Essentials in biore

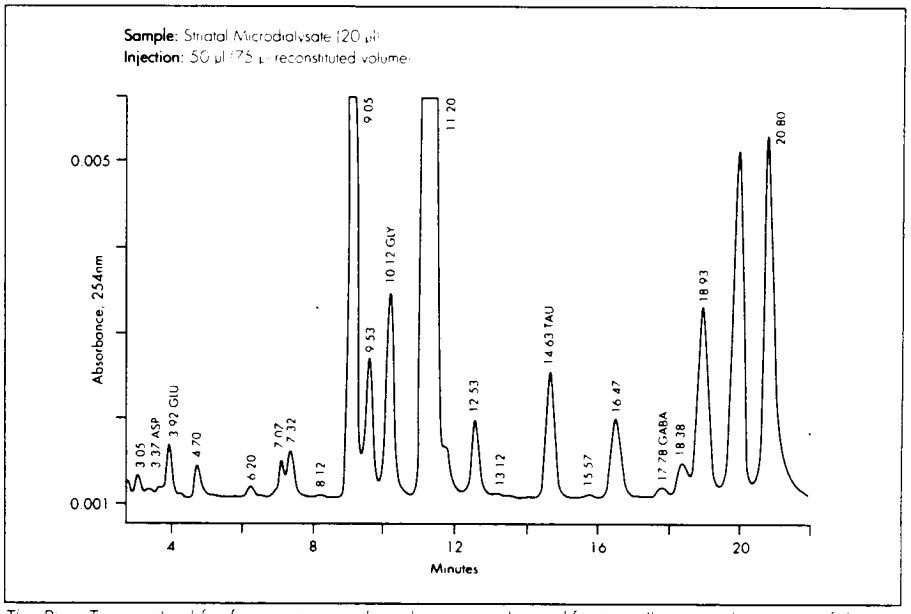
94-0417

Pico·Tag® Analysis of Neurotransmitter Amino Acids.

High sensitivity microdialysate analysis.

The analysis of neurotransmitter amino acids in conjunction with physiological manipulations within a living organism is often desirable when studying the central nervous system. Aspartic acid (ASP), glutamic acid (GLU) and gamma-aminobutyric acid (GABA) have been studied in depth while glycine (GLY), taurine (TAU) and several other amino acids have also been investigated[1]. In the past, experiments designed to understand the neurochemical significance of these amino acids were conducted on tissue slices or homogenates. Recently, the development of *in-vivo* microdialysis technology allows sampling of amino acids from a discrete area within living tissue. Through the use of a hollow fiber probe implant, the small molecular weight compounds contained in the extracellular fluid are allowed to diffuse into the fiber and the probe effluent can be analyzed to quantitate the level of these metabolites. The analysis of the probe effluent for amino acids requires a high sensitivity technique capable of quantitating nanomole/milliliter concentrations. Waters Pico•Tag method for free amino acid analysis provides sensitivity that is ideal for microdialysate analysis.

Figure 1: Basal Level of Neurotransmitter Amino Acids.



The Pico•Tag method for free amino acid analysis is easily modified to allow rapid analysis of the neurotransmitter amino acids present in rat brain microdialysate. This sample is collected prior to perfusion.

Measure neuronal response to potassium administration.

R.L. Burger, Jr., Q-S Yan, and S.M. Lasley at the University of Illinois College of Medicine have applied the Pico•Tag method to the analysis of neurotransmitter amino acid concentrations in rat brain microdialysate. Samples were collected at 20-minute intervals by perfusing artificial cerebral spinal fluid (ACSF) at 1 µl/min. through a microdialysis fiber. After monitoring basal release, the perfusate was altered to increase 50-fold the potassium ion concentration with an equimolar decrease in sodium ion concentration to retain isotonicity. Because the neurochemical system response is so rapid, short sample collection intervals are required in order to adequately document the response. This time limit restricts the amount of sample that can be collected and the corresponding amount of amino acids available for analysis. In all but a small percentage of samples, the Pico•Tag method provided the sensitivity required for the quantitation of amino acids in the microdialysate. Chromatograms of samples collected prior to and during elevated potassium perfusion are shown in Figures 1 and 2.

Waters Pico·Tag method detects neural cell amino acid production.

Studies have shown that greater than half of the ASP and GLU measurable in brain tissue are derived from non-neuronal sources such as glial cells[2-4], and thus emanate from general metabolic processes like protein synthesis. On the other hand, nearly all GABA comes from nerve cells. Nonetheless, the potassium-induced increase in the levels of these amino acids is characteristic of neurons. Since a sizable amount of ASP and GLU cannot be mobilized by elevated potassium these increased levels are smaller (5-7X) than those observed with GABA (up to 15X) (Figure 3).

Waters. The absolute essential for bioresearch.

Waters Chromatography Division
Millipore Corporation
34 Maple St.
Milford, MA 01757
(508) 478-2000

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Modified Pico•Tag Conditions.

Column: Waters Free Amino Acid Pico•Tag Column, 3.9mm x 300mm
Eluent A: Waters Pico•Tag Eluent 1
Eluent B: Waters Pico•Tag Eluent 2

Gradient: Time	%A	%B	Curve
Init	100	0	*
13.5	97	3	11
24.0	94	6	8
24.5	0	100	6
28.5	0	100	6
29.0	100	0	6

Flow Rate: 1.0 ml/min
Detection: UV at 254nm, 0.01 AUFS

High sensitivity Pico•Tag method.

