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A Novel, Mixed-Mode Weak Cation Exchange SPE Sorbent: Oasis® WCX

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The new Oasis® WCX solid-phase extraction sorbent is used to selectively extract three types of bases from rat plasma samples.

The new Oasis® WCX (Weak Cation eXchange) SPE material was developed to provide better sample preparation for strong bases and quaternary amines. The retention mechanism is mixed-mode, that is, both ion-exchange and reversed-phase, which improves retention for all types of basic analytes.

Experimental Conditions

Rat Plasma Samples

1. Spike rat plasma separately with valethamate, protriptyline, and atenolol (each 10 ng/mL). Acidify with H₃PO₄ (2% of total sample volume) for protriptyline only.
2. Condition and equilibrate Oasis® WCX μ Elution plate with 200 μ L each MeOH and water.
3. Load 150 μ L of spiked rat plasma samples onto the sorbent.
4. Wash with 200 μ L of 25 mM phosphate buffer, pH 7.0.
5. Wash with 200 μ L of MeOH.
6. Elute with 50 μ L (25 mL \times 2) of MeOH containing 2% formic acid.
7. Dilute and neutralize samples with 100 μ L of water containing 5% NH₄OH.

Saline Samples

1. Spike saline with valethamate, protriptyline, and atenolol (each 20 ng/mL).
2. Condition and equilibrate an Oasis® WCX and a commercial silica-based weak cation exchange 10 mg 96-well plate with 500 μ L each MeOH and water.
3. Load 0.25, 0.5, 1.0, and 2.0 mL of the spiked saline onto the sorbent.
4. Wash with 500 μ L of 25 mM phosphate buffer, pH 7.0.
5. Wash with 500 μ L of MeOH.
6. Elute with 250 μ L (125 μ L \times 2) of MeOH containing 2% formic acid.
7. Dilute and neutralize valethamate, protriptyline, and atenolol samples with water containing 5% NH₄OH, 250 μ L and 750 μ L, respectively.

Gradient: 5 to 95% B in 3 min

Flow Rate: 0.4 mL/min

Injection Volume: 10 μ L

Instruments: Waters 2777 Sample Manager, and Waters 1525 μ binary HPLC pump, Waters Micromass® Quattro Ultima™ (ESI in MRM)

Results

Figure 1 shows the LC-MS-MS chromatograms of the analytes extracted from rat plasma using Oasis® WCX sorbent. Recoveries are 100% for all three types of bases—a quaternary amine, a polar base, and a hydrophobic base. Additionally, using an XTerra® MS C₁₈ IS™ column at high pH results in good retention and peak shapes and a fast analysis.

Figure 2 shows a comparison of the SPE recovery results versus amount of saline loaded into the wells for Oasis® WCX and a commercially available silica-based WCX material. The silica-based material contains a carboxylpropyl cation exchange ligand. Excellent recoveries were seen for all three analytes under all loading volumes on the Oasis® WCX sorbent. However, on the silica-based WCX-type sorbent, the polar analyte was not retained during the load step, resulting in recoveries between 5 and 14%, and less than 80% SPE recoveries were observed for the quaternary amine and the hydrophobic base under similar conditions.

Conclusions

The Oasis® WCX mixed-mode sorbent provides a means of selective, fast, and robust sample preparation with high recoveries for quaternary amines, as well as polar and hydrophobic basic analytes. It outperforms existing commercial silica-based SPE WCX-type materials by eliminating the breakthrough of polar bases, as well as providing better overall recoveries for both quaternary amines and hydrophobic bases.

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Column: XTerra® MS C₁₈ IS™ 2.1 \times 20 mm, 3.5 μ m
Mobile Phase A: 10 mM NH₄HCO₃, pH 10
Mobile Phase B: MeOH with 10 mM NH₄HCO₃, pH 10

