notice effectiveness of the extraction. Once, the sample has passed through the cartridge, clozapine is clearly detected and quantifiable. Flurazepam is included as an internal standard.*

Solid Phase Extraction Method

- *Cartridge: 100 mg, 1 ml Zorbax SPE 18 EC (P/N 5184-3597, Box of 100)*
- *PRETREAT:*
  -1.0 mL Methanol
  -1.0 mL Deionized Water
- *LOAD:*
  -Sample (see sample preparation)
- *WASH:*
  -2.0 mL Deionized Water
  -1.0mL 50% Methanol/Water
- *ELUTE:*
  -1.0 mL Methanol
- *EVAPORATE and RECONSTITUTE:*
  -Dried at 40° C with nitrogen stream
  -Add 100 µL of mobile phase.

Sample Preparation

-200 µL Deionized Water
-200 µL Plasma
-50µL Flurazepam (as below)

Internal Standard,
-Flurazepam prepared as a 1800 ng/ml in Deionized Water

HPLC Method

LC Instrument:
HP 1090
Column:
Spherisorb C8
3.2mm x 100mm, 5µm
Eluent:
28% Acetonitrile in
25mM Potassium Phosphate
800µL Triethylamine/L, pH 3.0
UV: 245 nm
Flow: 0.5 mL/min.
Temperature: 40°C
Inj. Vol.: 20 µL

Recovery

- *Clozapine (200 µg/mL):*
  X = 103%, n = 10

- *Norclozapine (200 µg/mL):*
  X = 111%, n = 10

**Dr. Freeman is a professor in the Department of Medicine at the University of Western Ontario in London, Ontario Canada.*

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