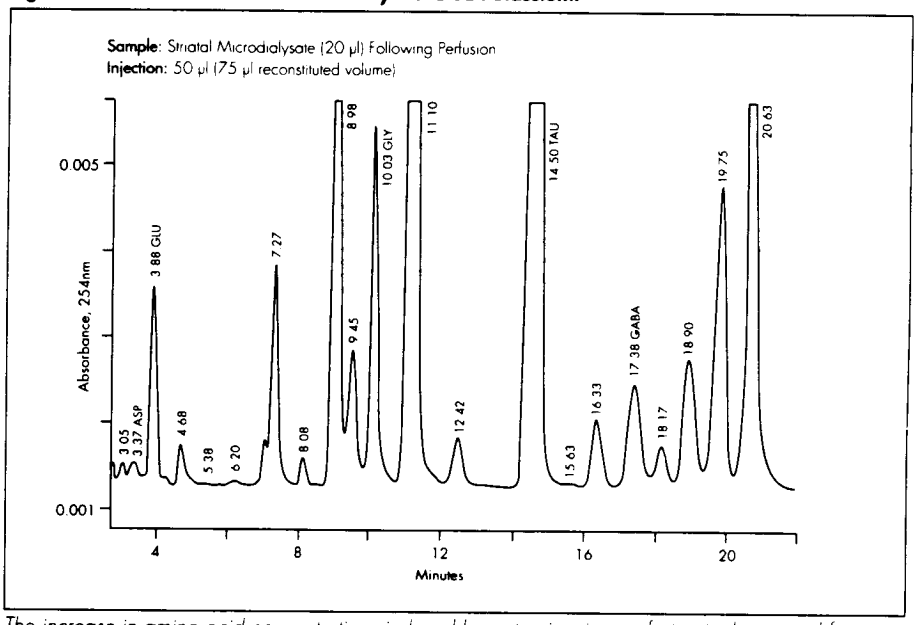
The PITC derivatization chemistry utilized by the Pico•Tag method is the first step in the familiar Edman Degradation. The PTC-amino acid derivatives formed are stable, amenable to high resolution reverse phase separation, and possess a strong UV absorbing chromophore. Sensitivity of the method is compatible with injection of 10-20 picomoles of amino acids on column. This performance is guaranteed by the integration of an optimized system configuration, packaged reagents and supplies, and comprehensive method documentation.

Unlike dedicated amino acid analyzers, Waters Pico•Tag method can be easily modified to accommodate unique samples or partial profiles. In the case of neurotransmitter analysis, the Pico•Tag method has been modified to focus on key amino acids. Using an abbreviated gradient [above], resolution and quantitation of ASP, GLU, GLY, TAU, and GABA are achieved in less than 25 minutes.

Versatile system for additional neuroscience applications.

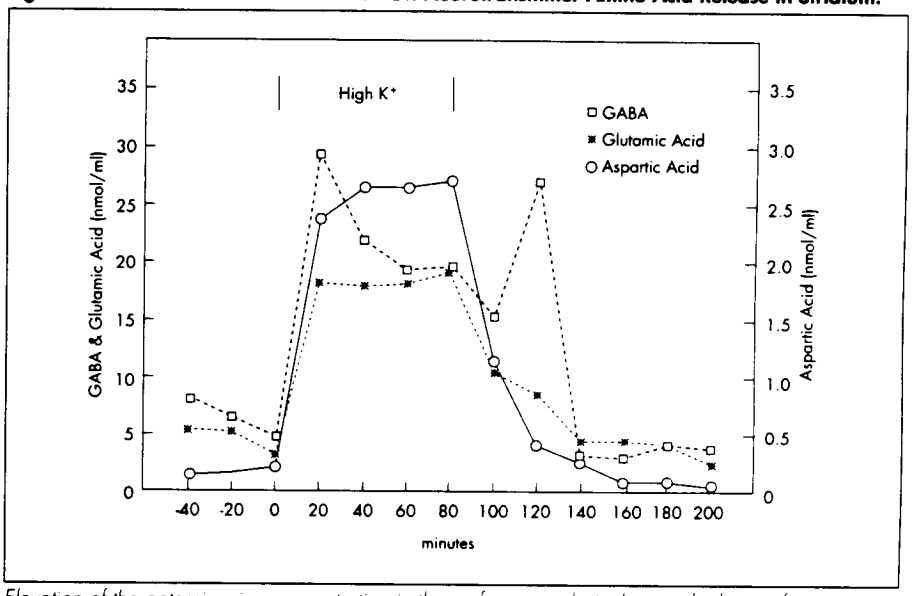
Waters Pico•Tag Amino Acid Analysis System is a versatile, gradient HPLC that can be used for a wide variety of bioresearch applications including neuropeptide analysis, protein purification, DNA characterization and therapeutic drug monitoring. With the addition of Waters 460 Electrochemical Detector, the high sensitivity analysis of monoamines and their metabolites can be accomplished.

Figure 2: Amino Acid Release Induced By Elevated Potassium.



The increase in amino acid concentrations induced by potassium ion perfusion is determined from only 20 µl microdialysate sample.

Figure 3: Effect of 150mM Potassium Ion On Neurotransmitter Amino Acid Release in Striatum.



Elevation of the potassium ion concentration in the perfusate results in the rapid release of neurotransmitter amino acids. The concentration increase is easily measured using the Waters Pico•Tag system.

Ordering Information

	Part No.
Waters Pico•Tag System and Chemistry Package for Free Amino Acid Analysis	91707
Waters Baseline 810 Chromatography Workstation	38612
Waters 460 Electrochemical Detector	41200

References

1. The Biochemical Basis of Neuropharmacology, F. E. Cooper [illegible] 1985, p. 124.
2. F. [illegible] Burgen [illegible] G. F. Yar & [illegible] Waters [illegible]
3. [illegible] Butcher & A. Hampson [illegible]
4. F. E. Fausen & [illegible] Lonnum [illegible]