CAPSULES
preliminary notes and applications from Bioanalytical Systems, Inc.

Determination of Morphine in Microdialysates Using SepStik Columns

Purpose

Determination of morphine (F1) in rat blood and brain dialysate samples.

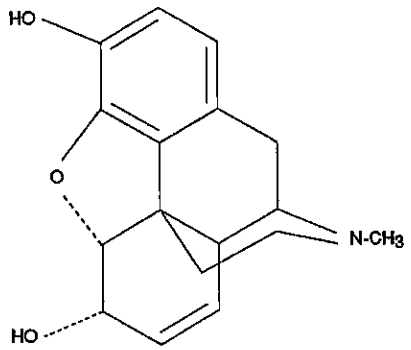

Figure 1. Structure of morphine

In order to separate and sensitively detect low morphine levels in microdialysates, a SepStik microbore column was used. The 1 mm internal diameter increases the concentration of the eluting morphine up to 21-fold compared to standard LC columns.

Existing Methods

Morphine can be analyzed by LCUV at 284 nm, but sensitivity is very low. LCEC with conventional columns provides higher sensitivity; however, the detection limit is still not low enough for some pharmacokinetic studies.

Conditions

System: BAS 200A Liquid Chromatograph

Column: SepStik Kit (BAS P/N MF-8949), ODS, 3 µm silica in a 100 x 1.0 mm bed volume.

Mobile Phase: The buffer contains 0.1 M sodium acetate and 0.5 mM EDTA. Adjust the buffer pH to 5.0 with 1 M acetic acid and mix with acetonitrile in the ratio of 95:5.

Flow Rate: 80 µL/min. This flow rate can be obtained in two ways: with a flow splitter or by using the pump's microbore function. If using the microbore function, set the flow rate at 80 mL/min. Connect a second microbore column (5µm SepStik, P/N MF-8901 or 8902) between the pump and the injection valve. Use a SepStik in-line filter before this column. The second column allows the system to achieve an optimum backpressure of 3200-4000 psi.

If using a splitter, use BAS P/N MF-8947 Flow Splitter Accessory Kit, and maintain about 2 mL/min flow rate at the pump. Adjust the splitter ratio to give 75-85 µL/min through the SepStik column by adjusting the pump flow rate, or if necessary, shortening the fused silica restrictor.

Temperature: 30 °C

Electrode: Glassy Carbon (BAS P/N MF-1000)

Potential: 700 mv vs. Ag/AgCl

Detection Limit: 5 pg injected yielded a S/N of 3. The injection volume was 5µL.

Sample Preparation: Dialysate was directly injected onto the system.

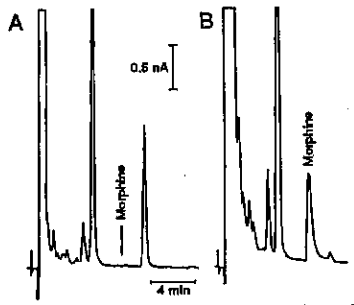

Figure 2. Chromatograms of rat blood microdialysates collected (A) 30 minutes before and (B) 160 minutes after morphine was injected subcutaneously.

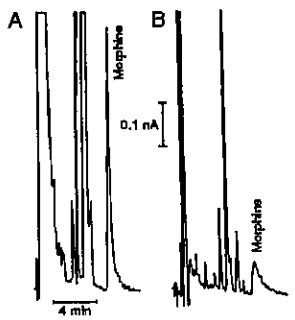

Figure 3. Comparison of (A) SepStik and (B) conventional column for assay of morphine in rat brain microdialysate collected 20 minutes after the drug was injected subcutaneously.