

Analysis of Ranitidine in Serum: Zorbax SPE C18 Cartridge

Application Brief
Pharmaceutical
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Ranitidine is a pharmaceutical compound used for the treatment and prevention of ulcers and for the relief of heartburn. It may be of interest to determine levels of ranitidine or similar compounds in the bloodstream with a reduced background from the 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 easily quantitated by reversed-phase HPLC. Recovery was high for the compounds of interest.

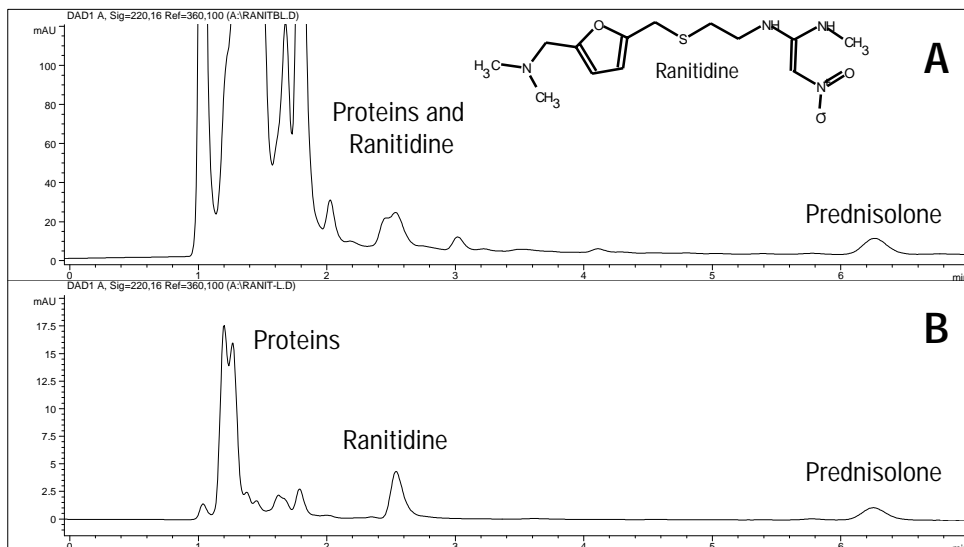


Figure 1. Comparison of ranitidine-spiked serum before (panel A) and after extraction (panel B) using the Zorbax SPE C18 cartridge. Notice effectiveness of the extraction. In panel A, the large amount of protein in the sample interferes with detection of ranitidine. Once the sample has passed through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

Solid Phase Extraction Method

- **CARTRIDGE:** 100 mg, 1 ml Zorbax SPE C18 (P/N 5184-3590, Box of 100)
- **PRETREAT:**
 - 1.0 mL Methanol
 - 1.0 mL Deionized Water
- **LOAD:**
 - 1.0 mL Sample (see sample preparation)
- **WASH:**
 - 1.0 mL Deionized Water
- **ELUTE:**
 - 1.0 mL Methanol
- **EVAPORATE and RECONSTITUTE:**
 - Room Temperature nitrogen stream
 - Add 200 μ L of Deionized Water with 10 μ L (200 μ g/mL) Prednisolone as internal standard.

Sample Preparation

- Ranitidine (1mg/mL each) in Water.
- Spike 5 μ L above solution
 - 8 mL bovine serum
 - 2 mL Deionized Water
- **Internal Standard:**
 - Prepare a 0.2 mg/ml solution of Prednisolone in Deionized Water.



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HPLC Method

LC Instrument:

Agilent 1100

Column:

Zorbax Bonus RP, 4.6 mm x 150mm, 5 μ m

(P/N 883688-904)

Eluent:

A: Methanol/40 mM Sodium Phosphate in water (pH 7)
50/50

UV: 220 nm

Flow: 1.0 mL/min.

Temperature: 35° C

Inj. Vol.: 20 μ L

Recovery

- Ranitidine (0.5 μ g/mL):

$X = 100\%$, $n = 3$

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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