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Simple and Fast SPE-UPLC–MS–MS Method for an Allergy Tablet Mixture of Acids and Bases

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The new Oasis Sorbent Selection Plate and UPLC™ technology enabled the rapid development of an SPE-UPLC–MS–MS method for a mixture of ibuprofen, pseudoephedrine and chlorpheniramine.

We have previously introduced a novel approach to selecting SPE methodology based on two protocols and four mixed-mode Oasis® sorbents. Proof of concept studies yielded positive results when knowledge of the samples' chemical properties were used to choose the correct sorbent and protocol. We have successfully taken this approach one step further and applied it to plasma samples containing the components of a commercial allergy and sinus combination tablet.

Experimental Conditions

The components of the tablets are ibuprofen (pKa 5.2), pseudoephedrine (pKa 9.9), and chlorpheniramine (pKa 9.2). This mixture represents a common analytical challenge of both acids and bases present in the same sample. Using the flow chart in Figure 1, we chose the Oasis® MCX sorbent, which is a mixed-mode sorbent, having both cation exchange and reversed-phase retention mechanisms. We followed Protocol 1.

SPE Protocol: Oasis® MCX 30-mg 96-well plate

Condition: 1 mL methanol followed by 1 mL water

Load: 1 mL sample (1:1 plasma: 4% H₃PO₄ in water)

Wash 1: 1 mL 2% formic acid in water

Elution 1: 2 × 250 µL methanol

Elution 2: 2 × 250 µL 5% NH₄OH in methanol

Dilution: 500 µL 2% formic acid in water (for elute 2 samples) or 100% water (for elute 1 samples)

ACQUITY UPLC™–MS Conditions

Column: ACQUITY™ UPLC BEH 1.7 µm 2.1 × 50 mm

Mobile Phase A: 0.1% formic acid in water

Mobile Phase B: 0.1% formic acid in methanol

Flow Rate: 0.3 mL/min

Gradient: 1 min initial hold at 95% A, followed by a 2 min linear gradient to 80% B, hold at 80% B for 1 min, and then return to initial conditions

Injection Volume: 10.0 µL

Column Temperature: 40 °C

Sample Temperature: 10 °C

Sample Diluent: 50/50 water/methanol

Sample Concentration in Plasma: Pseudoephedrine HCl 3 µg/mL,

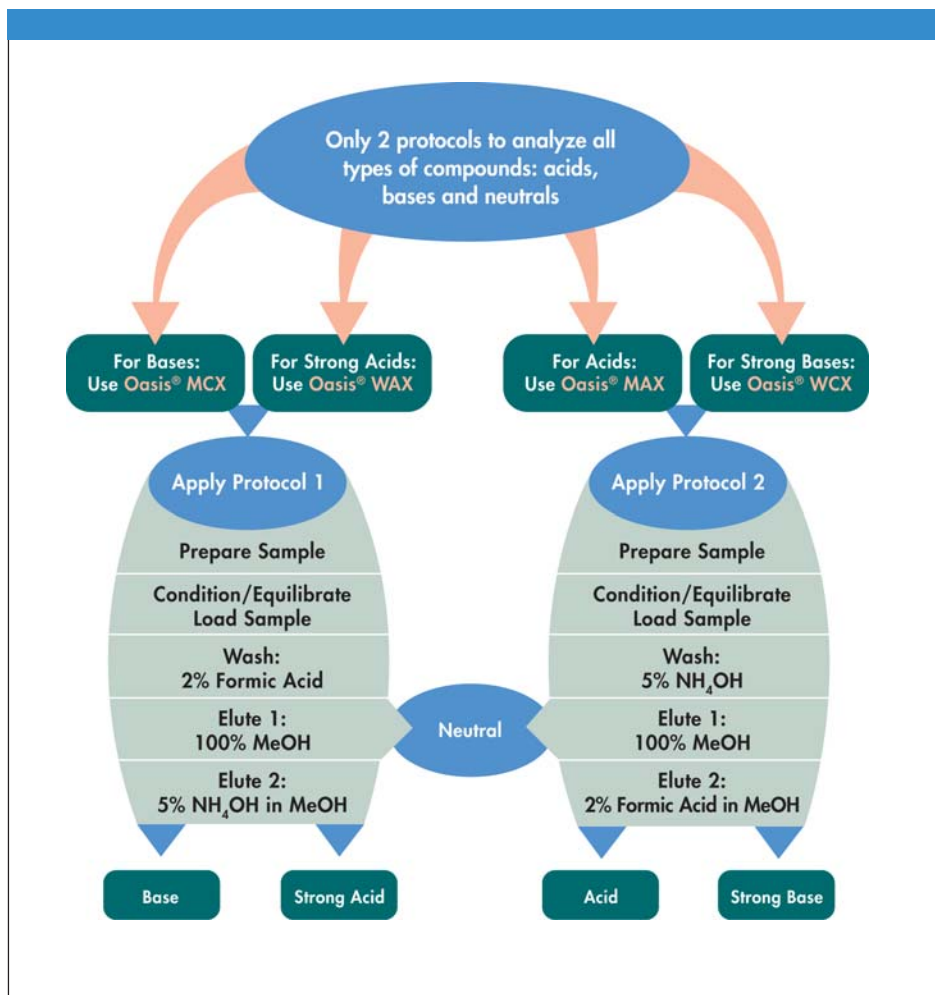


Figure 1: Decision tree outlining the systematic approach to selecting the appropriate Oasis®, SPE sorbent, and protocol.

