

HAMILTON

EDTA

Hamilton Company, Reno, Nevada

Hamilton Company is best known for manufacturing precision fluid measuring products, including syringes for HPLC and GC. Unknown to most people, for the last 25 years Hamilton Company has manufactured HPLC resins and columns while developing separations for customers. Two of the separations we developed are highlighted below.

EDTA is a chelating agent added to processed foods, pharmaceuticals, soaps, cosmetics, textiles, and household cleaners. Customers requested an HPLC method for the determination of EDTA in their product. A search of the literature turned up a few references for the separation of EDTA on PRP-1 reversed-phase columns. Unfortunately these methods required the use of ion pairing reagents or amperometric detection. Our goal was to develop a

method that utilized a simple mobile phase, easy sample preparation, and UV detection.

Experimental Conditions

Column: PRP-X100 anion-exchange, 10- μ m spherical particles, 4.6 \times 150 mm in size PEEK HPLC column (Part Number 79354).

Mobile phase: In Figure 1, hydrocortisone cream, a 30:70 3 mM sulfuric acid-methanol mobile phase was used for two reasons. First, the 3 mM sulfuric acid provided a strong driving ion to elute the EDTA copper complex in less than 3 min. Second, methanol is a strong enough solvent to elute the hydrophobic compounds present in the hydrocortisone cream.

In Figure 2, nasal spray, a 65:35 1.5 mM sulfuric acid-methanol mobile phase was used.

Detection Method

The detection method was chosen because of simplicity. UV detection at 254 nm is easy to use, with good sensitivity.

Detector: Milton Roy SpectraMonitor 3100 variable-wavelength UV detector.

Sample Preparation and Injection Size

In Figure 1, 8 g of cream was dispersed in 100 mL of deionized water. Four mL of this solution was mixed with 6 mL of 0.05 M cupric sulfate. This solution was filtered and 100 μ L was injected into the column.

In Figure 2, 4 mL of nasal spray was mixed with 6 mL of 0.05 M cupric sulfate. This solution was filtered and 100 μ L was injected into the column.

Results

The use of a PRP-X100 anion-exchange column allows the rapid quantitation of EDTA. The sulfuric acid mobile phase works well in these applications, as no interferences are seen. Organic solvent compatibility from 0 to 100% allows samples containing hydrophobic compounds to be analyzed with simple dilute, filter, and shoot methods. In Figures 1 and 2 the hydrophobic compounds elute at the column void.

Conclusions

For this application it was possible to improve upon the published methods and meet our goal of a robust method that utilizes a simple mobile phase, easy sample preparation, and UV detection.

For more information about Hamilton HPLC columns, contact the Hamilton Company or visit the web site.

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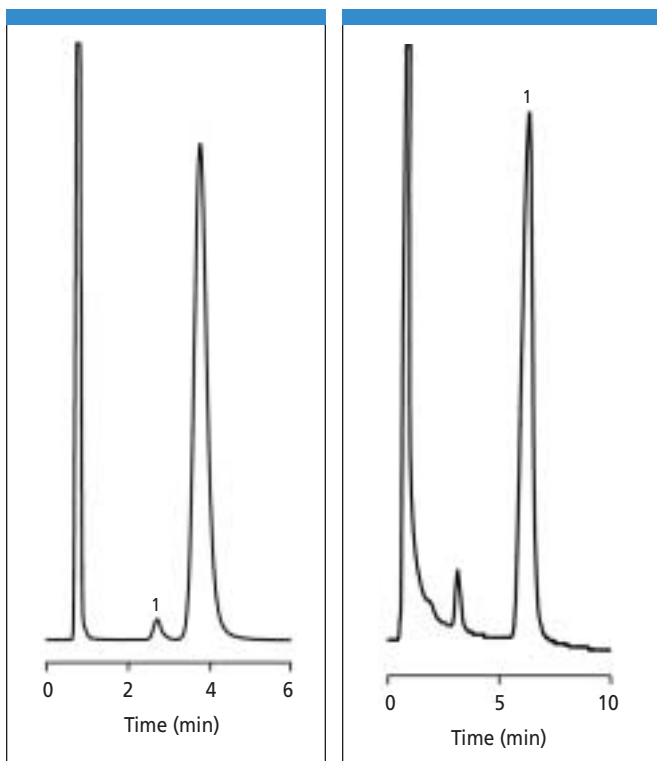


Figure 1: Hydrocortisone cream. PRP-X100, 10 μ m, 4.6 \times 150 mm. PEEK (Part Number 79354). Conditions: 70:30 3 mM sulfuric acid-methanol; 100 μ L injection; 2 mL/min; isocratic; ambient; UV at 254 nm. Peak 1 = EDTA copper complex.

Figure 2: Nasal spray. PRP-X100, 10 μ m, 4.6 \times 250 mm. (Part Number 79433). Conditions: 65:35 1.5 mM sulfuric acid-methanol; 100 μ L injection; 1.5 mL/min; isocratic; ambient; UV at 254 nm. Peak 1 = EDTA copper complex.