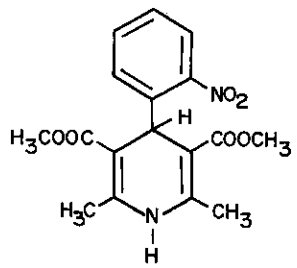
CAPSULES
preliminary notes and applications from Bioanalytical Systems, Inc.

Nifedipine in Serum

Purpose
Determination of nifedipine in serum.



NIFEDIPINE

Figure 1. Structure of nifedipine.

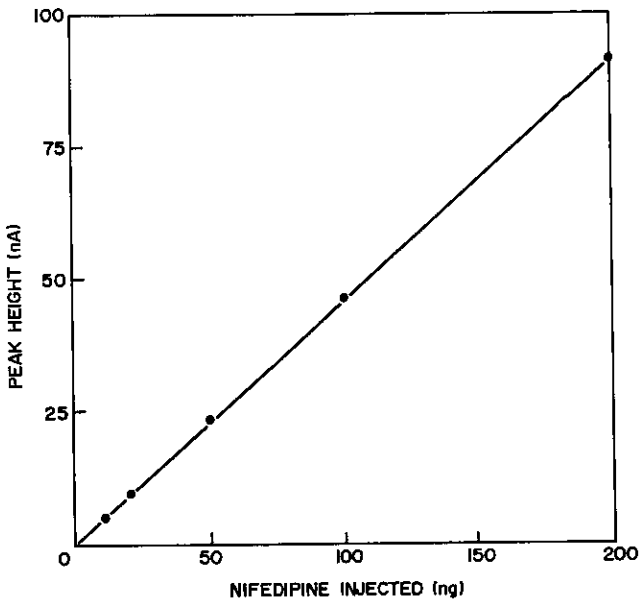


Figure 2. EC Calibration curve for nifedipine standards. Each point represents the mean of two determinations.

Nifedipine (methyl-1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridine dicarboxylate, F1) is a potent and long-lasting vasodilator belonging to the calcium antagonist class of pharmaceutical agents. Calcium antagonists inhibit the flow of calcium ions across the membranes of smooth muscle cells. As a result, muscle tone is relaxed and muscle spasms are diminished. Such relaxation is an effective treatment for vasospastic and effort-associated angina. Therapeutic concentrations of nifedipine range from 25-100 ng/mL blood [1].

Existing Methods
Nifedipine has been measured in biological fluids by fluorescence, TLC, GC-MS and GC-ECD. These methods may be non-specific, lack sensitivity, or require prior oxidation of nifedipine. Nifedipine also can be determined by LCUV [2] and by dual-series EC. The dual-series configuration first reduces nifedipine at the upstream electrode, then detects these products by oxidation at the downstream electrode. Sensitivity is enhanced and the need for rigorous deoxygenation of mobile phase and samples is eliminated by this procedure [3]. For single-detector EC systems, however, nifedipine may still be detected by either oxidation or reduction. Below we demonstrate oxidative EC and UV detection of nifedipine.

Conditions
System: BAS 400 Liquid Chromatograph
EC Detector: BAS LC-4B
Electrode: BAS glassy carbon
Potential: +0.95 V vs Ag/AgCl
UV Detector: BAS UV-108 variable wavelength (237 nm)
Column: BAS 3 µm Phase II ODS reverse-phase (100 x 3.2 mm) (PN MF-6213)
Mobile Phase: 65% (v:v) 0.05 M sodium acetate adjusted to pH 4 with glacial acetic acid, 35% acetonitrile. Flow rate was 1 mL/min.
Detection Limit: EC - 150 pg injected standard, 1.7 ng/mL serum (S/N = 3). UV - 250 pg injected standard, 3 ng/mL serum (S/N = 3).
Linear Range: EC: 150 pg to at least 200 ng injected standards, 5 to 100 ng/mL serum. UV:

