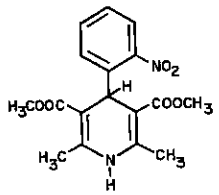
CAPSULES
preliminary notes and applications from Bioanalytical Systems, Inc.

Determination of Nifedipine in Plasma

Purpose
Determination of nifedipine in plasma.

Nifedipine is a potent and long lasting vasodilator



belonging to a group of calcium antagonists that are widely used as coronary vasodilators. The major therapeutic application is for angina pectoris and hypertension. Nifedipine has also been shown efficacious in the treatment of hypertrophic cardiomyopathy, rest angina refractory to β-blockers and nitrates, primary dysmenorrhea, acute pulmonary edema, and in structural preservation of myocardium.

Nifedipine is more than 90% absorbed from oral doses and almost completely metabolized in animals and man. Nifedipine is extremely light-sensitive, yielding nitrosopyridine or nitropyridine after exposure to vis or UV light, respectively. The metabolites and photodecomposition products are pharmacologically inactive.

Existing Methods
In biological fluids nifedipine and its metabolites have been predominantly determined by fluorescence, TLC, GC, and LCUV. The fluorometric method lacks selectivity while the TLC methods lack sensitivity. GC methods lack selectivity or specificity; precolumn and on-column oxidation of nifedipine makes the detection of minor metabolites almost impossible. LCUV methods lack sensitivity, requiring large amounts of plasma.

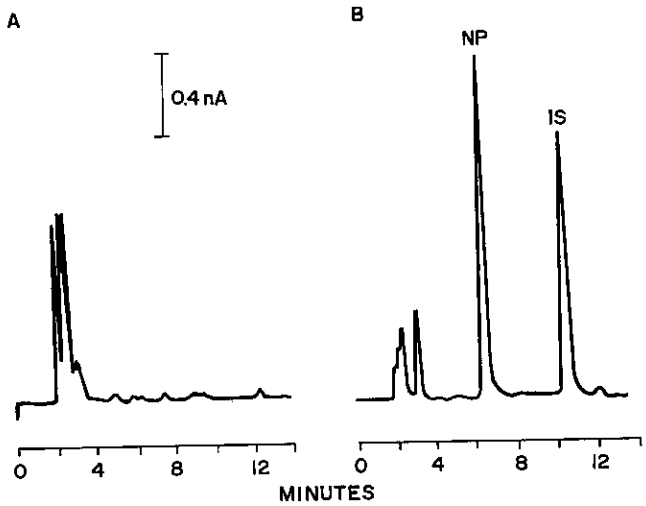


Figure 1. Chromatograms of an extract from A) human blank plasma, and B) human plasma after oral administration of nifedipine. Peaks: NP-nifedipine, IS - internal standard.

Reference
Determination of Nifedipine in Human Plasma by High-Performance Liquid Chromatography with Electrochemical Detection, H. Suzuki, S. Fujiwara, S. Kondo and I. Sugimoto, J. Chromatogr. 341(1985) 341-347.

Conditions
Electrode: Glassy Carbon
Potential: +0.95 V vs. Ag/AgCl
Column: 5 µm C-18 reverse-phase (150 x 4.6 mm)
Mobile Phase: Methanol:tetrahydrofuran:0.05 M phosphate buffer (pH 3.0), 66:1:33. Flow rate 0.8 mL/min.
Detection Limit: For a standard solution, 40 pg

injected. For a plasma sample (0.5 mL treated as outlined below), 2 ng/mL, corresponding to 100 pg injected.

Linear Range: 5-400 ng/mL in plasma samples.

Sample Preparation

To 0.5 mL of plasma containing internal standard, add 0.5 mL of 0.1 M borate buffer (pH 9.0) and vortex. Extract the alkalinized plasma with 6 mL of toluene. Transfer a 5 mL aliquot of the organic layer to a separate tube, evaporate to dryness, and dissolve in 100 µL of the mobile phase. Inject 10 µL into the chromatograph. All steps should be carried out in a dark room and samples shielded from exposure to direct light. Recovery of nifedipine for plasma was about 100%.

Clinical Application

Healthy volunteers were administered an oral dose of nifedipine and plasma concentrations were determined over a 7-hour period.

This method is sufficiently simple, sensitive, selective, and rapid to be used for routine clinical monitoring and in pharmacokinetic studies using small animals.

In the present report, twelve drugs were administered in combination with nifedipine and found not to interfere in the assay.

Notes

Nifedipine contains two redox centers, a dihydropyridine ring (oxidizable) and a nitro aromatic group (reducible). The first report dealing with the LCEC determination of nifedipine detected this compound via the reduction of the nitro aromatic group (see reference 1). This same report also detected the photodecomposition product of nifedipine via the reduction of the light-generated nitroso group. LCEC in the reduction mode is well suited for the determination of nifedipine, its photodecomposition products, and metabolites, because all of these compounds retain an easily reducible functionality, an aromatic nitro or nitroso group.

Reductive mode detection requires removal of dissolved oxygen from the sample prior to its injection into the LCEC system. Sample oxygen is reduced to H_2O_2 at the electrode, and will mask early eluting analytes. Removal of oxygen from small samples can be a problem, but published methods which work well are available (see references 2-4). The sample dissolved-oxygen problem can also be minimized by utilizing a series dual-electrode transducer (see references 1 and 2). The electroactive components of a sample, as well as dissolved oxygen, are reduced at the upstream electrode. The generated electroactive products, and other easily oxidizable components of a sample, are then quantitated (oxidized) at the downstream electrode. (The oxidizing potential of the downstream electrode must be set to discriminate against the oxidation of reduced oxygen, H_2O_2.)

The series dual-electrode technique was used by Bratin and Kissinger (reference 1) to determine nifedipine, and nitro and nitroso pyridine in plasma. As with the reduction mode, the series dual-electrode technique is well suited for the determination of nifedipine, its photodecomposition products, and metabolites: these compounds contain a functionality, an aromatic nitro or nitroso group, that can be reduced upstream and the reduction product detected downstream (at an oxidizing potential).

Analytes containing an aromatic nitro or nitroso group can be detected, utilizing the series dual-electrode techniques, in an LCEC system where the mobile phase and sample have not been deoxygenated (see reference 2 or consult BAS for more details).

Related References

1. The Electrochemical Behavior of Nifedipine and Related Compounds and the quantitation of Nifedipine in Plasma by LCEC, K. Bratin and P. Kissinger, Current Separations, 1982, Vol. 4, No. 1 (BAS Press).

2. Use of Series Dual Electrodes for Elimination of Sample Oxygen Interference, W. Jacobs, Current Separations, 1982, Vol. 4, No. 3 (BAS Press).

3. LCEC Forum, Current Separations, 1983, Vol. 5, No. 3, 53 (BAS Press).

4. *Effect of a Low-Dead-Volume Deoxygenator on Peak Broadening Using Reductive Electrochemical Detection, L.R. Taylor, LC, Liq. Chromatogr., HPLC Mag., 1986, Vol. 4, pg. 34.*

The information in this publication is supplied as a service to our customers. All of the chromatographic operations described here can be carried out on the BAS 200 Problem Solver. Performance of the literature methodology has not necessarily been verified by the BAS technical staff.