Rapid HPLC Purity Assessment of Radiotracers for PET Scanning.

HPLC as a tool for purity assessment.
High Performance Liquid Chromatography (HPLC) has long been regarded as an accurate, precise and adaptable analytical technique offering high speed separations with high sensitivity detection. HPLC has recently found a place in the Positron Emission Tomography (PET) laboratory for analysis of the radiotracers produced on site for in-vivo studies (see back page).

The need for radiotracer purity.
The radiotracer 2-FDG is synthesized from the commercially available substrate 1,3,4,6-tetra-O-acetyl-2-O-trifluoromethanesulfonyl-beta-D-mannopyranose. Side reactions that occur during the synthesis lead to products that may not be metabolized the same way as 2-FDG and may interfere with the imaging process. Because this preparation is for in-vivo use, it must be demonstrated to be sterile, pyrogen free, isotonic and radionuclideically, radiochemically and chemically pure. The FDA normally requires no more than 10% chemical or radiochemical contamination, but in practice, no more than 5% contamination is considered acceptable.

HPLC rapidly verifies radiotracer purity.
Assessment of radiotracer purity requires an easy, rapid analytical method. In the case of 2-FDG, the major radiochemical contaminants are fluorine-18 labelled 2-fluoro-2-deoxyacetate derivatives. The assay of 2-FDG radiochemical purity can be performed on a μBondapak™NH2 Column using a simple, single solvent elution method. Solutes are detected with a flowthrough sodium iodide radioactivity monitor. Figure 1 shows a sample of 2-FDG that is suitable for use in imaging. In Figure 2, the analysis of a sample that has 20% radiochemical contamination shows that it is unsuitable for use in ivivo imaging.

Figure 1: Quantitative analysis of radiotracer purity.

Figure 2: 2-FDG sample with unacceptable contamination.

A fast, easy analysis of a 2-FDG sample is accomplished on a μBondapak NH2 Column using a simple, single solvent elution method. Solutes are detected with a flowthrough sodium iodide radioactivity monitor. Figure 1 shows a sample of 2-FDG that is suitable for use in imaging. In Figure 2, the analysis of a sample that has 20% radiochemical contamination shows that it is unsuitable for use in ivivo imaging.

Chromatograms courtesy of Dr. Mark Goodman, University of Tennessee Medical Center.
Purity assessment requires simple HPLC instrumentation.

A simple HPLC system (Figure 3) configured to carry out purity assessment consists of a pump to deliver the solvent, an injector to introduce the sample to be analyzed, a column on which to perform the separation and a detector to "see" the compounds as they elute from the column. Starting with a simple isocratic HPLC system, one can add capabilities for multi-solvent gradient formation, alternative detection methods, or advanced data management to extend this technology to other radionuclides.

Recommended system.

A variety of HPLC configurations are appropriate for this analysis. Your Waters representative can help you determine which system is best suited to your assay needs.

The Emergence of PET Scanning.

Positron emission tomography imaging has been found to be a useful clinical and research tool for providing information concerning biochemical processes in diseased tissue in vivo. These include localization of tumors of the brain, breast, stomach and legs; the study of disorders such as stroke, epilepsy, schizophrenia, manic depression, Huntington's chorea and Alzheimer's disease; the determination of AZT effects in the brain; the diagnosis of coronary artery disease and evaluation of myocardial viability.

The utilization of PET for both clinical diagnosis and therapeutic monitoring is due in large part to the development of the radiotracer glucose analogue fluorine-18 labeled 2-fluoro-2-deoxy-D-glucose (2-FDG). Glucose is the primary energy source of the brain and also an important metabolic substrate for other tissues such as the heart under hypoxic conditions and tumors during proliferation. 2-FDG is transported across cell membranes by the protein carrier responsible for glucose transport. Once in the cell, 2-FDG is phosphorylated by hexokinase to 2-FDG-6-P. Once phosphorylation occurs, the molecule stays in the cell and undergoes no further metabolism. This allows the tissue to be imaged by PET.