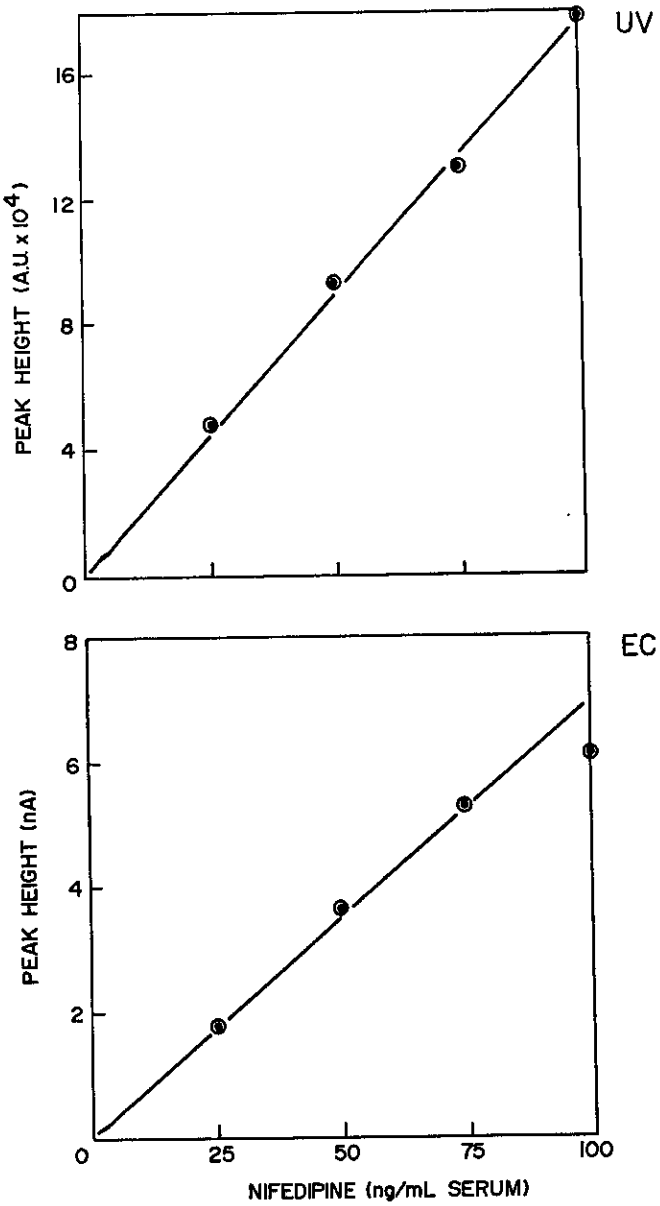


Figure 3. EC and UV calibration curves for spiked serum samples. Each point represents the mean of two determinations.

250 pg to at least 200 ng injected standards, 5 to 100 ng/mL serum.

Sample Preparation

1. To 15 mL centrifuge tubes add the following: 0.5 mL serum, 0.5 mL 1 M NaOH, appropriate quantities

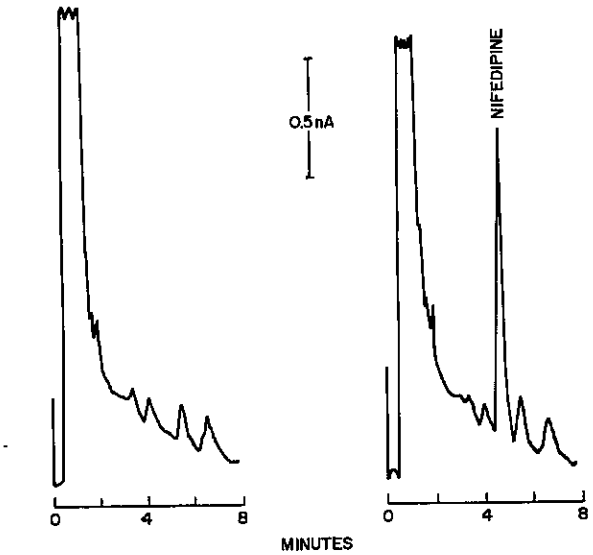


Figure 4. Chromatograms of blank (left) and spiked (100 ng/mL) serum samples.

of nifedipine or internal standard, and 5 mL pentane:dichloromethane (7:3) extraction solvent.