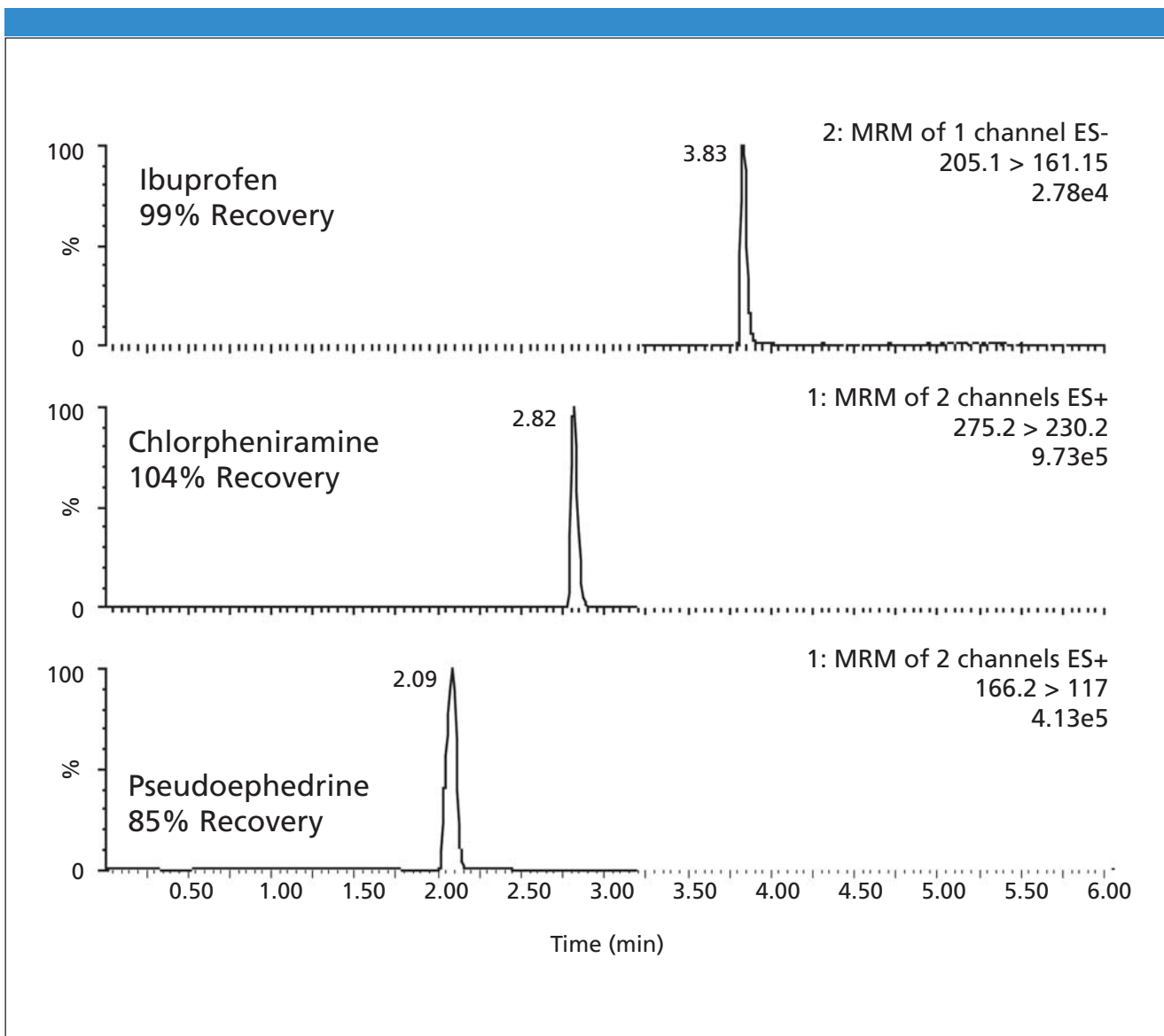


Figure 2: ACQUITY UPLC™/MS data and SPE recoveries for Oasis® MCX.

chlorpheniramine maleate 200 ng/mL, ibuprofen 20 µg/mL

Detection

Waters Micromass® Quattro Premier™

MRM transitions monitored:

Pseudoephedrine 166.2 > 117 ESI+

Chlorpheniramine 275.2 > 232.2 ESI+

Ibuprofen 205.1 > 161.1 ESI-

Results

ACQUITY UPLC™-MS data and SPE recoveries for each compound are shown in Figure 2. Ibuprofen, an acid (pKa 5.2), was found in elution 1 (methanol) as it interacts with the sorbent via reversed-phase retention. SPE recovery was 99%. The two bases, pseudoephedrine (pKa 9.9) and chlorpheniramine (pKa 9.2), were found in elution two (ammonium hydroxide in methanol) as they interact with the sorbent via ion exchange. The SPE recoveries were 85% and 104%, respectively.

Conclusions

We have taken a simplified approach to SPE sorbent and protocol selection and successfully applied it to a real world sample. With minimal knowledge of the samples' chemical properties, we were able to correctly choose the appropriate Oasis®, sorbent and SPE protocol and achieve excellent recoveries for both acids and bases with a single method, on the first set of conditions investigated.

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