

Analysis of Quinine in Serum: Zorbax SPE C18 Cartridge

Application Brief

Pharmaceutical

Wu Chen

Quinine and related compounds may be present in the bloodstream at various levels, both naturally, and as a result of medical treatment. It is of interest to determine levels of quinine and quinine-like compounds in the bloodstream with minimal background from the matrix (e.g., blood proteins). As shown below, it is possible to significantly reduce levels of matrix compounds through the use of a Zorbax SPE C18 cartridge. The analytes of interest are then easily quantitated by reversed-phase HPLC. Recovery of quinine was very high, see *Recovery*.

Solid Phase Extraction Method

- **CARTRIDGE:** 100 mg, 1 ml Zorbax SPE C18 (P/N 5184-3590, Box of 100)
- **CONDITION:**
 - 1.0 mL Methanol
 - 1.0 mL Deionized Water
- **LOAD:**
 - 1.0 mL Sample (see sample preparation)
- **WASH:**
 - 1.0 mL Deionized Water
 - 1.0 mL 10% Methanol
- **ELUTE:**
 - 1.0 mL 1% ammonia in Methanol
- **INTERNAL STANDARD:**
 - 10 μ L (1 mg/mL) nitrobenzene in Methanol as internal standard for High Load.
 - 10 μ L (0.05 mg/mL) nitrobenzene in Methanol as internal standard for Low Load.

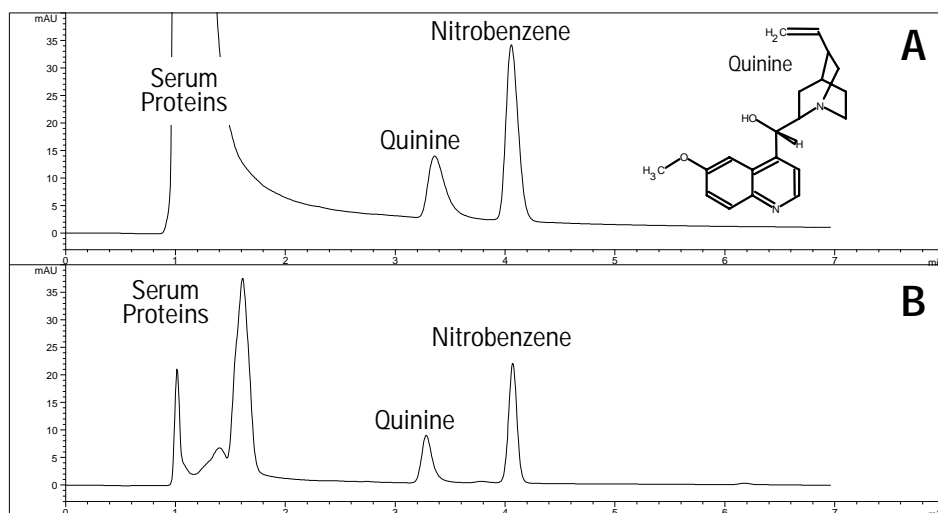


Figure 1. Comparison of quinine-spiked serum before (panel A) and after (panel B) extraction using the Zorbax SPE C18 cartridge. Notice effectiveness of the extraction. In panel A, the large amount of protein in the sample interferes with optimal quantitation of quinine. Once the sample has passed through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

Sample Preparation

- 1mg/mL quinine for High Load
- 0.05 mg/mL quinine for Low Load
- Spike 10 μ L above solutions
 - 0.5 mL bovine serum
 - 0.1mL Deionized Water
- Internal Standard:
 - see SPE Method, above.



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HPLC Method

LC Instrument:

Agilent 1100

Column:

Zorbax Bonus-RP, 4.6 mm x 150 mm, 5 μ m
(P/N 883668-904)

Eluent:

50% Acetonitrile, 50% K_2HPO_4 (18mM,
pH 6.85)

UV: 254 nm

Flow: 1.05 mL/min.

Temperature: 30 °C

Inj. Vol.: 5 μ L for High Load

30 μ L for Low Load

Recovery

- Quinine (20 μ g/mL):

$X = 91.6\%$, $n = 5$

- Quinine (1 μ g/mL)

$X = 86.4\%$, $n = 5$

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.

Dr. Wu Chen is a research chemist at Agilent Technologies, based in Wilmington, Delaware.

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Publication Number 5988-2527EN



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