2. Vortex the tubes for 60 seconds.

3. Centrifuge for 5 minutes at 2000 x g.

5. Transfer the organic layer to clean tubes and dry in a stream of nitrogen or a vacuum evaporator.

5. Redissolve the samples in 0.250 mL of mobile phase. Filter through 0.45 µm filters (PN MF-5655) by centifugation at 1600 x g in MF-1 microfiltration tubes (PN MF-5500) and inject 50 µL aliquots into the chromatograph.

Notes

Nifedipine is extremely light sensitive. It photodecomposes after exposure to UV or visible light [3]. All procedures must be carried out under reduced light.

11-Ketoprogesterone [1] and nitrendipine [2] have been used as internal standards.

A calibration curve for EC detection of injected standards is presented in F2. The UV detection curve was nearly identical.

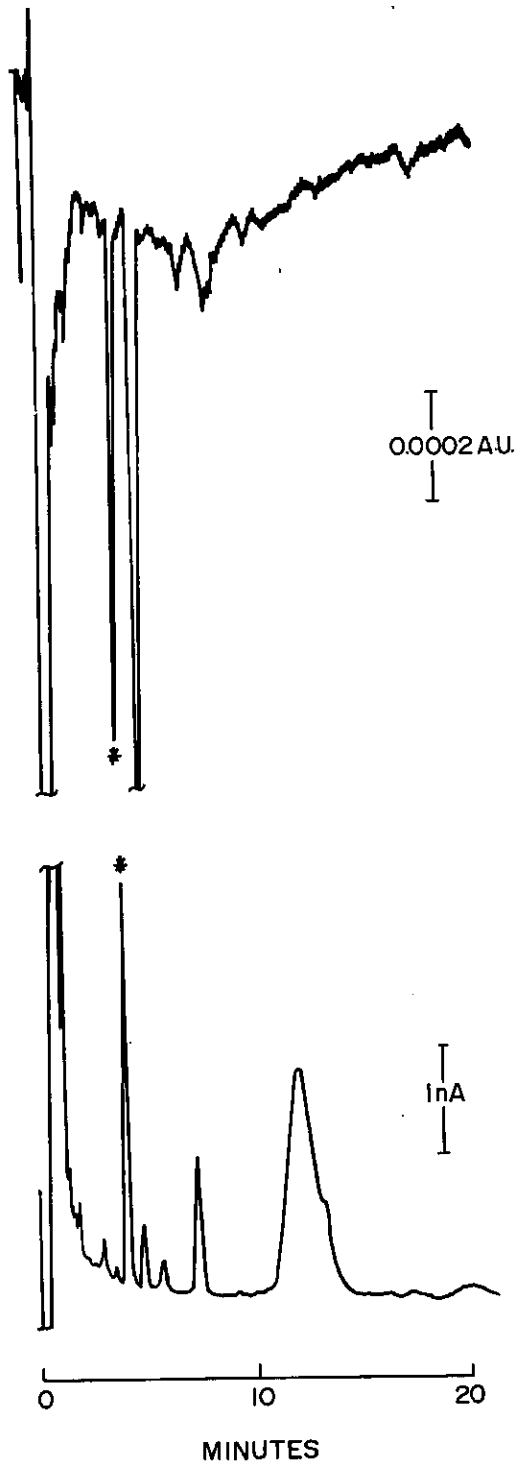


Figure 5. Comparison of UV (above) and EC chromatograms of 50 ng/mL serum samples. * = nifedipine.

Results of EC and UV determinations of nifedipine in spiked serum are presented in F3 and F4. Recovery of nifedipine from serum was 92%. Both detectors were sensitive enough to measure nifedipine at all therapeutic concentrations. Note however that the EC detector apparently became passivated (lost sensitivity) over time. Tests indicated that passivation was related to the number of serum samples that were injected, and not to the amount of nifedipine. (This observation does not apply to the use of either reductive LCEC or dual-series LCEC.) A chromatogram of an extended run (F5) shows the many EC active compounds present in serum extracts at the relatively high potential of +0.95 V used here. Notice that the UV detector is much less sensitive to these compounds.

References

1. P.R. Bach et al., Clin. Chem. 29 (1983) 1344-1348.

2. C.H. Kleinbloesem and J. Van Harten, J. Chromatogr. 308 (1984) 209-216.

3. K. Bratin and P.T. Kissinger, Current Separations 4 (1982) 4-8.