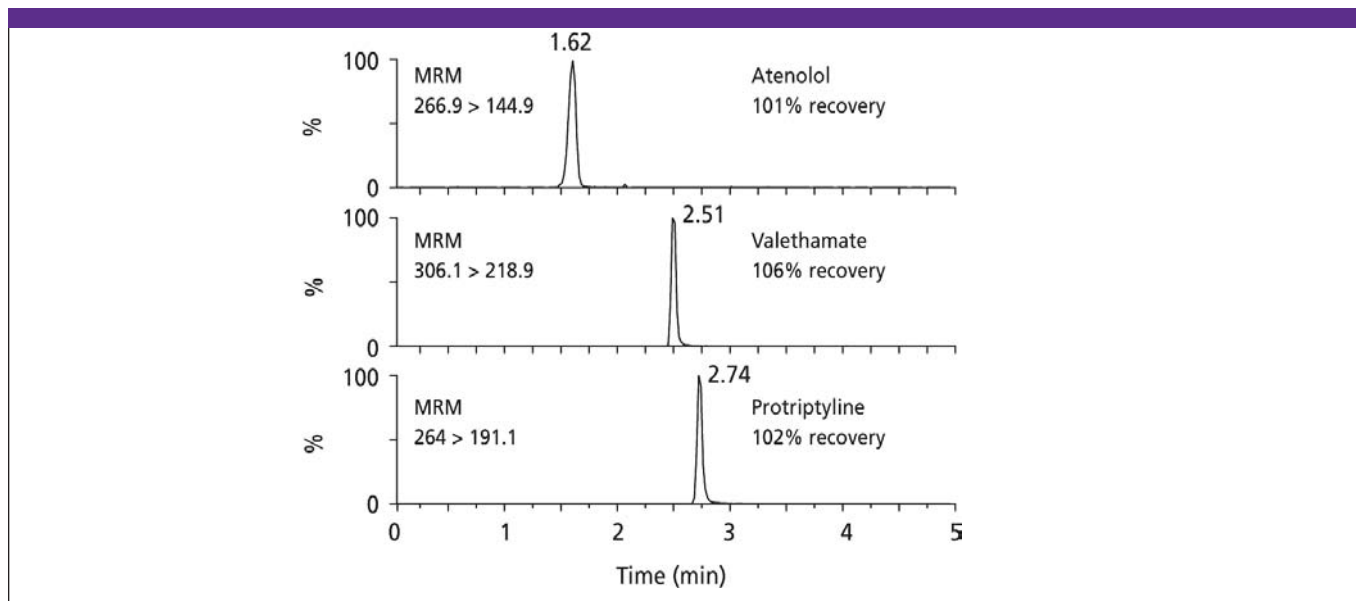


Figure 1: LC-MS-MS chromatograms of the analytes (polar base, quaternary amine, and hydrophobic base) extracted from rat plasma with Oasis® WCX 96-well plate. The SPE recoveries were calculated by comparison with postextracted spiked plasma samples.

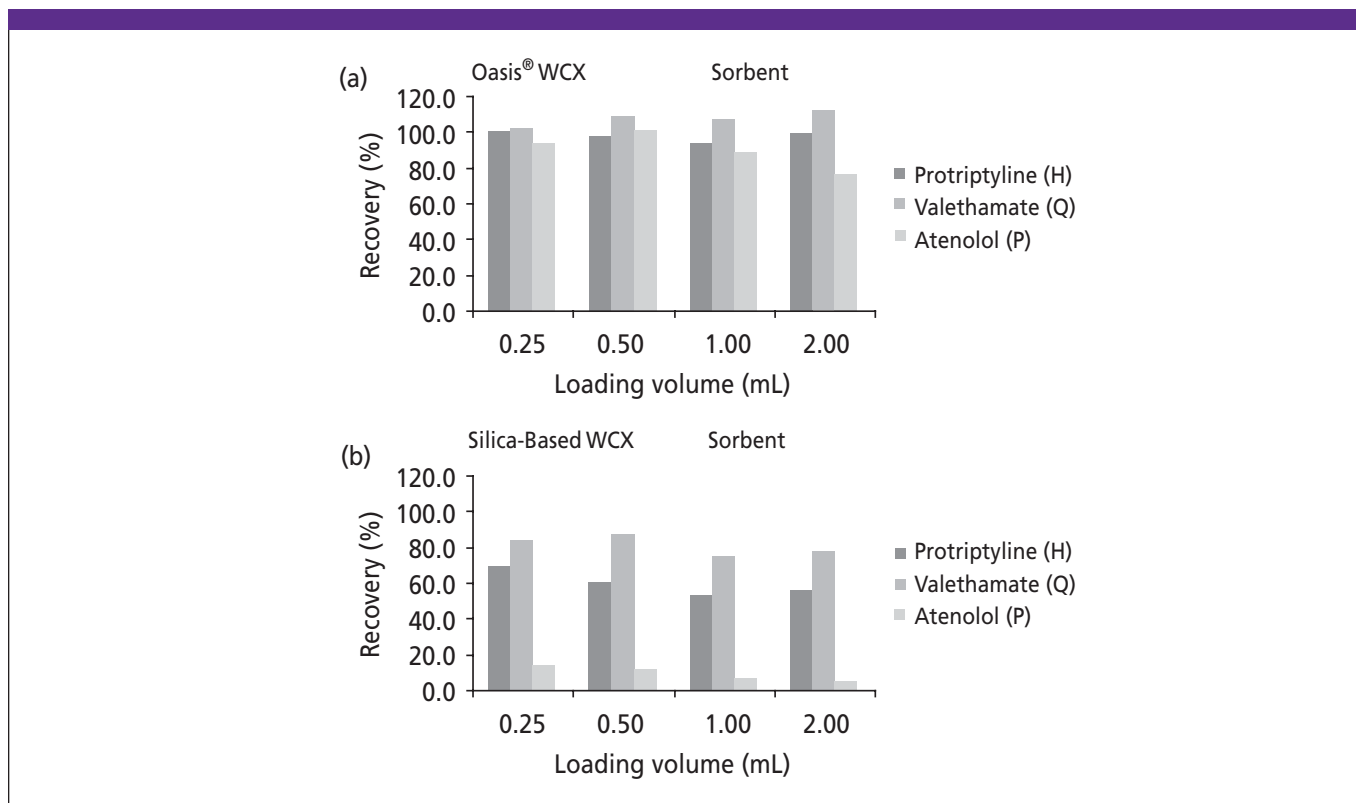


Figure 2: SPE recoveries for three types of bases versus volume of spiked saline solution loaded onto (a) the Oasis® WCX sorbent and (b) a commercially available silica-based WCX sorbent. H is hydrophobic base, Q is quaternary amine, and P is polar base.