CAPSULES
preliminary notes and applications from Bioanalytical Systems, Inc.

Glucuronidase Reactor for Fenoldopam Conjugates in Biofluids

Purpose

Demonstration of a post-columm reactor for converting electrochemically inactive drug conjugates to electrochemically active forms.

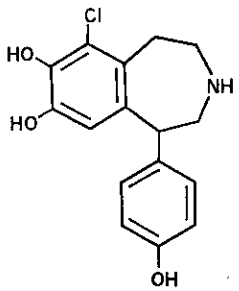

Figure 1. Structure of fenoldopam.

Fenoldopam [6-chloro-2,3,4,5-tetrahydro-1-(4-hydroxyphenyl)-1H-3-benzazepine-7,8-diol] is a therapeutic antihypertensive agent. The drug is metabolized to several glucuronides (F1) for excretion, but gut microflora may rehydrolyze these to the active aglycone, which is reabsorbed. This can be a significant consideration for dosage adjustment, so sensitive monitoring is needed. Although the aglycone is electrochemically active, the glucuronides are not. A post-column reactor containing β-glucuronidase (F2) was used to convert the glucuronides to fenoldopam, thus allowing sensitive EC detection.

Existing Methods

Chemical or enzymatic hydrolysis, followed by quantitation of the aglycone. These techniques are time consuming and can be imprecise.

Reference

Use of a Post-Column Immobilized β-Glucuronidase Enzyme Reactor for the Determination of Diastereomeric Glucuronides of Fenoldopam in Plasma and Urine by High- Performance Liquid Chromatography with Electrochemical Detection. V.K. Boppana, K.L. Fong, J.A. Ziemniak and R.K. Lynn, J. Chromatogr., 353 (1986) 231-247.

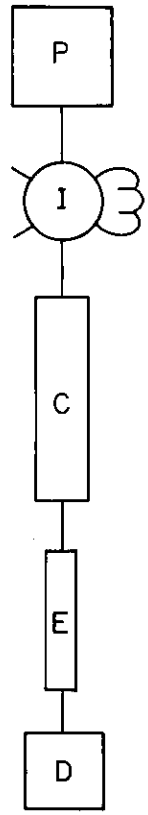

Figure 2. Diagram of LC system. P = pump, I = injector, C = column, E = enzyme reactor, D = electrochemical detector.

Conditions

Detector: BAS LC-4A
Electrode: BAS Glassy Carbon
Potential: +0.65 V vs Ag/AgCl
Column: 5 µm, C 18 reverse-phase (250 x 4.6 mm)
Mobile Phase: A: 11.0 g sodium acetate trihydrate, 10.5 g citric acid monohydrate, 4.9 g sodium hydroxide, 0.335 g disodium EDTA and 37.5 mL

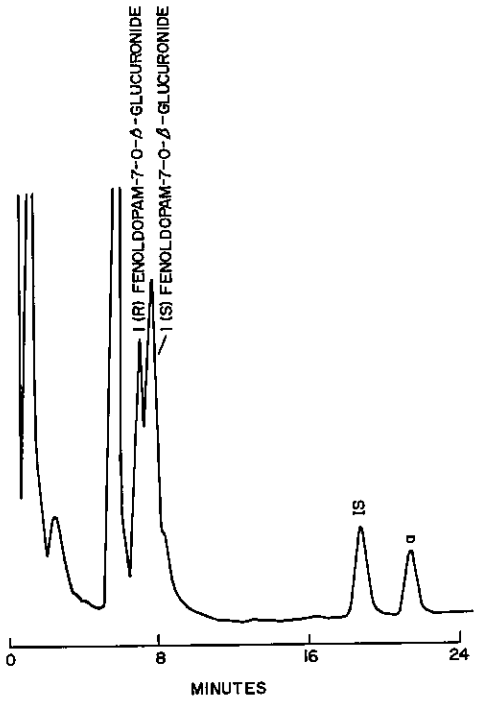

Figure 3. Chromatogram of plasma from voluteer, 1/2 hour after oral administration of fenoldopam. IS = internal standard, a = diastereomer of IS.

acetic acid per liter of water, pH 4.0. B: methanol. Combine 80 parts A with 20 parts B. Flow rate was 1 mL/min.

Linear Range: 0.4 - 200 ng fenoldopam glucuronide injected

Sample Preparation

Plasma samples (F3) were combined with acetic acid, phosphate buffer and internal standard, then loaded onto a disposable preparative column. The sample was eluted with acetic acid in methanol, dried, dissolved in acetate- methanol buffer, then injected in 20-50 µL aliquots. Urine samples were simply diluted 1:100 with water and injected in 50 µL aliquots.

Notes

The immobilized enzyme reactor was prepared by mixing β-glucuronidase with glutaraldehyde-treated glass beads, then slurry-packing the beads into a stainless-steel column (50 x 2.1 mm I.D.).

The reactor was stable during 4 months of usage and 6 months of storage.

The determination of fenoldopam presented in this report can be duplicated with the BAS 400 Liquid Chromatograph or the BAS 200 Problem Solver.

The information in this publication is supplied as a service to our customers. Performance of the methodology has not necessarily been verified by BAS technical staff.