Waters
Lab Highlights
An Internal Communication of Applications and Techniques
LAH 0325 3/86

ANALYSIS OF CHOLINE AND ACETYLCHOLINE BY UV-VISUALIZATION LC

The physiological significance of choline and acetylcholine esters in plants and animals has been thoroughly demonstrated (1). However, analytical methods for the detection of these compounds have been insensitive, non-specific or required some type of derivatization reaction (1). LC coupled with a UV-visualization method makes derivatization superfluous. A method utilizing this technique for detecting choline, acetylcholine and two related esters has been described (1). As a prerequisite for better understanding the assay procedure, a short review of the UV-visualization technique is in order.

UV-visualization (2,3,4) affords quantitation of non-UV-absorbing ionic compounds with a UV-sensitive detector by the addition of a UV-absorbing paired ion chromatography reagent to the mobile phase. In essence, the paired ion chromatography reagent travels as the co-ion of the analyte ion to facilitate its detection.

UV-visualization has been described previously for the analysis of alkyl sulfonates (2,3,4) and sodium valproate (5), an anti-epileptic drug. This technique is well suited, therefore, to the non-UV-absorbing choline and acetylcholine esters.

FOR RESEARCH USE ONLY

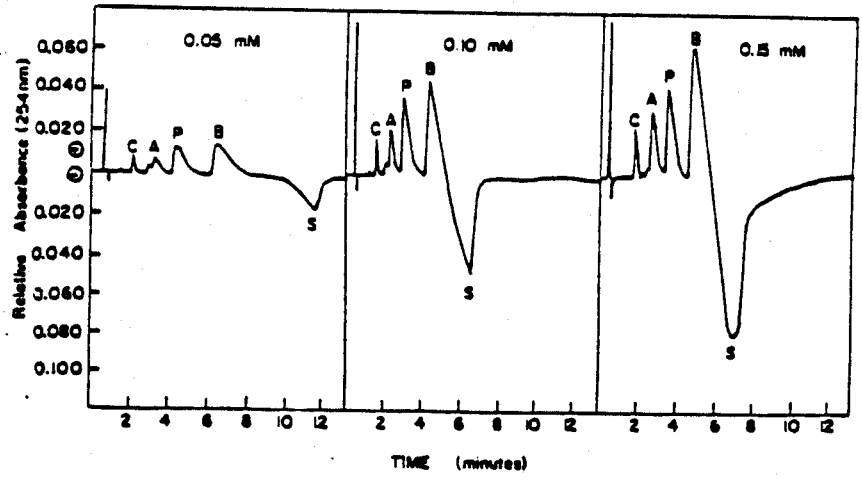

Figure 1. Chromatograms of 40-nmol equimolar mixtures of acetyl-choline (A), butyrylcholine (B), choline (C), and propionylcholine (P) using three different mobile phase PEP concentrations; system peak (S). Mobile phase conditions in text. 10 µl Injection Volume.

MILLIPORE
Waters Chromatography Division
34 Maple St. / Milford, MA 01757 / 617-478-2000
Carmen Santasania

The LC column used was a μBondapak™ C_{18} Radial-Pak™ cartridge (10cm X 8mm ID) in an RCM-100® Radial Compression Module with only one handle down. The mobile phase consisted of 0.15 mM 1-phenethyl-2-picolinium bromide (PEP, the paired ion chromatography reagent) in butanol: methanol: acetic acid: water (8:4:2:86). The flow rate was maintained at 3.0 ml/min with detection at 254 nm. Approximately 150 ml of mobile phase is passed through the system to establish equilibrium. Background UV absorbance due to the paired ion chromatography reagent was offset electronically.

Figure 1 shows chromatograms of the separation of choline esters with three concentrations of PEP. All analyte ions produced positive peaks followed by one negative "system" peak. The retention time of the large negative "system" peak corresponds with the retention time of PEP when it is injected as a sample.

The authors (1) were unable to successfully develop a quantitative extraction procedure from plant tissue extracts probably due to phenolic compounds known to be present in substantial amounts in plant tissue, however, they are currently investigating alternative extraction procedures for determining choline and acetylcholine in plant extracts.

1. Jones, R. S.; Stutte, C. A. *J. Chromatogr.* **1985**, *319*, 454.
2. Bidlingmeyer, B. A.; Warren, Jr., F. V. *Anal Chem.* **1982**, *54*, 2351.
3. Bidlingmeyer, B. A. *J. Chromatogr. Sci.* **1980**, *18*, 525.
4. LAH #0075, 0099, and 0108.
5. Bidlingmeyer, B. A.; Warren, Jr., F. V. *Anal. Chem.* **1984**, *56*, 487.