

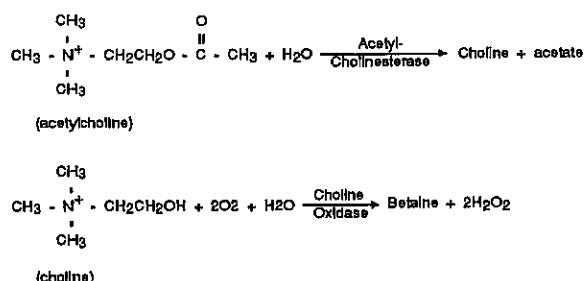
# CAPSULES

preliminary notes and applications from Bioanalytical Systems, Inc.

## Determination of Acetylcholine and Choline in Microdialysates Using SepStik™ Columns

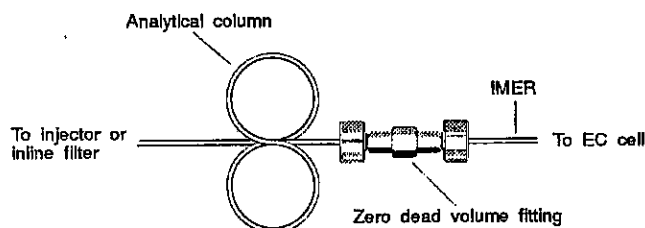
### Purpose

Determination of acetylcholine and choline in microdialysates



**Figure 1.** Scheme for enzyme-mediated LCEC determination of acetylcholine and choline.

A special SepStik™ acetylcholine and choline microbore analytical column (F2) is used for the efficient and sensitive separation of acetylcholine and choline. The two analytes are converted to hydrogen peroxide on a postcolumn SepStik™ immobilized enzyme reactor. The bands of hydrogen peroxide are detected amperometrically on a Pt electrode.



**Figure 2.** Diagram of SepStik™ ACh/Ch column coupled to immobilized enzyme reactor (IMER) containing acetylcholinesterase and choline oxidase.

### Conditions

System: BAS 481 liquid chromatograph

Column: SepStik™ ACh/Ch Kit (BAS P/N MF-8908) contains one analytical column (530 x 1.0 mm, 10 μm), and 2 enzyme reactors (50 x 1.0mm, 10 μm). Related fittings, Pt electrode and standard chemicals are included in this kit.

Mobile Phase: 50 mM Na<sub>2</sub>HPO<sub>4</sub> (pH 8.5) containing 5 mL/L Kathon (BAS P/N CF-2150) reagent.

Flow Rate: 130 μL/min. actual measured flowrate.

For conventional pumps, use BAS P/N MF-8947 Flow Splitter Accessory Kit, and maintain a 2 mL/min. flowrate at the pump. Adjust splitter ratio to give 120-140 μL/min. through the microbore columns.

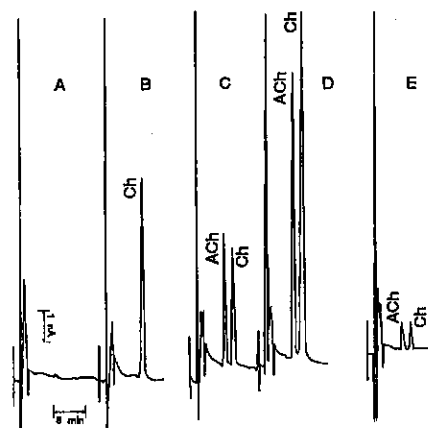
Electrode: Pt, (BAS P/N MF-1012)

Potential: 500 mV vs. Ag/AgCl

Detection Limit: 75 femtomoles injected yielded a S/N of 3. The injection volume was between 5 to 20 μL.

### Sample Preparation

Dialysates can be directly injected onto the system. However, a precolumn inline filter (BAS P/N MF-8952) is recommended to prevent potential clogging problems.



**Figure 3.** Acetylcholine and choline in rat striatum microdialysate using MF-8908 ACh/Ch SepStik™ kit

A: Blank (Ringer's solution)

B: Dialysate collected before neostigmine was added to perfusion solution

C and D: Dialysate collected during perfusion of Ringer's solution containing neostigmine, an acetylcholinesterase inhibitor

E: 1 pmole standards