

Waters

Lab Highlights

PROTEINS

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PREP SCALE PURIFICATION OF ADRENODOXIN USING PREP 500/I-125

Adrenodoxin, present in the mitochondria of bovine cortex adrenalis is physiologically important in the biosynthesis of steroid hormones and exhibits a molecular weight of roughly 13,000. The Prep 500 and an experimental I-125 Protein Cartridge was used to successfully separate this component from a matrix containing NADPH-Cyt. P-450 reductase. Figure 1 shows the analytical run of the crude extract showing adrenodoxin assigned as the last peak. A scale-up to 10 ml of injected volume in Figure 2 and subsequent check on the analytical column (Figure 3) shows better than 90% purity after Prep 500 separation.

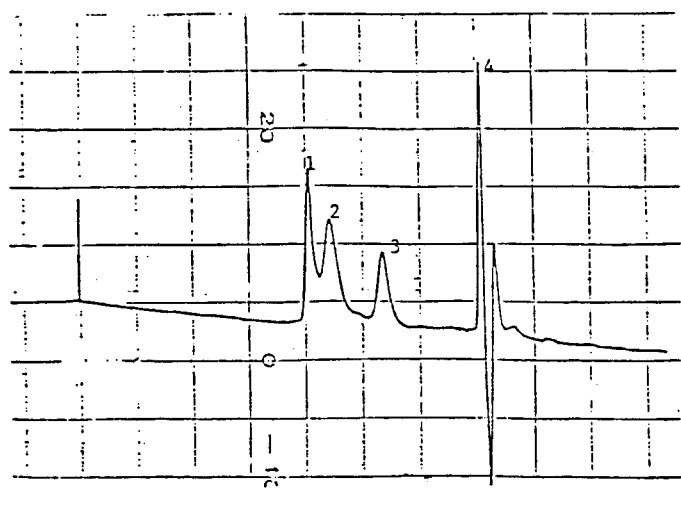


Figure 1

Column: Protein I-125
(7.8mm ID X 30cm X 2)
Solvent: 0.15M K_2HPO_4 pH 7.4
Flow Rate: 1.5 ml/min
Detector: UV 280nm 0.08 AUFS

Peak #	Component
1	NADPH-Cyt. P-450 reductase MW: 79,000
4	Adrenodoxin MW: 13,000

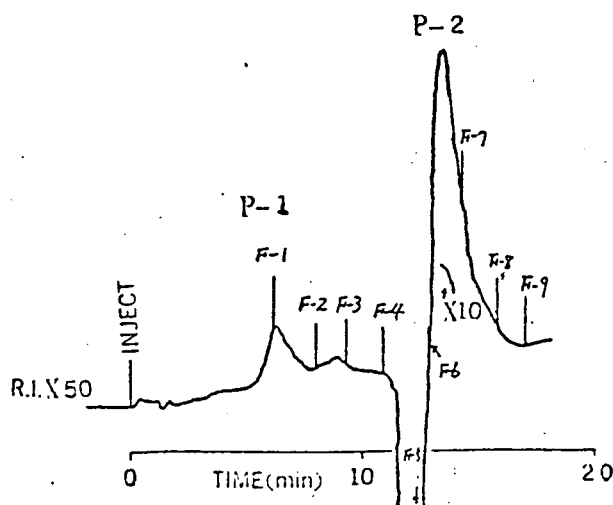


Figure 2

Column: Prep PAK 500/I-125
(5.7cm ID X 30cm X 2)
Solvent: 0.15M K_2HPO_4 pH 7.4
Flow Rate: 50 ml/min
Sample Vol: 10 ml
R.I.: X 10

Peak #	Component
1	NADPH-Cyt. P-450 reductase MW: 79,000
2	Adrenodoxin MW: 13,000

Chromatogram on back of page...



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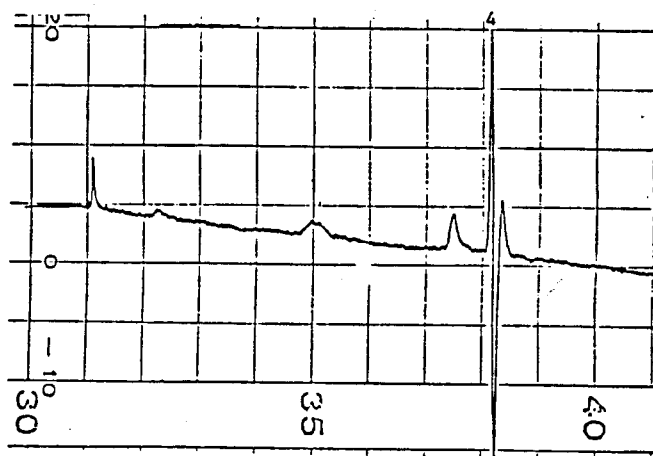


Figure 3

Column: Protein I-125
(7.8mm ID X 30cm X 2)

Solvent: 0.15M K_2HPO_4 pH 7.4

Flow Rate 1.5 ml/min

Detector: UV 280nm 0.04AUFS

Peak #	Component
4	Adrenodoxin MW: 13,000